Introduction

1.1   Oil palm

1.1.1   Taxonomy

The African oil palm, *Elaeis guineensis*Jacq., is placed in the Arecaceae family along with coconut,the American oil palm and date palms. There are three naturally occurring forms of the oil palm fruit, termed *Dura, Tenera*, and *Pisifera*. Most cultivars are the *Tenera* form which produces fruit with higher oil content (Duke, 1983).

1.1.2   Botanical description

Oil palm can reach 60-80 ft in height in nature, but is rarely more than 20 or 30 ft in cultivation. Leaf bases are persistent for years, and prominent leaf scars are arranged spirally on the trunk of mature palms where bases have fallen. Leaves are up to 25 ft in length, with leaflets numbering 200-300 per leaf, about 3-4 ft long and 1.5-2.0" wide, with entire margins. The leaflets are 60-120 cm long, 3.5-5 cm broad; with a very strong central nerve, especially at the base. The flower-stalks from lower leaf-axils, 10-30 cm long and broad male flowers on short furry branches 10-15 cm long. The flower set close to the trunk on short pedicels; female flowers and consequently fruits in large clusters of 200-300. These clusters close to trunk on short heavy pedicels, each fruit plum-like, ovoid-oblong to 3.5 cm long and about 2 cm wide.The fruit black when ripe, red at base, with thick ivory-white flesh and small cavity in center; kernel encased in a mesocarp which contains the oil. About 5 female inflorescences are produced per year; each inflorescence weighing about 8 kg, the fruits weighing about 3.5 g each (Duke, 1983).

The oil palm is a vertical monocot plant that produces separate male and female inflorescences in leaf axils. The inflorescence of both sexes is a compound spadix with 100-200 branches, initially enclosed in a spathe or bract that splits 2 weeks prior to anthesis (Duke, 1983).

1.1.4 Pollination

Oil palm is cross-pollinated and the key pollinating agent is the weevil, *Elaeidobius kamerunicus* Faust. Earlier, oil palm was thought to be wind pollinated. Owing to the low level of natural pollination, assisted pollination is a standard management practice in plantations.Earlier, oil palm tree was thought to be wind pollinated owing to the low level of natural pollination.This practice was discontinued following the discovery that oil palm was insect pollinated and the introduction of *E. kamerunicus* from the Cameroons, West Africa in 1982 (Syed et al., 1982; Jaligotet al., 2001; Mayes et al., 2008).

1.1.5 Fruit

As in many palms, oil palm fruits are drupes. The mesocarp and endocarp vary in thickness, with *Dura* types having thick endocarps and less mesocarp, and *Tenera* types the opposite. The exocarp color is green changing to orange at maturity in the *virescens* types, and orange with brown or black cheek colors in the *nigrescens* types. The fruit ranges in size from <1 cm to 2 cm, and are obovoid in shape. The mesocarp, from which palm oil is derived, is fibrous and oily, and the seed is opaque white, encased in a brown endocarp; palm kernel oil is derived from seeds. The female infructescence contains 200-300 fruit, and fruit set is 50-70%. The fruit usually ripens at about 5-6 months after pollination (Duke, 1983). Figure 1.1 (A & B) shows the oil palm tree and fruit.
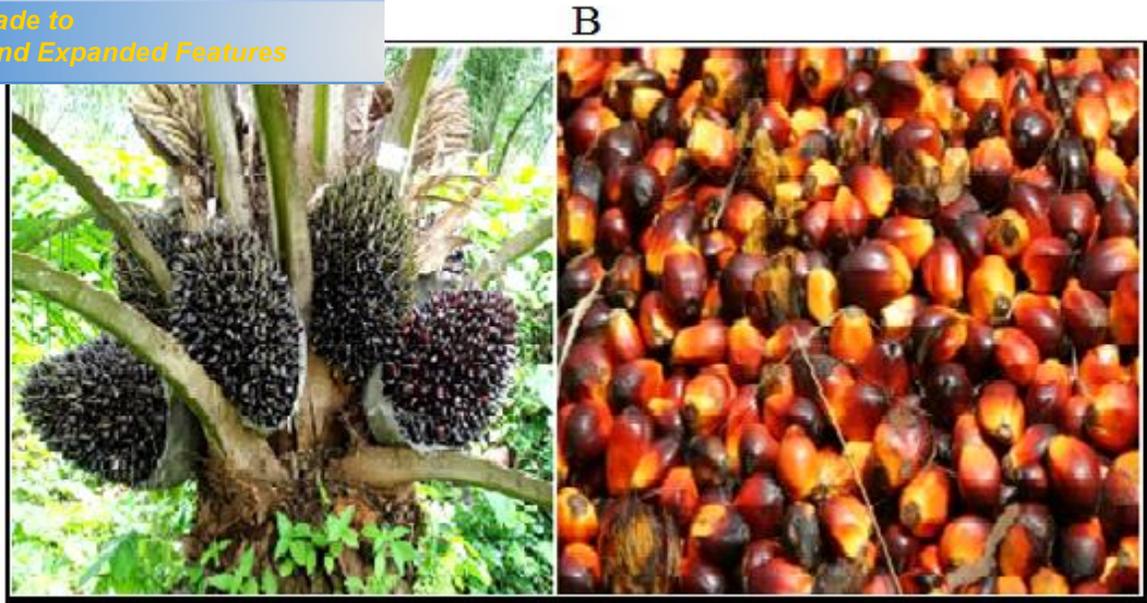
Figure1.1: Oil palm tree (A) and fruits (B).

1.1.6 History

The oil palm, *Elaeis guineensis* Jacq.originated from West Africa where the main palm belt ran through Sierra Leone, Liberia, the Ivory Coast, Ghana and Cameroon in the equatorial regions of the Republics of Congo and the Democratic Republic of the Congo (Hartley, 1988).

The development of oil palm as a plantation crop started in Southeast Asia when four seedlings of the African oil palm from Amsterdam were planted in the Botanic Gardens in Bogor-Indonesia in 1848. The business of oil palm plantation was first established in Sumatra-Indonesia by M. A. Hallet, an agronomist from Belgium. The development of the industry in Malaysia was attributed to a Frenchman, Henri Fauconnier, who was an associate of Hallet. About 1870, the seedlings were transferred to the Singapore botanical gardens and then to Malaysia in 1875. The African oil palm has also been taken to central

was first cultivated in Brazil, and later to Colombia and other neighboring countries (Hartley, 1988).

In 1911, Fauconnier visited Hallet's oil palm development in Sumatra and had purchased some oil palm seeds and these were planted at his Rantau Panjang Estate in the state of Selangor. He returned to Sumatra the following year to obtain seeds that he had selected together with Hallet from Tanjong Morawa Kiri Estate for further planting. With seedlings obtained from the year 1911 and 1912 importation, Fauconnier established the first commercial oil palm planting at the Tennamaram Estate, to replace an unsuccessful planting of coffee bushes (Tate, 1996).

Elaeis guineensis Jacq.is consideras the most important species in the genus *Elaeis* which belongs to the family Palmae. The second species is *Elaeis oleifera* (H.B.K) Cortes which is found in South and Central America and is called the American Oil Palm. Although clearly lower in oil-to-bunch content than its African equivalent, *E. oleifera* has a higher level of unsaturated fatty acids and has been used for production of inter-specific hybrids with *E. guineensis*.The African oil palm (*Elaeis guineensis*) has become one of the most important oil production crops, as it is used in a wide variety of ways throughout the world. It is a source of materials for foodstuffs and medicines. Oil palm blossom liquid is used to make palm wine, and its fibers are also used for various purposes. Their most important purpose until now has been the extraction of palm oil (from the flesh of the oil palm fruit) and palm kernel oil (from its kernel or seed) for the manufacturing of edible and industrial oils. The increasing demand for palm oil in 2010 saw world exports increase to 38.8 million tones out of which Malaysia exported about 17 million tones. Oil palm plantations feed a growing global demand for cheap vegetable oil used in the

4

and fuel or biodiesel from crude palm oil (Laurance, 2003). Compared to the year 2000, demand for palm oil is predicted to more than double by 2030 and to triple by 2050(Brown and Michael, 2005).

Each oil palm tree produces compact bunches weighing between 10 and 25 kilograms with 1000 to 3000 fruitlets per bunch. Each fruitlet is almost spherical or elongated in shape; the oil palm tree will start bearing fruits after 30 months of field planting and will continue to be productive for the next 20 to 30 years; thus ensuring a consistent supply of oils. Each fruit bunch which weighs between 15 and 25 kg, are made up of between 1000 and 4000 oval-shaped fruits, each measuring about some 3 to 5 cm long. At the time of harvesting, the fleshy part of the fruit is converted into oil through a series of processes, while the palm kernel oil is extracted from the nut itself. Processing of the crude oil ends with three different products: 1) Food products (cooking oil, margarine, sweets); 2) manufactured goods (cosmetics, soaps, detergents, candles, lubricants) and 3) fuel (biodiesel) (Ricardo, 2006).

1.1.7   Plantations around the world

The commercial oil palm, *Elaeis guineensis*, is believed to be indigenous to West Africa (the specific name, *guineensis* shows that the first specimen described was collected in Guinea, West Africa). There is a general idea that commercially planted palms in Indonesia, Malaysia and other Southeast Asian locations were derived from four West African palm varieties. The history of oil palm as a commercial crop is rather short, dating back to 1807 on the West African coast where its cultivation commenced (Tan et al., 2009).

...ablished first and foremost in tropical regions, where they covered a total of about 6.5 million hectares in 1997 and produced 17.5 million tons of palm oil and 2.1 million tons of palm kernel oil. By the year 2005, the area filled by oil palm plantations had grown to 12 million hectares and palm oil production had reached about 30 million tons. In other words, both the area occupied by plantations and oil production had almost doubled in less than 10 years. Presently, Indonesia and Malaysia are the biggest producers of palm oil, together accounting for about 83% of the world's total production and 89% of the world's export (Dimelu and Anyaiwe, 2011).

The most important development in the recent history of the crop was the discovery of the inheritance of shell thickness. The recognition that the *Tenera* fruit form was a hybrid between the thick-shelled *Dura* andtheshell-less*Pisifera*forms allowed *Tenera* planting material to be produced by controlled pollination. As the *Tenera* yields some 30% more oil than *Dura* at no extra cost, profitability of the crop was transformed and rapid expansion of plantings occurred from the nineteen sixties onwards. Extensive breeding and agronomic research has been done by institutes such as CIRAD-CP in France, the Nigerian Institute for Oil Palm Research (NIFOR) and the Malaysian Palm Oil Board (MPOB), and also by private plantation companies. As a result, yields have more than quadrupled since the early days. The best fields in Indonesia give peak yields of 30-35 tons FFB per year, with an oil extraction rate of 25%, to giveover 7.5 tons oil/ha per year, together with 1.5 tons of kernels (Abubakar, 2004).

Research on vegetative propagation by tissue culture has been in progress for many years, but as yet only a few hectares of clonal palms have been planted because of somatic variation issues (Jaligot et al., 2011).

...nical distribution.

The natural habitat of the oil palm lies between latitude 10° north and south of the equator. It is within this region that the biggest areas of semi-wild *Elaeis guineensis* groves in the low land regions of Southeast Asia and Western Africa lie. This region has a marked dry season lasting up to four to five months (Western Africa only). While the oil palm is ecologically suited to such conditions, physiologically it gives lower yields. Under plantation conditions, the cultivation of oil palm has been extended from the natural habitat of 10ºN and 10ºS of the equator to 23ºN and 23ºS, i.e. between the tropics of Cancer and Capricorn.

Annual rainfall in these regions ranges from a low of 1500 mm in the derived Savannah to a high of 3000 mm in the tropical rain forest (Abubakar, 2004). Oil palm yields more oil per hectare than any other oil crops (Corley and Tinker, 2003). It is grown in several tropical countries under a wide range of rainfall conditions. The optimum annual rainfall for oil palm to achieve its maximum production capacity is 1,800 mm, well distributed throughout the year (Nodichao et al., 2011).

1.1.9   Climate and soil requirements

It has been found that adequate soil moisture is more important than nutrient supply for oil palm growth, which can be supplied artificially. A mean minimum temperature of 20.0-23.0°C and a mean maximum temperature of 28°C-32°C, which is peculiar to tropical countries, are best suited. If the temperature falls below the range, particularly at night, bunch development is affected and yield reduced. Growth in young seedlings stops at temperature of below 15°C.

7

...t least 5 hrs of sunshine per day in all months is desirable, rising to 7 hrs per day in some months. Tropical climates with ample sunshine, about 5-7 hrs day in all months with high CO_2 concentration is good for oil palm tree to grow. Oil palm can be grown on a wide range of soils, the best being the coastal alluvial clay, riverine and coastal alluvial and soils of volcanic origin (Abubakar, 2004).

1.1.10 Crop varieties

The Oil Palm (*Elaeis guineesis*) is an unbranched monoeciousplant. It can grow to a length of 20 meters – 30 meters and lives up to 200 years. The oil palm has a typical adventitious root system. All mature plants have a solitary columnar stem with persistent frond bases, where the stem supports a crown of fronds (Mahmud, 2009).

The frond consists of leaflets, each with a lamina and midrib, a central rachis to which the leaflets are attached, a petiole and a frond sheath. The oil palm produces either a male or female or at certain stages hermaphrodite inflorescence in each of the frond axil during the mature stage. The proportion of female inflorescences to total inflorescences (sex ratio) determines the yield. High yield tend to be obtained with a high sex ratio. The female inflorescence, when its flowers are pollinated and fertilized, becomes the fresh fruit bunch (FFB) of commerce. The fertilized flower produces a fruit, which grows and ripens over about 6 months. The oil palm fruit is a sessile drupe and consists essentially of an exocarp (skin), a fleshly mesocarp which contains palm oil, a hard stony endocarp (shell) and kernel (seed) the source of the kernel oil and meal.

three main varieties of oil palm distinguished by their fruit characteristics. These are: *Dura*, which has a thick shell separating the pulp from the kernel,thekernel tends to be large comprising 7% - 20% fruit weight;*Tenera,* which has a thin shell between pulp and kernel, together with a fibrous layer round the nut. The kernel is usually smaller, comprising 3% - 15% of fruit. The oil content is higher at 24% - 32%;*Pisifera*, which has no shell and is very frequently female, is sterile. As a result of their very marked tendency to female sterility, *Pisifera* palm are not used for commercial planting. The pericarp is composed of a large number of oil cells, which are full of oil when the fruit is freshly ripe. Cement binds the cells to one another and to a skeleton of hard fibers running lengthwise through the pericarp and keeping it together (Maizura, 1999).

The most cultivated high yielding oil palm variety, the thin shell *Tenera* (oil:bunch (O/B) >20%)is produced when the thick shell *Dura* (O/B ~ 17%) crosses with the shell-less *Pisifera*.The *Pisifera,*which is female sterile is used as the pollen source. Previously, seed production solely relied on the Deli *Dura* as the maternal parent with the exclusive use of the AVROS *Pisifera* as the pollen source. Collection of oil palm genetic materials were carried out by Malaysian researchers to widen the genetic base for breeding and to ensure conservation of palm genetic resources

The collection for *Elaeis guineensis* started in Nigeria early in the 1970's followed by other countries in Western and Central Africa and the island of Madagacar (Rajanaidu and Jalani, 1994 a, b),while*Elaeis oleifera* genetic resources from six Central and South American countries were also collected (Rajanaidu and Jalani, 1994b).The development of oil palm growing went hand in hand with the establishment of research organisations,

neighboring planting regions in the 1900–1920s to build up germplasm collections. The genetic base constituted in that way and the selected materials resulting from it bear the name of those organisations (Corley and Tinker, 2003). This set forms the genetic base for oil palm breeding. One of the factors that causes a significant increase in oil palm yields was the discovery of the better production potential displayed by crosses between the African "origins" and the Deli introduced and improved in Southeast Asia (Cochard et al., 2009).

For a better understanding for the complementarily existing between the Africa and "Asia" origins, and to diversify the genetic base taking this constraint into account, it is necessary to describe the structure of the genetic resources involved in the history of oil palm breeding in relation to the subspontaneous African origins. This would also enable the determination of integration into breeding programmes and the selection strategies should be adapted.

Previously, the results from genetic marker and genome methylation studies showed that the tissue culture abnormalities in oil palm arise from interaction of genetics and epigenetic mechanism. Different efforts are geared towards developing diagnostic tools for predicting genetic predisposition to abnormality(Tregear et al., 2002).These include large-scale gene expression analysis via DNA microarray, genetic mapping and the candidate gene approach. It is anticipated that an effective screening process, will provide greater confidence to the industry in producing and utilizing clones (Cheah, 2003). Considerable advances have also been made in oil palm genetic engineering over the last 10 years.

Virtually most of the genes and promoters required to modify the fatty acid biosynthetic pathway have been obtained. Currently, technology is available to produce three novel

eate, high in strearate and high in palmitoleic. Nevertheless, there are many pressing issues that need to be addressed in the immediate future such as developing a better transformation method with low copies of trans-genes, and assurance of bio-safety, and public acceptance (Mohd et al., 2004).

MPOB has the biggest oil palm germplasm collection in the world (Mohd et al., 2004). Molecular fingerprinting (using RFLP) studies in oil palmshowed genetic variation in the oil palm population, which can be used to explore the germplasm collections, particularly those that gave higher level of diversity for further characterization and exploitation. The final aim is to seeknovel and valuable genes to diversify the oil palm crop and palm oil products (Maizura et al., 2006).

1.1.11 Oil production and importance

Global interest in sustainable agriculture requires a review of how the oil palm plantations have evolved in ensuring that palm oil is produced according to the set standards of responsible production practices. Many inherent advantages are already within the oil palm system of Plant Physiologyogy of high productivity and efficient carbon assimilation (FAOSTAT, 2007).

With the growing global demand for agricultural products, landscapes throughout the tropics are increasingly dominated by agriculture (Jaligot et al., 2011). The increase in oil palm (*Elaeis guineensis*) cultivation is cited as a major driver of deforestation and biodiversity loss in tropical countries (Donald, 2004; Koh and Wilcove, 2007, 2008) and it also has major impacts on global carbon balance (Danielsen et al., 2008). Oil palm plantations expanded almost exponentially in recent decades (Donald, 2004), covering over

...utheast Asia (Danielsen et al., 2008). Palm oil is now the world's most traded oilseed crop with uses from cooking oil to biofuel (Carter et al., 2007) and with over 37 million metric tons produced in 2005 (Turner et al., 2008). In 2006, 85% of the global oil palm crop was produced by Indonesia (43%) and Malaysia (42%) (FAOSTAT, 2007).

The oil palm is the highest yielding oil seed crop. It yields on the average 3-4 metric tonnes of palm oil per hectare/year while other competing oil seeds yield less than 1 metric tonnes/hectare/year (Mohd et al., 2004). In terms of production, it has exceeded all other oil seeds since 1980. By 2005, annual oil palm production stood at 15.4 million tones contributing a disproportionate 95% of total oil seed production. The expansion of oil palm plantings in Malaysia during the past 41 years has been phenomenal. From a 0.26 million ha in 1970, the oil palm planted area had expanded to 4.5 million hectares by 2008, occupying 60% of the agricultural land in the country (Figure 1.2 and Figure 1.3). About 60% of the planted area is in Peninsular Malaysia; 30% in Sabah and 10% in Sarawak.
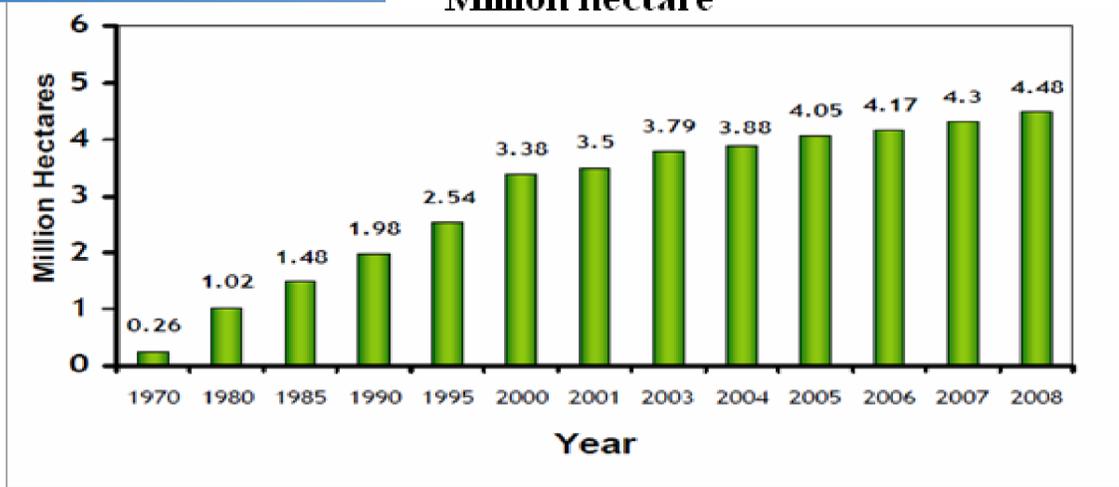
Figure 1.2: Expansion of oil palm plantations in Malaysia from 1970-2008 (MPOB and APOC, 2010)
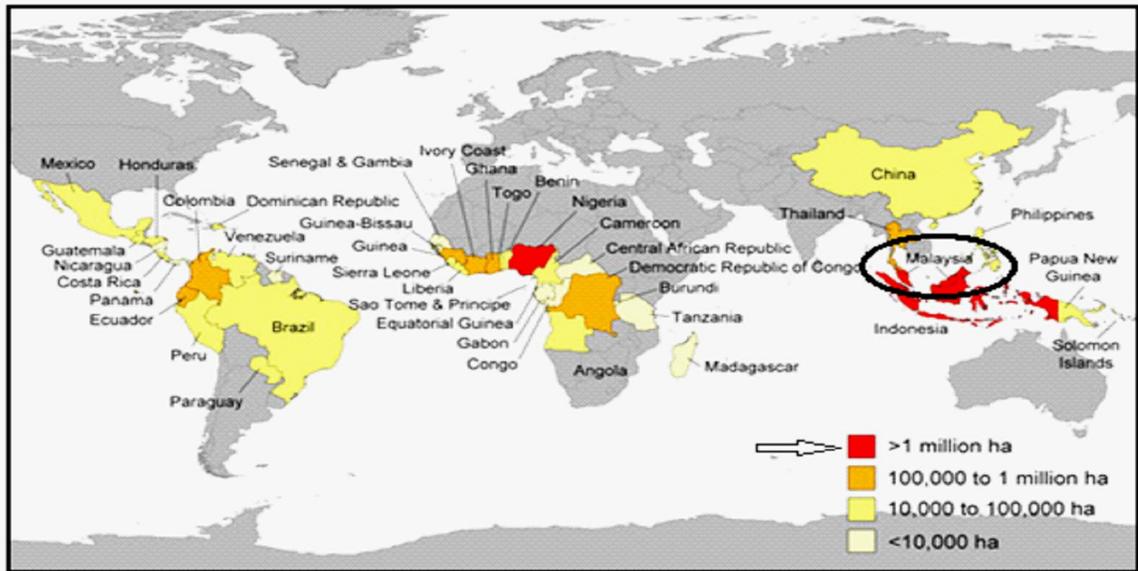

Figure 1.3: Map showing the extent of oil palm cultivation in 43 oil palm-producing countries in 2006 (FAO 2007).

the world's leading exporter and producer of palm oil in 1966 and 1971, respectively (Gopal, 2001; Harcharan, 1976). By 1986, when the Industrial Master Plan (IMP) was launched, oil palm had become Malaysia's leading agricultural commodity and third-largest export earner.

Malaysia now accounts for about half of the world production of palm oil; its plantations, processors, and manufacturers are generally regarded as operating at the industry's technological frontier. Malaysia evolved from simple cultivation and crude oil processing to become the industry's leading innovator (Rasiah, 2006; Dimelu and Anyaiwe., 2011). The relatively low priced oil is used for a variety of purposes. The world demand for palm oil has soared in the last two decades, first for its use in food, consumer products and more recently as the raw material for bio-fuel. The growing affluence of India and China, the world's top two importing nations, will increase demand of edible vegetable oils. In the US, a recent wave of dietary focus on the trans-fat issues has led to increased consumption. In addition to being less expensive, palm oil is semi-solid at room temperature, making it ideal for baking and food production (Steenblik, 2007).

The oil palm fruit is unique in producing two types of oils from the same fruit – palm oil from the flesh or mesocarp and palm kernel oil from the seed or kernel inside the hard-shell mesocarp (Henson, 1999). The oil palm kernel also yields a residual product known as palm kernel meal, which is mostly used for animal feed. The two oils are extracted by careful separation at certain stages of the milling process. Palm kernel production is about 10% of the quantity of palm oil produced. The two oils have very different applications and market outlets. Palm oil is used mainly for food, while palm kernel oil goes mainly

for making soaps, detergents and toiletry products (Yusof, 2006)and is used to make specialty fats for various food products (Khoo, 2001).

Palm oil, is a well balanced healthy edible oil, is widely acknowledged as a versatile and nutritious vegetable oil;trans fat free, with a rich content of vitamins and antioxidants (Xu et al., 2011). Palm oil is a major ingredient in many foods. In many margarines, for example, palm oil provides the body or texture such that no further modification of the oil is necessary, a superb bonus in the current health conscious world. Margarines containing mostly partially hydrogenated fats are being phased out for natural formulations and palm oil has become a favored ingredient.

Often, palm oil is incorporated as the preferred solid fat to achieve dated "zero-trans" formulation. By suitable modifying palm oil through fractionation and interesterification of blending, it is possible to produce a wide variety of margarine incorporating various palm fractions and thus achieving zero-trans content. Reduced fat spreads are formulated to contain 40-80% fat content and are sold as soft tubs, stick, liquid and whipped forms.

Besides its use as an edible vegetable oil, the current interest in biodiesel has placed it in high demand and palm oil high prices all time (Billotte et al., 2005). In addition, palm oil is the largest natural source of tocotrienol, a member of the vitamin E family, and is also high in vitamin K and dietary magnesium (Agrawalet al., 2009).

Palm oil comprises show two component oils obtained from the kernel and the mesocarp, respectively, and with different acid compositions (Figure 1.4). Palm oil contains saturated palmitic acid (44.3%), oleic acid (38.7%), and linoleic acid (10.5%), giving it higher unsaturated acid and long-chain fatty acid triglyceride content (Cater et al., 1997).

ils obtained from coconut (*Cocos nucifera*) and Wright's waxweed (*Cuphea wrighti*), palm kernel oil has a high content of saturated acids and medium-chain fatty acid triglycerides, mainly lauric acid (48.2%) (Larson et al., 2003).These two types of palm oil provide a good model for the exploration of the mechanism of biosynthesis of fatty acids.

Figure 1.4: Dissected mature nut of oil palm (*E. guineensis* Jacq.) (Xu et al., 2011).

Many food manufacturers are trying to find alternatives to trans-fat, partially hydrogenated oils, which contribute to heart disease and other medical problems. The focus on the trans-fat issue has resulted in palm oil being considered more healthful than some other fats. The other major factor of palm production is its role in sustainable energy campaigns around the globe. European countries have promoted the use of palm oil by injecting hundreds of millions of dollars into national subsidies towards bio-diesel. Europe is now a leading importer of palm oil. Through the subsidizing of bio-fuels, European governments have accelerated the demand for palm oil in Europe, and as a consequence have

large areas of rainforest in Southeast Asia (USDA, Foreign

Agriculture Service 2009).

Many economists predict it will be the leading internationally traded edible oil by the year

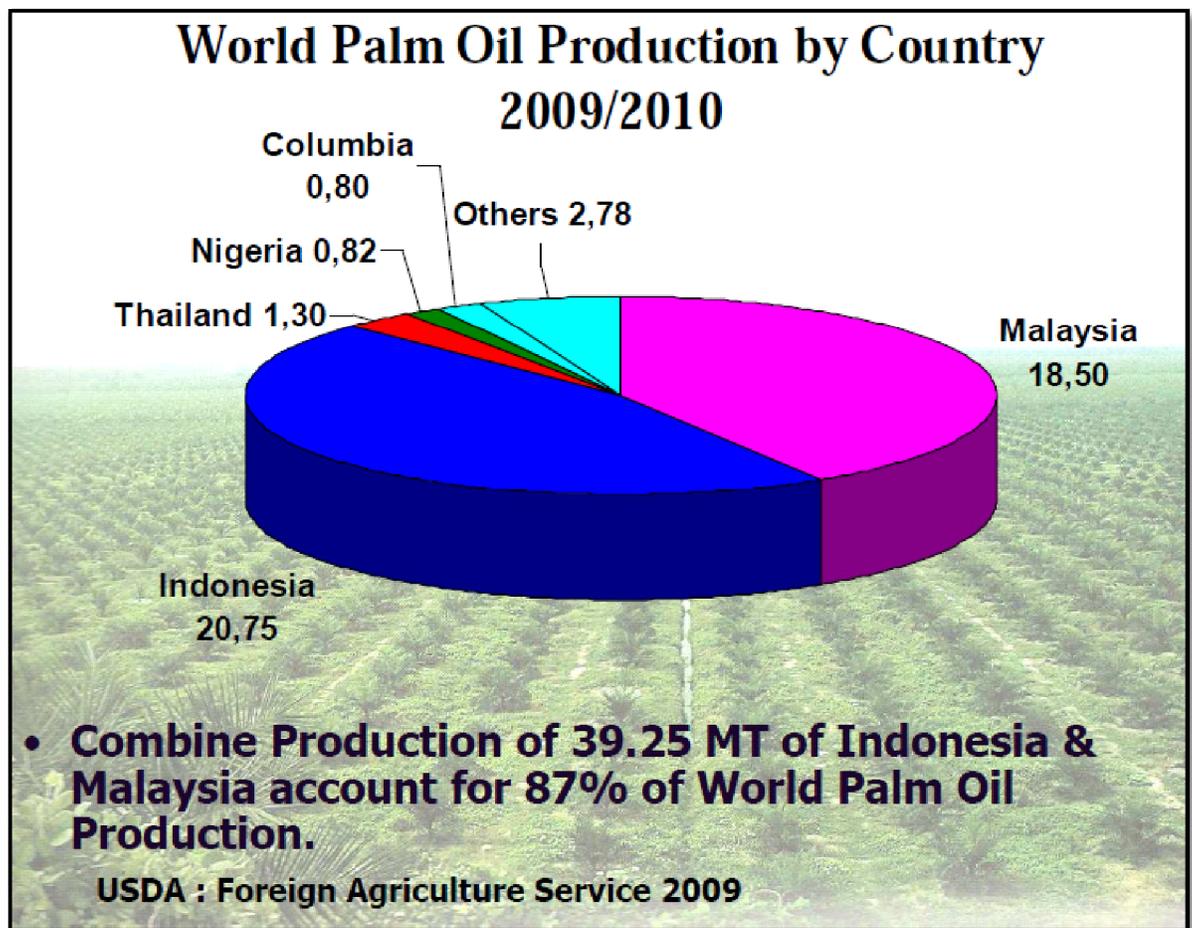2012. Today, Malaysia and Indonesia account for about 87% of world production (Figure

2.5).

Figure 1.5: World palm oil Production 2010 (Metric ton), USDA, Foreign Agriculture
Service 2009.

Successful infection needs complicated signal exchanges at the plant surface and the intercellular space interface in plant–fungus interactions (Müller et al., 2011). In the first stages of infection, receiving of external signals plays an important role in stimulating developmental and morphogenetic processes following incursion of the host epidermis. Signal transferring, differentiation and growth of the structure and manipulation of the host plant cell are facilitated throughout a variety of extracellular effector molecules and proteins. These kinds of molecules are usually secreted into the intercellular space between fungal cell and the host plant or delivered inside the plant cell (Lucas, 2004). The analysis of genome sequences of plant-infecting fungi supports the particular importance of these secreted proteins, and there remains a lot of work targeting the identification of genes coding extracellular proteins despitemany years of research (Dean et al., 2005).

So far, researche on plant–fungal interactions mainly focused on the molecular changes related to fungal attack and to plant response. Severaldefense factors and signal molecules have been identified in plants. Nevertheless, the molecular bases of multiple-player systems that may produce beneficial effects on plant health are largely not fully understood and represent an area of research and discovery (Marra et al., 2006).

Plant-fungal interactions are very complex because of the high diversity of interactions that can occur between the pathogen and the host. Biotrophic fungal pathogens, which often reprogram host metabolism as they obtain all of their nutrients from living plant cells through productionof cell wall-degrading enzymes which rapidalydestroy the plant tissue.

been achieved in the last few years in the identification of the determinants of plant-fungus interactions. Currently, more than 25 fungal genomes have been elucidated, including human and plant pathogens, such as *Aspergillus fumigatus and Magnaporthe grisea*, respectively (Mehta et al., 2008).

A key challenge in modern fungal biology is to analyse the expression, function and regulation of the entire set of proteins encoded by the revealed fungal genomes. When pathogenic fungi start the infection process, secreted and intracellular proteins are up- or down-regulated, improving the predation ability of the fungi (Murad et al., 2006; Murad et al., 2007). In this field, several proteomic studies have been carried out in order to understand fungal pathogenicity. These include pioneering studies, aimed at understanding the dimorphic transition from budding to filamentous growth (Bohmer et al., 2007), as well as appressorium construction (Grenville-Briggs et al., 2005). Appressorium formation is a very important event in the establishment of a successful interaction between the pathogen *Phytophtora infestans* and its host plant potato (Grenville-Briggs et al., 2005).Proteomic analyses have also been used to study wheat leaf rust, caused by the fungus *Puccinia triticina* (Rampitsch et al., 2006). Rust diseases cause a significant annual decrease in the yield of cereal crops worldwide (Webb and Fellers, 2006).

Fungal diseases are, in nature, more the exception than the rule. Thus, only a limited number of fungal species are able to go through inside the host cell and invade host tissues, avoiding recognition and plant defense responses, in order to obtain nutrients from them, causing disease and sometimes host death. In agriculture, annual crop losses due to pre- and post-harvest fungal diseases exceed 200 billion Euros, and, in the United Stated alone, over $600 million are annually spent on fungicides (Arora et al., 2004).

Basal stem rot (BSR) is the major threat for oil palm (*Elaeis guineensis*) in Southeast Asia especially Malaysia and Indonesia and is caused by the fungus *G. boninense* and it is the most damaging disease for oil palm,and it can cause up to 50% lose of the palms in plantation (Santoso et al., 2011).

The BSR disease, which usually affects matured palms, is lethal and incurable though it has been identified more than fifty years ago (Figure 2.6). It has been found to infect oil palms as early as 12 to 24 months after planting, with increased incidence on 4 to 5 years old palms, particularly in replanted areas (Henson and Mohd, 2003), or areas under planted with coconut palm trees (Ambak and Melling,1999).

BSR has been referred to as a ''significant constraint'' for oil palm production in Southeast Asia (Miller et al., 1999). It has been stated that BSR has been a serious disease of oil palm for over 80 years with severe economic loss in Malaysia and North Sumatra (Flood et al., 2000). Basal stem rot levels of 10–20% have little effect on the total yield in term of fruit bunches due to compensatory growth, again reducing the economic significance of the rot. In spite of these factors, BSR caused by *G. boninense* is still claimed to be the single major disease constraint to oil palm in the Southeast Asia region (Flood et al., 2000).

The fungus was reported to infect 1–2 years after planting with increases in 4–5 year old palms (Ariffin et al., 2000). Occurrence appeared high in oil palm grown inland. The symptoms of the infection comprise a mottling or yellowing of fronds followed by necrosis. Spear leaves eventually remain unopened. It is assumed that, when foliar symptoms were observed, at least one-half of the basal stem has been killed by the fungus. Young palms

ears to die after the first symptoms, but mature palms take about two to three years. The oil palm tree eventually collapses leaving diseased bole tissues in the ground. Basidiomata may develop at the stem base, leaf base, or infected root; the location provides a guide to the diseased area inside the palm.

G. boninense is spread by spores and grows in the non-living tissues, which weakens the tree making it susceptible to wind damage (Paterson et al., 2000). *G. boninense* has been found even on young plants and the disease has been observed at the seedling stage. The fungus has shown to be soil borne, although it can be spread by spores (Paterson et al., 2007).

Figure1.6: Palms afflicted by BSR (Toh, 2008).

Figure 1.7: *Ganoderma boninense* mushrooms at the base of infected oil palm (Markom et al., 2009).

...ction has spread notably through the plant, resulting in its death. The bracket is hard and woody, semicircular in shape and quite large (up to 15 cm in diameter and 5 cm thick). Brackets range in colour from dark to chocolate brown and even orange, often with creamy white edges. The top of the bracket might look glossy and the underside look white. Brackets are generally found at the base of the trunk either singularly or in a cluster, but this is not always present. In the end, the disease is particularly common in aged and clearly stressed plants, which are in natural decline (golden canes) or have reached the end of their life expectancy (black wattles) (Steyaert, 1975). Previous studies showed enzyme activities from *G. boninense* covering some of the structural polymers of oil palm cell walls (Rees, 2006).For successful penetration and degradation of intact roots, production of an array of cell wall degrading enzymes (CWDE) is probably required to go through to the outermost tissues, comprising cellulose, lignin and suberin.

When the pathogen has adhered to the palm roots, transmission electron microscopy (TEM) assay of infected roots one month after inoculation suggested that degrading enzymes are important in the extensive degradation of oil palm cell walls during pathogenesis by *G. boninense* and are among the proposed pathogenicity factors for the *G. boninense* interaction. Degradation of the cell wall occured at separated locations and will invade of all wall layers, including the middle lamella region, which in secondary walls is high in lignin content (Rees et al*., 2009).

The lignin components in plants are the rate-limiting step to biolysis. It is a secondary metabolic process from which more energy is expended in degradation than can be obtained from utilization. Lignin keeps the more weak cellulose and hemi-celluloses away

ning straight chemical bonds. The chemical structure of the polymeric lignin is complex (Figure 2.8).
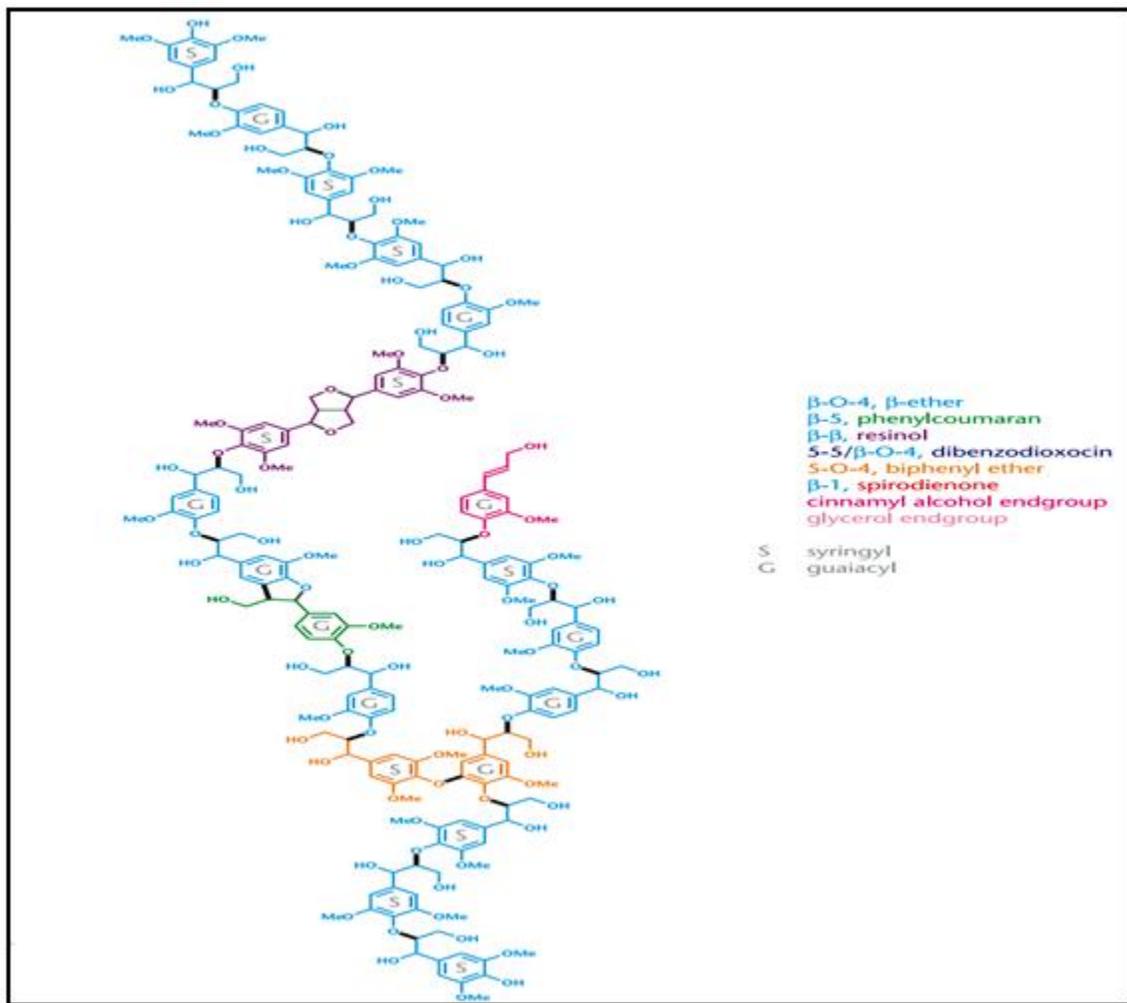
Figure 1.8: Chemical structure of lignin (Boerjan et al., 2003).

Areas of cell wall attack are sometimes not adjacent to fungal hyphae. Attack of plant cell walls, resulting in development of holes through all cell wall layers is indicative of simultaneous wood decay with the pathogen producing enzymes that can attack all cell wall layers. Similar strategies of cell wall attack have been observed from simultaneous degradation of *Laurelia philipiana* wood by a *Ganoderma* sp. (Agosin, 1990) and of date

a colossum and *Phanerochaete. Chrysosporium* (Blanchette, 1984; Adaskaveg et al., 1991).

Unfortunately, little information is known about the biology and genetics of *G. boninense* until recently. Epidemiological studies of the pathogen are also lacking. It has been stated that the major constraints to BSR disease control is the lack of sufficient information on variation in *Ganoderma* species associated with disease and their mode of reproduction (Rolph et al., 2000).Limited studies have been done to investigate the spatial patterns of BSR and how BSR is spread throughout the area of plantation. Such information is needed to fully understand the dynamics of the disease. For these reasons, there is a need for an effective and comprehensive BSR management plan for the area of oil palm plantation (Azhar et al., 2011).

The available control methods for BSR disease such as cultural practices and mechanical and chemical treatment have been proven unsatisfactory due to the fact that *G. boninense* has various resting stages such as melanised mycelium, basidiospores and pseudosclerotia. The failure in controlling the disease is due to the characteristics of *G. boninense*. The fungus is soil borne and so many fungicides may not be effective because of the degradation in the soil before they can reach their target (Susanto et al., 2005).

1.4   Plant "omics''

The complete set of the genomic information from key species of the model plants such as *Arabidopsis thaliana* and *Oryza sativa* is now available and will further improve the application of a range of new technologies to functional plant gene analysis, since the functional genome studies of plants has entered the high-throughput stage (French et al., 2009). In order to identify functions of unknown genes, different fast and multi-parallel

25

...ing developed and used. These new technologies and approaches are based on known methods but are adapted and improved to accommodate inclusive, large-scale gene analysis. Such techniques are novel in the sense that their design allows researchers to analyze many genes at the same time and at an extraordinary pace.

Such methods allow analysis of the different constituents of the cell that help to figure out gene functions, namely the transcripts, proteins and metabolites. Similarly the phenotypic variations of the entire mutant collections can now be analyzed in a much faster and more efficient way than before. These different methodologies have been developed to form their own fields within the functional genomics technological platform and are termed transcriptomics, proteomics, metabolomics and phenomics. Gene functions, however, cannot be easily inferred by only one approach. It requires the integration of all information collected by the different functional genomics tools that one will be able to clearly assign functions to the unknown plant genes(Koltai and Volpin, 2003).

Transcriptomics, which aims to profile gene transcription in a cell or a tissue globally, is mainly applied either by *in situ* synthesis of oligonucleotides ('oligonucleotide microarrays') or by deposition of pre-synthesized DNA fragments ('cDNA microarrays') on solid surfaces and their hybridization with labeled gene transcription products. The direct proteomics approach may combine 2-dimensional gel electrophoresis (2DE) of proteins with mass spectrometry (MS) or with liquid chromatography-tandem mass spectrometry (LC-MS/MS), to generate a list of expressed proteins (Kersten et al., 2002), whereas reverse proteomics may globally map protein interactions by means of yeast two-hybrid (Y2H) system (Fang et al., 2002).

arious gene expression investigations is a powerful tool for discovering novel genes or for gaining additional information about certain biological processes in a genomic scale. Transcriptional profiling using microarrays have developed into the most prominent tool for functional genomics and have realistically demonstrated how information from raw sequence data can be converted into a broad understanding of gene function. The synthesis is the first step towards protein synthesis and, for many genes changes in abundance are related to changes in protein levels (figure 2.9). Changing the transcriptional rate of genes is mostly related to changes in steady state levels. Such fluctuations in relative amounts are indicative of changes in the environment and developmental stages or reflect responses to all kinds of stimuli. Monitoring the transcriptome, i.e. the complement of all transcribed mRNAs of an organism, by measuring concentrations of selected genes in a multi-parallel and quantitative way allows assigning of functions to a multitude of unknown genes.For a correct understanding of gene function it is essential to discover other genes which are co-regulated with the gene of interest. It is also important to know when, where, and to what level a gene is expressed.

Constant improvement of the technology will offer even more perspectives for the fast and comprehensive analysis of plant gene functions. (Holtorf et al., 2002).

Phenotype

Phenome
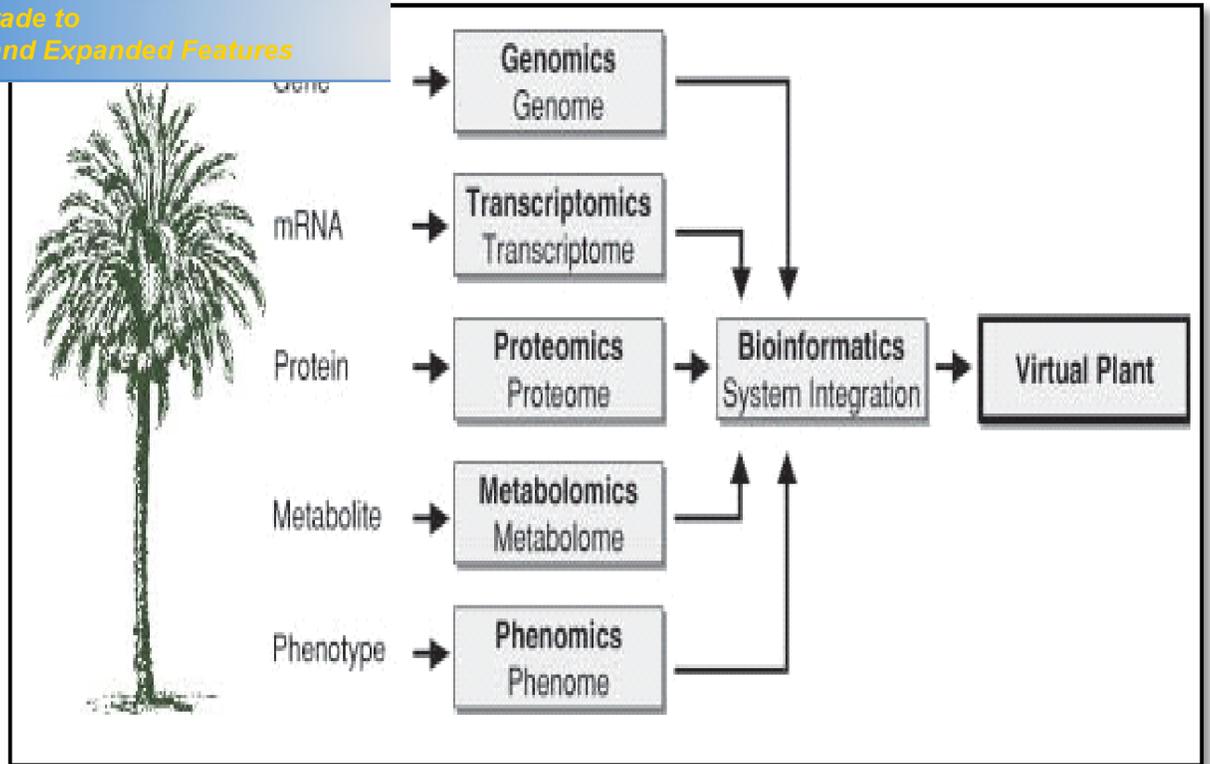
Figure 1.9: Scheme showing how the integration of results from different technological levels of functional genomics leads to construction of a virtual plant (Holtorf et al., 2002)

1.5   Defense genes of plants

Plants respond to pathogen infection by enhancing the antifungal gene expression. Many genes are induced both at the site of infection and in distal parts of the plant, leading to development of the hypersensitive actions (HA) and gene expression, respectively. The expression levels of several genes are often used as indices of plant resistance to pathogen infection (Hyun et al., 2011).

Active resistance of plants to pathogens depends on recognition of the pathogens and initiation of defense mechanisms. Despite the identification of the defense related genes of many plants, little information is known about the signal transduction pathway that gives

sequent events that occur during the interaction between pathogenic organism and a compatible plant, and that establishes and maintains infection (Dodds and Rathjen, 2010). Traditionally, studies of the interactions of pathogens with plants were focused on one or only a few components of the interaction, and thus, yielded only limited information on the genetic networks involved. However, from relatively few extensive functional genomics studies, an impressively large amount of information has been gained on plant-pathogen interactions.

Root tissues represent an important structure for plant growth and development, and are continuously invaded by microorganisms, which cause deleterious effects that culminate in the development of disease and/or death of the plant. Roots are intrinsically complex tissues to explore, most of the studies done previously focused on the developmental aspects of roots and with the increase in the availability of expressed sequence tag (EST) sequences, genes involved in cell cycle, cell growth, a biotic stresses and hormone signalling, while slow progress has been made in discovering plant-genes and proteins in the tissues governing the interaction of plant pathogens and their hosts (Scheres, 2002; Kohler et al., 2003; Jiang and Deyholos, 2006; Kwasniewski and Szarejko, 2006; Mouchel et al., 2006).Nutrient acquisition is an essential process for the survival of all heterotrophic organisms.As primary nutrient source, plants represent an important carbon source for a wide number of organisms. Several species of microorganisms live in close association with plants colonizing the surface (epiphytic colonization) or tissues (endophytic colonization), and sometimes causing diseases (pathogenic colonization).

The events that trigger the establishment of these interactions are activated by the recognition of specific signal molecules associated to plants and microorganisms, which

eins. Upon detection of these stimuli, there is a continuous process of response between plants and microorganisms that characterizes the plant-microorganism interaction.

Several plant factors such as pathogen recognition receptors and phenolic compounds as well as pathogen proteins and molecules including virulence factors and exopolysaccharides play important roles in plantmicroorganism relation and are determinants in the nature of the interaction (Brencic and Winans , 2005)

Extensive genomic studies have revealed several resistance and defense-related genes expressed during pathogen infection. After the recognition of the pathogen by the plant, signal transduction pathways are triggered, which result in the production of reactive oxygen species, accumulation of pathogenesis-related proteins and phytoalexins, as well as localized cell death (McDowell and Dangl, 2000;da Cunha et al., 2006). Among the defence-related genes reported are chitinases, β-1,3-glucanases, chalcone synthases and peroxidases. Managing root diseases of agriculturally important crops is a considerable challenge. Root diseases are caused primarily by pathogens from four higher-order lineages: fungi, straminipila, metazoa, and eubacteria (Agrios, 1997). These pathogens are difficult to control due to the protective environment of the soil and because reduced-risk pesticide chemistries are often only effective against organisms from a single phylum at best. Soil fumigants with broad spectrum biocidal activity face increased regulation and, in the case of methyl-bromide, are scheduled for imminent phase out (Cox et al., 2003).

Fungal pathogens cause some of the most serious and devastating diseases of crop plants (Strange and Scott, 2005). The costs of control are high, and approximately 22% of global agrochemicals used in plant production are fungicides, and these have a net annual worth

n, 2005). In oil palm, the basal and upper stem rot diseases caused by *G. boninense,* and vascular wilt disease caused by *Fusarium oxysporum* f.sp. *elaeidis,* can cause substantial losses of palms (Durand-Gasselin et al., 2005).Higher plants have developed a wide range of systems to protect themselves from damage related to biotic and abiotic stress. Certain developmental stages are particularly susceptible and must be afforded extra protection in order to allow successful completion of the reproductive cycle. A good example is the flower, which often contains tissues rich in macromolecules, which may be a target for invading herbivores or germs (Lotan et al., 1989). In order to protect floral organs from attack by microorganism, pests and herbivores, a range of protective molecules could be synthesized to provide a chemical defence, including chitinases (Leung, 1992), ß-glucanases (Lotan et al., 1989), hydroxyproline-rich proteins (Chen et al., 1992), and proteinase inhibitors (Atkinson et al., 1993). Accumulation of defense-related proteins can be subjected to both tissue-specific and environmental control, i.e. they may be activated as part of a predestined developmental program or be inducible. Considerable studies have been made earlier in the understanding of the pathways controlling the activation of higher plant genes by biotic and abiotic stress (Maleck and Dietrich, 1999; Rojo et al., 1999; Kovtun et al., 2000; Meskiene et al., 1998; Liu et al., 1998).

The isolation and characterization of disease resistance or stress responsive genes through molecular biology are the initial steps in the commencement of a systemsbiology approach for studying of oil palm towards an understanding of the defense and stress response mechanisms. It is essential todefine as many possible differences in gene expression between two types of cells or between two conditions. In this study, three genes that are

st the fungal infection have been selected based on their known function in other plants to be investigated in the oil palm. Comparing the patterns of gene expression for these genes and protein profiling of *G. boninense* infected (in different time of infection), and normal palms as a control were carried out in this study to better define the early response of the Oil Palm to this fungus.

The gene expression and protein profiles will correlate with the defense mechanism of the palms before identifying candidate gene(s) and proteins with potential characteristic fordeveloping strategies to control *G. boninense*infection. Understanding gene roles and gene expression profiling can be approached via several different techniques. Quantitative methods for global and synchronized analysis of expression profiles, such as Real-Time PCR would be powerful in giving overall understanding of the molecular and biochemical basis of the response to infection by *G. boninense* (Ravigadevi et al., 2005).A possible strategy to control plant pathogens is the development of natural plant defense mechanisms against the tools that pathogens commonly use to penetrate and colonize the host plant tissue.

Protein separation using two-dimensional gel electrophoresis and protein identification are the most useful approach for a poorly characterized plant like oil palm. The strategy enables to study the changes in gene expression patterns of *G. boninense*infected oil palm in Malaysia. As previously stated,plants respond to fungal attack by eliciting different mechanisms, the main one among them is the plant defence response. This information may may assist in the development of diagnostic tools and the identification of resistant or less susceptible oil palm varieties.

is represented by the host plants ability to inhibit the pathogenesis capacity to degrade plant cell wall polysaccharides (King et al., 2011).

1.5.1 Polygalacturonase-inhibiting proteins (PGIP)

Polygalacturonase-inhibiting proteins (PGIPs) are plant defense cell wall glycoproteins that inhibit the activity of fungal endopolygalacturonase (endo-PGs) and modulate their activity (Janni et al., 2008). PGIPs are considered typical defense proteins that are able to limit the growth of those fungal producing PG during tissue colonization. The involvement of PGIP in limiting Plant diseaseease has been demonstrated in transgenic tomato, arabidopsis, tobacco, and grape plants, where over expression of PGIP was associated with the reduction of symptoms caused by the fungal pathogen *Botrytis cinerea* (Powell et al., 2000; Ferrari et al., 2003; Manfredini et al., 2005; Aguero et al., 2005). PGIPs are glycoproteins present in the plant cell wall of vegetative as well as fruit tissues. Genes encoding PGIPs have been cloned from various plants (Cervone et al.,1987; Deo &Shastri,2003; Favaron et al.,1994; Gotoh et al.,2002; Mahalingam et al., 1999;Yao et al.,1999).

PGIPs belong to a family of leucine-rich repeat (LRR) protein and have an important role in plant–pathogen interactions. Several Plant diseaseease-related genes, such as Cf-2 and Xa21, which encode the member of the LRR protein family, have high homology to PGIPs (De Lorenzo et al., 2001).

The cell wall is the first barrier that plant cells use to oppose the attack of pathogens (De Lorenzo et al., 2001). Polygalacturonase (PG) is the first cell-wall-degrading enzyme synthesized by phytopathogenic fungi cultured on isolated cell walls (Cooper and Wood, 1975).Polygalacturonases (PGs) are produced by many plant fungal pathogens and are the

be secreted. They can degrade plantpoly-galacturonic acid, cause cell wall collapse and provide nourishment for fungi (Hu et al., 2011). PGs that break down the polygalacturonate chain in a random manner into small chains of oligogalacturonate are called Exo-PG and other PGs that cleave the polygalacturonate chain in terminal manner and release monomeric products, i.e. galacturonic acids are called exopolygalacturonase (Bateman and Basham, 1976). They are noticeable in the initial stages of plant infection and produced in abundant amounts during host colonization (Cooper and Wood, 1975). PGs have been related with diseases characterized by tissue maceration and soft reek (Bateman and Bashman ,1976). Furthermore, leucine-rich repeat receptor-like protein kinase (LRPKm1) that is likely to participate in defense-related signalling has been isolated by PGIP probe in apple (Komjanc et al., 1999). The role of PGIP in monocots has been feebly investigated andtheir activity has been characterised only in pectin-rich species such as *Allium cepa* (Favaron et al., 1993) and *A. porrum* (Favaron et al., 1997; Favaron, 2001). PGIPs have been characterized in wheat, although their N-terminal sequences (Lin and Li, 2002; Kemp et al., 2003) do not show any similarity with the typical PGIP sequences determined from purified proteins or deduced from gene sequences (De Lorenzo et al., 2001). Moreover, rice PGIP gene has been reported to be involved in flower development (Jang et al., 2003).

The demonstration that PG is a pathogenicity factor in *Claviceps purpurea* during the infection of rye (Oeser et al., 2002) has reinforced the interest for a defence role of PGIP also in monocots. Pectin is a main component of the primary walls of dicotyledonous and non-graminaceous monocotyledonous cells. During pathogenesis, cell walls of the plant cells act as the first line of defense that pathogens encounter when attempt to colonize the

nal requirements. A pathogen has to break the pectin layer for its ramification.

A wide range of enzymes, including exo- and endo-polygalacturonase, pectatelyase, pectin methyl esterase and betagalactosidase are involved in pectin modifications (De Vries and Visser, 2001). Pectin-degrading enzymes, including endo-polygalacturonase (EPG), are among the first glycanases to be secreted during fungal infection.Plants secrete proteins that specifically bind to PGs and modify their enzyme action. Cervone et al. (1987) hypothesized that PGIP benefits the plant by retarding the hydrolytic activity of PG-catalysed hydrolysis of polypectate and lead to the formation of oligomers with a degree of polymerization greater than four. Such oligomers elicit active defence mechanism in plants. Therefore, the action of PGIP *in vivo* is to counteract fungal invasion by causing PGs to increase their elicitation of plant defence responses. The PGIP family of proteins, present in the walls of dicots and non-graminaceous monocots, are in some cases capable of inhibiting greater than 92% of the activity of fungal PGs (Berger et al., 2000) and sometimes even to 100%(Gao and Shain, 1995). The main goal in plant pathology is to understand the molecular basis of pathogen recognition by plants. Though PGIP is one among the few well-elucidated, pathogenesis-related (PR) proteins of known ligand–protein binding model, research on this group of PR proteins has not been reviewed.Analyses of PG genes in *Botrytis cinerea* (*Bcpg1* gene) and in *Aspergillus flavus* (*P2c* gene) showed that PG contributes significantly to virulence of these fungi (Have et al., 1998).PGs play a significant role in tissue maceration, colonization, and virulence. However, it is not known if different PGs are produced in different hosts during postharvest decay. *P. expansum* produces at least five PG enzymes in culture (Jurick et al.,

by plants, and in pear, it has been shown to be involved in fruit ripening and softening via degradation of cortical cell walls (Sekine et al., 2006; Jurick et al., 2010).

EPG (poly-*a*-1,4-galacturonide glycanohydrolase, EC 3.2.1.15) degrades unesterified regions of homogalacturonan (HGA), the 1,4 linked *a*-D-galactosyluronic acid polymer found in pectin of plant cell wall. There are also strong correlative evidences supporting the involvement of EPG in causing symptoms in diseases characterized by soft-rotting or tissue maceration (Bateman and Basham, 1976; Isshiki et al., 2001; Huang and Allen, 2000). EPG fragmentation of HGA results in the transient formation of elicitor-active oligogalacturonoides (OGAs) with degrees of polymerization between 9 and 15. These OGAs are rapidly (within 15 min) converted into smaller, biologically inactive fragments by EPG. Therefore, factors that limit fungal EPG action are likely to increase the lifetimes and concentrations of biologically active OGAs and may result in enhanced or prolonged plant defence responses (Gomathi and Gnanamanickam, 2004).

Generally, the inactivation of pectinolytic enzymes was thought to be due to the action of non-proteinaceous and non-specific inhibitors like phenolics(Gao and Shain, 1995). Contrary to this, some researchers found that phenolics do not inhibit EPG. For example, concentration as high as 200 µg ml–1 *p* coumaric acid and ferulic acid of tomato did not inhibit the PG of *Botrytiscinerea* (Glazener, 1992).It has been reported that proteins extracted from the cell walls of red kidney bean hypocotyls, tomato stems, and suspension-cultured sycamore cells can totally inhibit the activity of PGs secreted by the fungal plant pathogens *Colletotrichum lindimuthianum*, *Fusarium oxysporum* and *Sclerotium rolsfii* (Niture, 2008). They also found that the inhibitors showed varying degrees of inhibition on

and their physical properties were similar to those of phytohemmaglutinins and plant glycoproteins capable of agglutinating transformed animal cells. During the 1970s, not much research had focused on PGIP, except for the contributions of Albersheim and his colleagues at the Department of Chemistry, University of Colorado (Fisher et al., 1973; Glazener, 1992). Figure 2.10 depicts the model proposed by Albersheim and co-workers on the role of oligosaccharide and PGIPs in the induction of plant defense response.

Ion fluxes
Protein phosphorylation
Active oxygen production
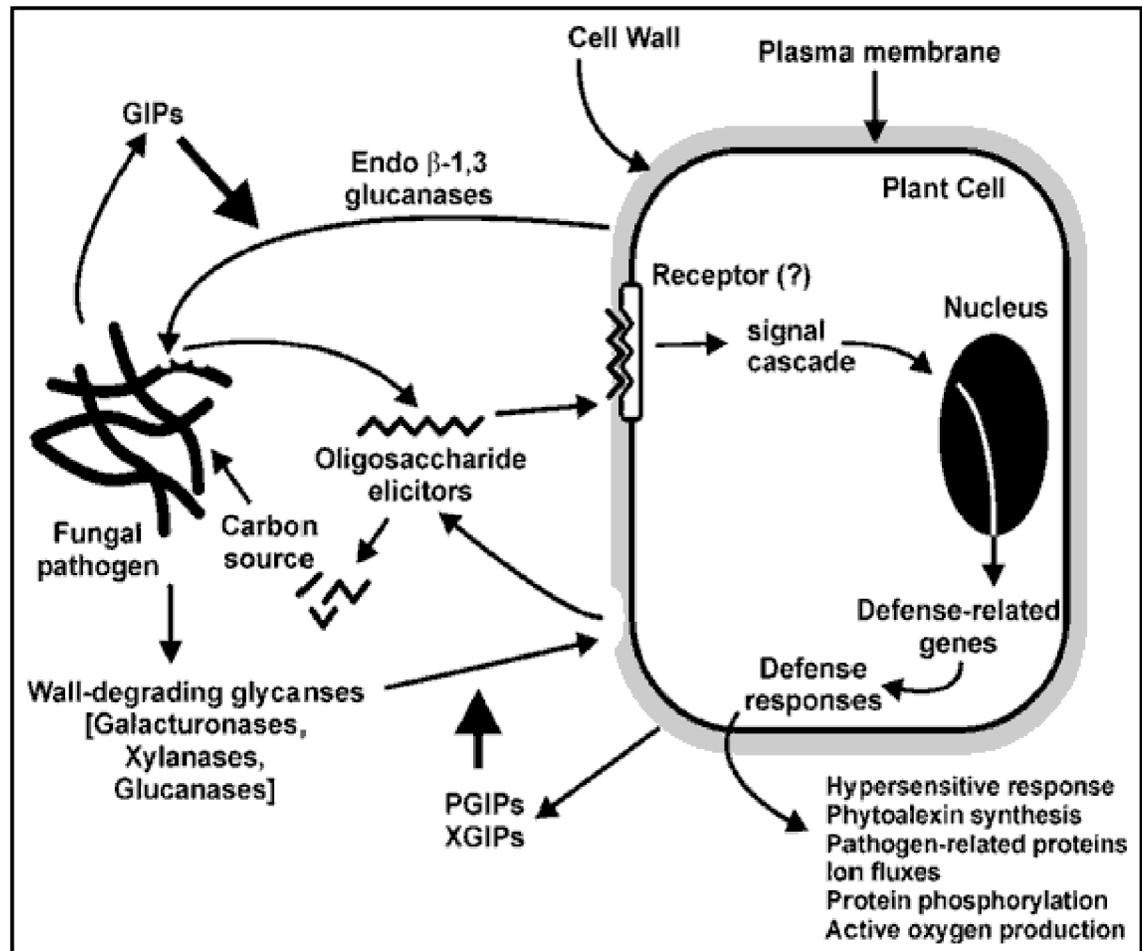
Figure1.10: Model depicting the role of oligosaccharide and *PGIPs* in the induction of plant defense response (adapted from Gomathi and Gnanamanickam, 2004).

as detected in crude extracts of peach tissue infected with *Monilinia* spp., though its presence was confirmed by isoelectric focusing after fractionation. Further experiments confirmed the presence of proteinaceous inhibitors present in apple and peach tissues infected with *Monilinia* spp (Fielding, 1981). While assessing susceptibility of pear fruit to three potential pathogenic fungi in relation to its maturity, a non-phenolic inhibitor was identified and was found to be cell-wall-bound proteins (Abugoukh et al., 1983).

In the 1980s, PGIPs from different plants have been characterized biochemically, but little progress was made on the elucidation of structure and regulation of their expression, which is crucial for the understanding of the *in vivo* function of this class of proteins. A major study on the inhibition of pectinolytic enzymes has been correlated with phenolic compounds and phenol oxidases. Previous studies on the inhibition of enzymes responsible of pectin lyses , polymethyl galacturonase, and pectate lyase of *Colletotrichum capsici* and *C. Gloeosporioides* (pathogenic fungi of chilli) by fungitoxicants have shown better inhibition *in vivo* than *in vitro*; PG was not inhibited *in vitro*, but was inhibited by the same fungitoxicant *in vivo* (Gomathi, 2001). *Capsicum annuum* (chilli) plants treated with fungitoxic chemicals showed less phenolic content and less activity of peroxidases than the untreated and infected plants (Gomathi, 2001). PG activity, however, showed a reverse pattern. These results suggested that phenols are not involved in the inhibition of PG and this led us to search for proteinaceous inhibitors in chilli plants. . In general, the proteinaceous inhibitors for PG were isolated only from dicotyledonous plant species.

Previously, and for the first time, PGIP from a monocotyledonous species was purified from a non-graminaceous plant, *Alliumporrum* (Favaron et al., 1997), and from a

(Gomathi and Gnanamanickam, 2004). Its presence was confirmed by biochemical and immunological evidences (Kemp et al., 2003). But the levels of PGIP were low in wheat seedlings, and this can be explained by the presence of one-tenth the amount of pectin in wheat cell wall compared to dicot plants.PGIPs are specific inhibitors. They do not inhibit other cell-wall degrading enzymes, and the inhibition percentage of different fungal PG differs. PGIP of pear inhibited only PGs secreted by pathogenic fungi of pear and did not inhibit PGs of *Aspergillus niger*and *Fusarium. oxysporum*(Abugoukh et al.,1983). This suggests that PGIPs can discriminate between PGs. The degree of susceptibility of PGs to PGIPs depends on the mode of action of the fungal PG. PGs exhibiting strictly endo mode of cleavage are selectively inhibited only by certain PGIPs, but those cleaving pectin in between the exo and the endo modes and the exo-polygalacturonases are easily inhibited by many PGIPs. PGs of *C. lindemuthianum*, *Cochliobolussativus*, *Cryphonectria parasitica* and *A. niger*exhibited an intermediate substrate degradation and these exo/endo PGs were inhibited greater than 90% by all the four PGIPs. However, PG of *A. niger*and *Fusarium moniliforme* were inhibited by PGIPs extracted from Pinto and Blue Lake cultivars of bean, but not of tomato and pear. PG of *Postia placenta* was not inhibited by any of these PGIPs (Cook et al., 1999).

Although PGIP is considered as a constitutive protein; the amount of *PGIP* gene transcripts varies at different stages of maturity and at different distances from the diseased region. In apple for example, high expression level of *PGIP* gene was expressed in the decayed area and in adjacent areas of the fruit inoculated with *Penicillium expansum* and *B. cinerea*.

nt increase in PGIP in tissues away from the decayed region, suggesting that the *PGIP* gene is readily activated by fungal infection (Conway, 1998).In general, it appears that PGIPs from various plants show inhibition of PGs from fungi to which the plant was not exposed. This observation is strengthened by a report that bean PGIP-2 interacts strongly with a PG of maize pathogen, *Stenocarpella maydis* that causes epidemics in USA (Berger, 2000).

PGIP isolated from *Glycine max* was found to effectively inhibit PGs of soybean cyst nematode (Mahalingam et al., 1999). PGIPs do not inhibit EPGs of plant origin. For example, PGIP of *Phaseolus vulgaris* did not inhibit EPG of tomato and exo-PG of pollens of water oak. However, it inhibited the exo-PG of corn pollen and sorghum (Cervone et al., 1990). Likewise, pear PGIP inhibited *Pencillium expansum*, *Botrytis cinerea* and *Dothiorellagregaria*, while it did not inhibit PG of pear fruit (Abugoukh et al., 1983). Many plants possess more than one PGIP with differential abilities to inhibit different PGs of pathogens (Cook et al., 1999).

Specificity of PG basically based on the existence of the difference in molecular forms of PGIP in a single plant. In *A. porrum*, more than 20 isoforms (Favaron, F. 2001) and in bean five isoforms have been detected (Desiderio et al., 1997). Expression of PGIPs in tissues of plants is differential: PGIPs in tomato, apple, and raspberry were predominately expressed in fruit tissues (Johnston et al., 1993; Stotz et al., 1994; Yao et al., 1999), whereas in others such as bean, it was expressed in the vegetative parts (Devoto et al., 1998; Salvi et al., 1990).

(LTP)

Plant lipid transfer proteins (LTPs) are low molecular mass, abundant, ubiquitous, and mostly basic proteins extensively studied in higher plants. LTPs are defined by their ability to facilitate the transfer of phospholipids between membranes *in vitro*. The proteins contain eight cysteine residues with four conserved disulfide bridges that exert different functions in defending itself against biotic stresses such as fungal and bacterial pathogens as well as abiotic stresses, including high salinity,drought, and wounding and low temperatures(Zavallo et al.,2010; Qin et al., 2011).LTPs are synthesized as precursors with a putative signal peptide and are located extra-cellularly or are connected with the cell wall. These proteins are alpha-helical proteins: their four alpha helices, linked by flexible loops, form a hydrophobic cavity that ensures the binding of lipids and hydrophobic molecules. They are defined by their capability to transfer phospholipids between the plasma membranes *in vitro*. They have been characterized in a large variety of organisms including mammalian tissues, plants, yeast, fungi and some bacteria. There was a suggestion - but not yet well confirmed - that these proteins participate in extracellular flux of lipids, essential for the biogenesis and renewal of endomembranes (Wirtz, 1991).

The situation is more complicated for plant LTPs for which is still being in search of an *in vivo* role. It is now clear that these proteins are present in numerous plants and share in common structural and functional properties: small size (about 9 kDa), high isoelectric point (Douady et al.,1986; Sossountzov et al.,1991), presence of eight cysteine residues, between 90–93 amino acids, located in conserved positions and forming four disulfide bridges (Kader,1996,1997;George and Parida ,2010). Structural studies by NMR and X-

carried out on plant LTPs. These proteins comprise a single compact domain with four alpha-helices and a long C-terminal region.

They have a tunnel-like hydrophobic cavity able to accommodate fatty acids (Shin et al., 1995; Gomar et al., 1996). A complex between maize LTP and palmitic acid has been indeed recently crystallized (George and Parida, 2010). This recent structural model of LTPs gives a basis for understanding the function of LTPs in relation to the transfer and binding of molecules containing acyl chains (phospholipids, fatty acids, acyl CoA or other acyl group containing molecules).

In higher plants, both specific and nonspecific lipid transfer proteins have been characterized. The basic lipid transfer proteins from maize seedlings, spinach leaves, and castor bean seedlings are nonspecific. All nonspecific plant lipid transfer proteins are soluble proteins which may account for as much as 4% of the total soluble protein. The intracellular location of the lipid transfer proteins has not been established but they are located outside the chloroplast and have been thought to be cytosolic. The most thoroughly characterized proteins have a pI of about 9 and a molecular mass of about 9 kDa.

The amino acid sequences have been determined for the spinach leaf and castor seedling protein. In addition, on the basis of amino acid sequence identity to known LTPs, several polypeptides from barley and finger millet, which were originally described as probable amylase inhibitors, were identified as LTPs. ThecDNA of the maize LTPhas been characterized. Comparison of the deduced amino acid sequences of cDNA clones encoding the maize and barley LTPswith the directly determined amino terminal sequences of the mature proteins showed that these proteins are synthesized as precursors containing 25 or 27 additional N-terminal amino acids, respectively (Werner et al., 1991).

as being involved in many other biological functions. LTP is involved in long-distance signaling during systematically acquired resistance in *Arabidopsis thaliana* (Maldonado et al., 2002).Subsequently, it was shown that they all possess an N-terminal signal peptide that directs the LTP to the endoplasmic reticulum for extracellular export via the secretory pathway. Therefore, other functions have been suggested, including involvement in biosynthesis or deposition of hydrophobic monomers which form the waxy and polymeric cutin layers of most aerial organs, or participation in embryogenesis and transportation of fatty acids and acyl coenzyme A as carrier proteins(Kader, 1996). LTP also have been implicated in plant responses to salinity in tomato and wheat, drought in tomato and wheat and low temperatures in barley. Cabbage cryoprotectin, an LTP unable to bind any lipids, has been implicated in the stabilization of plant membranes during cold stress (Sun et al., 2008).LTPs are also similar in their structure with elicitin. Elicitin is a small, cysteine-rich, lipid-binding protein which is secreted by the plant-infecting oomycetes, Phytophthora and Pythium, and which triggers the hypersensitive response (HR) and systemic acquired resistance (SAR) in tobacco. Elicitin binds the plant sterols that are necessary for successful reproduction by the oomycete. Recent results have revealed that a lipid based molecule may act as the complete transportable signal for SAR (Sarowar et al., 2009).

...ein 10 (PR10 proteins)

Plants are induced to express a group of genes at increased levels in response to infection by a variety of pathogens. The defense strategy of plants against infection factors involves a large number of tools, including various types of stress proteins with putative protective functions. A group of plant-coded proteins induced by different stress stimuli, named "pathogenesis related proteins" (PRs) is assigned an important role in plant defence against pathogenic constraints and in general adaptation to stressful environment. PR proteins are well-known proteins established to be induced by pathogens and other related abiotic stresses (Figure 2.11) (Van Loon et al., 1994, 1999). Based on their structural and functional properties, up to 17 different families of PR proteins have been identified thus far in mono- and dicotyledonous plants. These PR families have a wide range of function from cell wall rigidification to signal transduction and antimicrobial activity (Christensen et al., 2002; Van Loon, 2006).
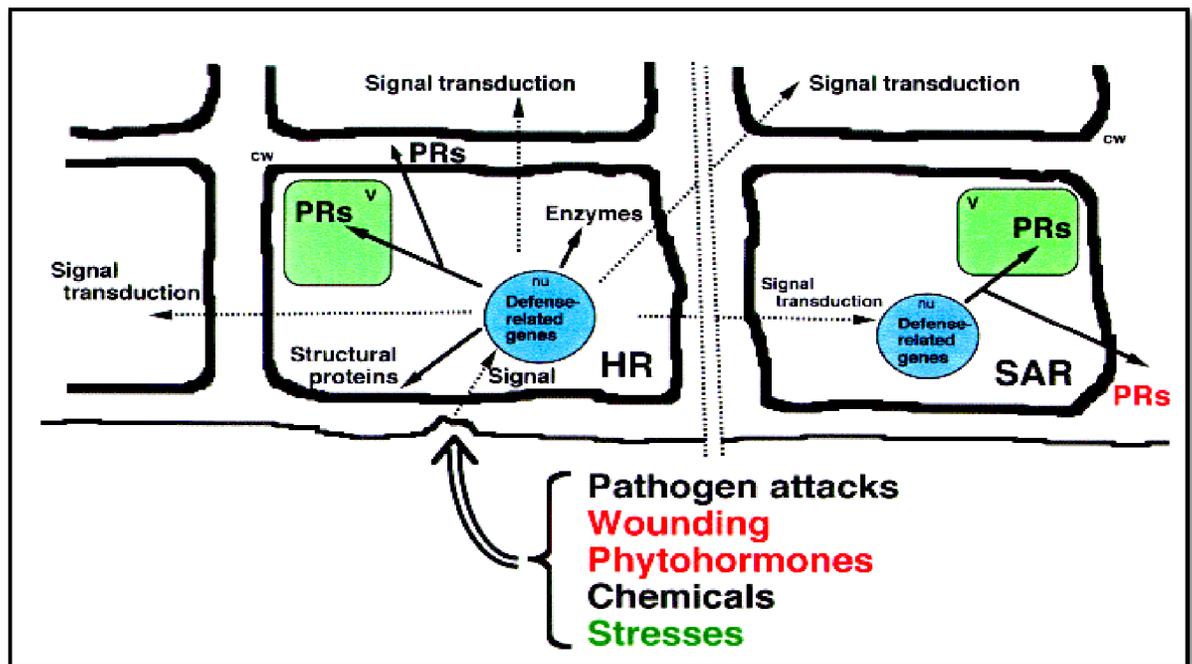
Figure 1.11: The Mechanism of Pathogen Related Protein PRs during Plant Defense responses (Bowles, 1990).

Somssich et al., 1986; 1988) is a typical member of the PR-10 family. PR10 proteins have been identified in a wide range of flowering plants. At the transcriptional level, many biotic and abiotic stresses have been shown to activate PR10 protein, signifying their important roles during plant-pathogen response (Liu et al., 2003). The complexity of the multi-gene family of PR10 proteins and their possible roles under usual and unusual physiological conditions encouraged researchers to investigate the gene regulation for the spatial and temporal expression pattern of PR10 genes in a conifer species (Liu et al., 2003).The PR-10 proteins are regulated according to development and environment and they are widely distributed in seed plants. Based on similarities in their amino acid sequences, sub-cellular location, and putative function, the PR-10 family is divided into two distinct groups: intracellular pathogenesis related proteins (IPR) with homology to ribonucleases (Van Loon et al., 1994), and (S)-norcoclaurine synthases (NCS, EC 4.2.1.78) (Samanani et al., 2004). The role of PRs has been a highly dynamic study areasince their discovery. Putative plasma membrane localized receptors of PR10 inducers are suggested, and secondary signals of PR10 induction, such as salicylic acid (SA), jasmonic acid and ethylene, have been established.Many of these derivative signals are known as inducers of PR10 expression. PRs are well-known by species specificity, thus allowing their application as markers in taxonomical, phylogenetical and evolutionary studies. Pathogenesis related proteins comprise 17 groups, most are associated with plant defense response. Although most of them are induced by stress, biotic or abiotic, they show diverse functions (Hoffmann, 2002).

PR10 are distinguished by specific biochemical properties. They are low-molecular proteins (6-43 kDa), extractable and stable at low pH (<3), thermostable, and highly

on, 1999). The protein contains four α-helices and four β-strands arranged antiparallel between helices. The tight packing of the α-helices on both sides of the central β-sheet (α−β−α sandwich structure) results in a compact, bipartite molecular core, which is stabilized by hydrophobic interactions and multiple hydrogen bonds (Fernandez et al., 1997). This compact structure probably determines the high stability of PRs and their insensitivity to proteases.Since *PR10* genes were originally identified in peas expressing resistance to fungi (Riggleman et al., 1985).PR10 has also been reported as responding to stress and abscisic acid, as a pollen allergen and has been shown to be constitutively expressed in roots. The specific role of PR10 in these processes has remained elusive although PR10 proteins from ginseng, white lupin and hot pepper have been demonstrated to possess RNase activities (Park et al., 2004). However, molecular experiments have also suggested that a PR10 protein from white lupin may have the abilty to bind cytokinins (Biesiadka et al., 2002) although this remains to be confirmed experimentally. While expression of pea PR10 in potato conferred resistance to early dying disease (Chang et al., 1993), constitutive expression of this gene in transgenic canola did not result in enhanced resistance to blackleg (Wang et al., 1999).The PR10 subfamily of this group includes a number of low molecular weight proteins ranging from 15 to 20 kDa, with different biochemical characteristics.

In general, PR10 proteins exhibit allergenic, anti-fungal and ribonuclease activities (Chadha and Das, 2006; Liu et al., 2006; Srivastava et al., 2006b).Just like other PR proteins, PR10 is induced by both biotic (Koistinen et al., 2002) and abiotic stress (Hashimoto et al., 2004) such as drought (Dubos and Plomion, 2001), wounding and cold-hardening (Liu et al., 2003). In addition, the PR10 homolog in mulberry, WAP18, is

reezing tolerance (Ukaji et al., 2004). On the other hand, the constitutive expression of pea PR10 in *Brassica napus* resulted in enhanced seed germination and improved growth under saline condition (Srivastava et al., 2006a).

1.6   Plant proteomics

A Proteomics study (protein match expressed by a genome) hasencouraged as a consequences of genomic sequencing projects to deal with issues relating to the function and regulation of the sequenced genes. There are several high through put RNA measurement tools such as differential display, transcript imaging, and microarrays that have been developed for the analysis of the transcriptome, but these techniques do not really provide insight into the quantitative and qualitative understanding of the final gene products, or ''proteins''. The amount of protein as an end product is not always related to its amount. In addition, a lot of proteins go through post-translational modifications, like single peptide removal, phosphorylation and glycosylation, which play a significant role in their movement and sub-cellular localization. As a result, the study of proteins themselves is the only way to provide information at a certain time and under certain conditions, about the protein amount and their activities (Zivy and de Vienne, 2000).

Basically the conception of proteome is based on a relatively traditional technique of protein separation. Two-dimensional electrophoresis (2-DE) (O'Farrell, 1975), which separates the protein according to their isoelectrical point in the first dimension and in the second dimension to their molecular weight (SDS-PAGE). Proteomics has been for many years the most powerful technique for the separation of protein complex. 2-DE allowed large-scale studies of gene expression and genetic variation long before the appearance of transcriptome tools by allowing the visualization of several hundreds of proteins on a

limited, however, by the difficulty of identifying the proteins (Zivy and de Vienne, 2000).In recent years, the application of proteomic approaches as a tool for global expression analysis and protein identification has been highly efficient in the field of protein research. Improvements in the main techniques and equipments for 2-DE and mass spectrometry have allowed a high throughput analysis of protein expression in different fields. While the proteomics research is greatly advanced in animals, plant proteomics has not yet reached a sufficient level of complexity to identify and study plant proteins involved during the pathogen-host plant interactions. Many proteins involved in the mechanisms of response to biotic/abiotic stress signals are present in low abundance and thus are not easily detectable in crude extracts. Major studies in plant proteomics involve subcellular proteomes, including chloroplasts, mitochondria, nuclei and plasma membranes (Peltier et al., 2000;Ferro et al., 2003; Werhahn and Braun, 2002; Bae et al., 2003; Kawamura and Uemura, 2003; Gonzalez-Camacho, 2007) and most of them still rely on 2-DE separations of crude cellular extracts. The study of proteins has also become progressively more important since it is now well established that there is little correspondence between the transcript and protein levels (Gygi et al., 1999; Chen et al., 2002). Post-translational modifications such as the removal of signal peptides, phosphorylation, glycosylation, ubiquitination, among others are important processes for protein function and subcellular localization which are not accounted for using genomic strategies (Jensen, 2006).

Therefore, proteomics is playing an increasingly important role in addressing these issues and has become a necessary and complementary approach in the post-genomic era. Furthermore, by analyzing the proteins being expressed during a specific condition,

es/proteins that are co-regulated and act together in response to a given stress can be identified. Nevertheless, the root proteomics (rooteomics) analysis is still less well developed when compared to other plant tissues and even more when compared to other organisms such as prokaryotes, yeasts and mammalians (Agrawal and Rakwal, 2006).

The proteomics science is basically linked to genomics since the information about proteins can be derived from the information contained in the genome of an organism. However, while the genome is static for a particular cell, the proteome which is derived from the genome varies greatly depending upon the cell type as well as its functional condition (Archana, 2006). 2-DE is the most common technique used in proteomics. For over 25 years, it has been the technique of choice for analyzing the protein composition of cells.Several plant proteomics reviews have been published; including sub-cellular proteomics (Wang et al., 2008).

One of the major factors responsible for the limited data regarding root proteomics is related to low protein and tissue amounts. Interestingly, Mooney et al.(2006) showed that proteins involved in disease resistance represented 13% of root proteins compared to 7% identified in leaves.These results indicate that root tissue is an excellent target to study plant pathogen-interactions and the use of differential proteomic studies can aid in the discovery of resistance- and defence-related proteins.

It is true thatthe results obtained from plant-proteomics research are below the expectations generated at first and that the results have shown that the vitality, variability, and behavior of proteins are more complex than had ever been imagined, especially as refers to a number of protein species per gene as a result of alternative splicing, reading frame, and

s, trafficking, and interactions, and considering that protein complexes, rather than individual proteins, are the actual functional units of the biological systems (Gonzalez-Fernandez and Jorrin-Novo,2012). On the other hand, and differently from other biological systems, like yeast (Picotti et al., 2009) and humans (Anderson et al., 2009), the full potential of proteomics is far from being fully exploited in plant-fungal pathogen research, and that is because of the low number of fungal pathogen species under investigation at the proteomic level, the low proteome coverage in those species investigated, and the almost unique use of classical, first generation techniques, those based on 2-DE coupled to MS (Fernandez et al., 1997).The proteome has been defined as the entire complement of proteins present in a cell, organism, or tissue under certain conditions. Accordingly, proteomics is the study of this complement expressed at a given time or under certain environmental conditions (Wilkins et al., 1996). There are three main areas for the proteomics study.First is the large-scale identification of proteins by linking structural information, such as internal peptide sequences, amino acid composition, isoelectric point, molecular mass, sequence tags of selected peptides to genomic or protein databases. The second area is the research about comparison of variations in protein patterns due to stimulation by hormones, environmental changes and/or genetic mutations, followed by identification of proteins which show changes in concentration or posttranslational modifications, and third by studies of protein–protein interactions using techniques such as mass spectrometry (Pandey and Mann, 2000).

Although some authors have suggested that bi-dimensional gel electrophoresis (2-DE) is an ancient and surpassed technique, this procedure has been frequently utilized in plant

omising alternative technologies such as multidimensional protein separation, protein arrays and others have emerged recently.

However, 2-DE is currently the only technique that can be routinely applied for quantitative expression profiling of large sets of complex protein mixtures such a root cell lysates (Gorg, 2004). Although 2-DE technology is not cheap, the equipment is more accessible than mass spectrometers. This makes proteomic studies possible for research groups located in developing countries, spreading the proteomic science around the world. It is true that protein identification is an important step in proteomic analysis and mass spectrometry-based strategies has contributed enormously in this aspect. Thus, the enthusiasm in proteomic studies is a result of the union of techniques based on 2-DE and those focused on mass spectrometry, and this union has been responsible for the increase in the functional assignment of proteins and genes in various organisms, including plants (Cullis, 2004).Immobilized pH gradients and gels, as well as power supplies and gel supports are now commercially available and have clearly improved reproducibility and decreased wasted time. By using these benefits, good quality 2-DE gels have been obtained, generating valuable information for the understanding of root metabolism and physiology. Initially, model plants such as *Arabidopsis thaliana* and *M. truncatula* were used to develop extraction and gel protocols. Therefore, 2-DE associated to peptide mass fingerprinting was used to investigate the natural variation in the root proteome among eight *A. thaliana* ecotypes (Chevalier, 2004). Comparison of 2-DE maps demonstrated that only one-quarter of spots were shared by all accessions, suggesting that ''rooteomics'' could be a valuable tool to understand natural variations of plants and also to compare susceptible and resistant varieties to isolate resistance proteins. The proteome knowledge

applied to crop plants, aiming to solve agricultural problems and find biotechnological targets and/or tools. However, some other studies have using 2-DE gels focused on developmental aspectsof primary maize roots, aiming to identify proteins that are differentially accumulated during root growth (Sheffield et al., 2006). Differential spots were identified by MS showing that two proteins that were shown to accumulate differentially between wild-type and mutant roots can be linked to lignin metabolism, giving clues on genes that might be involved in the developmental switch that results in the initiation of lateral roots (Liu et al., 2006). The cassava (*Manihot esculenta* Crantz) root proteome was also evaluated using high-resolution 2-DE, (Sheffield et al., 2006). Gel image analyses revealed an average of 1467 electrophoretically resolved spots on fibres and 1595 spots on tuber in the pH 3-10 range. As cassava is a major source of energy in the diet of more than 700 million people, particularly in the developing countries of South America, Africa and Asia, the proteomic approach could be useful for a better understanding of protein and carbohydrate accumulation in tuber. Therefore, results reported by Sheffield et al. (2006) not only facilitate insights into the molecular processes underlying root physiology and differentiation, but can also give valuable information for cassava genetic improvement programs in order to solve some problems as disease susceptibility, low production and reduced nutritional quality.

New improvements in electrophoresis tools such as the development of algorithms for gel image analysis (Blueggel, 2004) and difference gel electrophoresis (DIGE) that facilitates complex protein comparison analysis by labeling different samples with fluorescents dyes (Ornstein and Petricoin, 2004**)** and dilute Immobilinegels for the resolution of large proteins (Bruschi, 2005) are already being used in biomedical research.

hniques in plant research could contribute towards the development of plant proteomics, specifically for proteomic processes involving root pathogen interaction.Although by no means perfect, 2-DE coupled with mass spectrometry remains the core technology for separating and identifying complex protein mixtures in proteomic projects at least for the foreseeable future (Gorg et al., 2004). Rooteomic studies aiming at the identification of agronomically important proteins is a field that can be largely explored using both these techniques. It is certain that an array of novel interesting proteins will be discovered using these approaches.Proteomics has become a main field of practical genomics after the successful completion of many genome sequencing projects and the advance of many analytical methods for protein characterization. The applications are currently expand to analyze different functional aspects of proteins like, protein-protein interactions activities and structures, posttranslational modifications.

With the aim of profiling proteins in biological samples, proteomics has long been associated with the techniques of two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) (Shevchenko et al., 1996; Wilkins et al., 1996). Introduction of thedry strip immobilized pH gradient (IPG), helped mostly to overcome the Classical 2-DE limitations in resolution and reproducibility of gels (Gorg, 1991). The improved techniques in 2-DE allowed inclusive protein visualization on 2D gels.Proteomics was further advanced by the development of biological MS and the availability of searchable sequence databases.

The MS techniques that were developed for the ionization of proteins and peptides include matrix-associated laser desorption ionization (MALDI) and electro spray ionization (ESI) (Karas and Hillenkamp, 1988; Fenn et al., 1989). Combined with time of flight (TOF), ion

lem MS (MS/MS) spectrometers, these techniques offer high sensitivity and mass accuracy (Aebersold and Mann, 2003).

Large numbers of groups have published protein reference maps for specific tissues that include roots, stems, flowers, seed pods and cell suspension cultures. Additional analysis of proteomics from crops have been reported for specific stages of seed filling and seed development (Gallardo et al., 2003) and for somatic embryogenic tissue culture cells (Imin et al., 2004).The proteomic analysis of the plasma membrane is a difficult one for reproducible and complete mapping due to its hydrophobic nature. Specific strategies have been developed to recover the hydrophobic membrane proteins on 2-D gels (Santoni et al., 2000). Efforts have been employed to construct 2-DE reference maps of the plasma membrane under optimized solubilising conditions (Rouquie et al., 1997; Santoni et al., 1998). In order to identify *Arabidopsis* plasma membrane proteins that are associated with the early stage of cold acclimation, highly-purified plasma membrane fractions from non-acclimated and cold-acclimated *Arabidopsis* leaves were analyzed using MALDI-TOF MS (Kawamura and Uemura, 2003).

The interaction between root and infectious fungi is considered the most studied area in the proteomics analysis of root-pathogen association. Several reports have shown the use of proteomics for the isolation of proteins of agronomic application including pathogenesis-related proteins, chitinases, among others. However, still little proteomic information is available regarding the biochemical and physiological interactions in the host-parasite system. Moreover, the molecular bases of the interaction between roots and pathogenic fungi are not well understood yet (Mehta et al., 2008).The proteomic tools offer an excellent way to evaluate the host proteome during the host-parasite interaction process.

publications on the proteomics of symbiotic interactions (nitrogen-fixing and arbuscular mycorrhizal), the use of this strategy appears rather limited in the area of plant responses to pathogens. The available amount of root tissue for analysis is commonly a main problem for more detailed studies. Similar difficulties have been reported in the symbiotic root-fungi interactions in *M. truncatula* (Dumas-Galdot et al., 2004). These authors reported that few studies have been performed in the appresoria stage, in which limited amounts of root are available; making proteomic researches a complex task. The extraction of proteins using an efficient methods producing high protein yield from the same sample is one of the alternatives to overcome this problem. Techniques that allow this analysis have been developed for root-fungi interaction and have been used successfully (Dumas-Galdot et al., 2004).

This strategy could be applied for other plant-microbial interactions in order to optimize the results obtained. In addition, plants are able to synthesize and secrete enzymes that are able to degrade the cell wall of the invading pathogen and these enzymes are often classified as pathogenesis-related proteins because they are highly expressed upon infection. For example, the root protein profiles of *M. truncatula* have been analyzed using 2-DE after being infected by the *Aphanomyces euteiches* pathogen (Colditz et al., 2004). Several differentially expressed proteins in response to the infection were identified by MALDI-TOF-MS and the majority of the induced proteins belonged to the family of the class 10 of PR proteins. Other protein spots were also identified as putative cell wall proteins and enzymes of the phenylpropanoidisoflavonoid pathway, including an isoliquiritigenin (chalcone) 2-O-methyltransferase (COMT) and a chalcone reductase (CHR), which are required for the production of medicarpin, the major phytoalexin

ngal pathogens. Previously, a comparison of *M. Trunculata* lines with different susceptibility to the *A. euteiches* was performed.

Another strategy that can be applied for Plant diseaseease control is the analysis of the pathogen proteins, which can be identified and used as targets. Pathogenic fungi have several effector proteins that play a key role in parasitic colonization of plants (Kamoun, 2005). Apoplastic effectors are secreted into the plant extracellular space, where they interact with extracellular targets and surface receptors. By using a proteomic approach, Apoga and Tunlid (2001) investigated the role of the extracellular matrix secreted by the cereal pathogenic fungus *Bipolaris sorokiniana*. However, the proteins isolated from 2-DE gels and analyzed by MS revealed no significant matches to sequences available in public databases. Protein identification by using the MS technology is highly dependent on the public databases, and the amount of information constantly deposited is impressively large. However, attention must be drawn to the fact that a large number of proteins with unknown functions or not yet isolated can be completely ignored when protein homology is searched. This is also true for root proteins.

Fizames and his group (2004) obtained interesting results when they analyzed root transcriptomics of *Arabidopsis* plants using the SAGE (Serial Analysis of Gene Expression) technique, these authors reported 6000 different transcript tags, which showed no match to the *Arabidopsis* genes and suggest that a significant amount of transcripts present in roots originated from unknown or wrongly annotated genes. This is an intriguing result and may reflect the difficulty in root protein identification.Several proteomic studies on symbiotic fungus-root interactions have also been conducted and these organisms, although beneficial to the plant, seem to induce an array of defense-related proteins during

is has identified proteins involved in the development and functioning of mycorrhiza, including proteins involved in defense response and root physiology (Bestel-Corre et al., 2002). An interesting case is that of *Trichoderma*, which is a fungus that can increase root growth, control deleterious non-pathogenic root microflora, destroy toxic metabolites produced by deleterious microflora and control root pathogens (Harman et al., 2004).

Several studies have shown that root colonization by *Trichoderma* strains results in increased levels of defense-related plant enzymes, including various peroxidases, chitinases, β-1,3-glucanases, and the lipoxygenase-pathway.*Trichoderma* species have been reported as potentially useful for biological control, since they are able to attack or inhibit the growth of plant pathogens directly. Proteomic studies of the interaction of *Trichoderma* and maize seedlings grown from treated- and non-treated seeds were investigated by 2-DE. About 40% of the proteins observed in the presence of *Trichoderma* were not detected in untreated plants (Harman et al., 2004).Similarly, Marra (2006) identified 29 proteins related to the triple interaction among bean roots (*Phaseolus vulgaris*) with the fungi *Botrytis cinerea* and *Rhizoctonia solani* and its antagonist *Trichoderma*. Proteins with differential expression patterns were evaluated by MS and in root proteome.

PR proteins and other factors related to resistance were associated to the interaction between the plant and the two pathogenic fungi as well as with the antagonist. These results are in agreement with the findings of Harman et al.(2004), which showed that *Trichoderma* has the property of inducing resistance to many pathogenic microorganisms.Another important approach is the analysis of the *Trichoderma* proteome in response to the interaction with the plant. The proteins identified interaction process

processes that trigger plant defense, and may be used for the induction of resistance in plants. Some efforts have been made in this direction. Suarez et al. (2005) analyzed the extracellular proteome secreted by *Trichodermaharzianum* in the presence of different fungal cell walls. A combination of liquid chromatography and mass spectrometry allowed the identification of a novel aspartic protease, which showed 44% identity with the aspartic proteinase polyporopepsin (EC 3.4.23.29) from the basidiomycete *Irpex lacteus*. In summary, the results discussed exemplify the range of proteomic applications that can successfully be applied to the study of fungi-root molecular interactions.

1.7   Plant-pathogen interactions in the light of proteomic studies

In the case of pathogens, several of the proteins involved in pathogenicity are secretory proteins, which were observed in bacteria, fungi and nematodes, and were mainly identified by secretomic studies. These proteins include proteases, cellulases and pectate lyases, which are important,cell wall degrading enzymes, crucial for host plant colonization (Figure 1.12). These results clearly show the importance of secretomic studies when searching for pathogenic proteins. In addition to these well-known enzymes, other proteins, such as SODs and oxidases have also been reported in the different pathogens, and are associated with protection against the oxidative stress response by the plant on infection. A similar situation was observed with regard to defence-related proteins in plants. The most reported defense-related proteins are PR proteins, including thaumatins, glucanases, peroxidases and chitinases, observed in several pathosystems described in (Figure 1.12) (Mehta et al.,2008).

...teins in plant defence has been established; however, their direct role in resistance enhancement still needs to be verified. The general biotic stress response represents another class of regulated proteins, which include glutathione superoxide dismutase, S-transferase, and heat shock proteins (Karim et al., 2011).
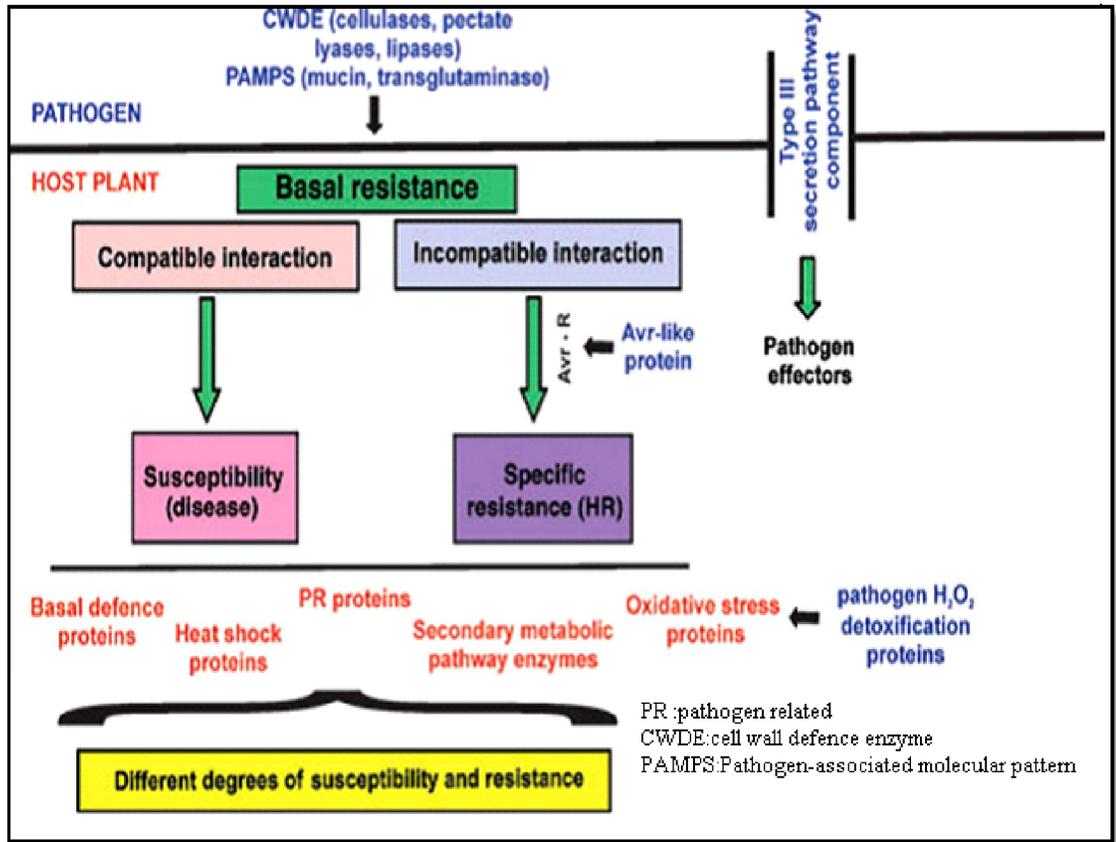
Figure1.12: Plant–pathogen interactions and perceptions into proteomic studies of the proteins involved in these processes (adapted from Mehta et al., 2008).

For plant-pathogen interaction studies, comparative proteomics proves to be the best approach as it has emerged as a promising tool for global analyses of protein expression levels in the recent past (Ca´novas et al., 2004). Different aspects of biological processes including protein identification, post-translational modifications (PTMs), protein expression profiles under stress/infection conditions during plant development and

could be successfully analyzed using proteomics-based approaches (Hashiguchi et al., 2010). Drought and other different stress condition have been investigated in various plants using proteomics-based dissection of differentially expressed proteins under different environmental factors including (Salekdeh et al., 2002; Yan et al., 2005;Yamaguchi et al., 2010; Sengupta et al., 2011). A recent review on plant proteomics emphasized the importance of undertaking root proteomics-based analyses under various stress conditions (Mehta et al., 2008).Proteomics is a latent tool for dissecting molecular mechanisms underlying fungal interactions. Proteomic changes in the host plant as it becomes infected or resists the pathogen can be traced back to the interaction between a particular host and pathogen gene pair if the genetics of the host-pathogen interaction follows the gene-for-gene model. The following biochemical changes are of big importance because they possibly will give close into crucial 'switch points' in the defence-related pathways that could be manipulated to engineer host plants with improved resistance or defence to the pathogen. This approach may also help to show the more complex mechanisms underlying activation of plant defence responses against biotrophic, necrotrophic and hemibiotrophic fungal and oomycete phytopathogens. Proteomics can also be employed to decipher molecular processes that occur during the recognition of pathogens by plants (basal defense) irrespective of the eventual outcome of the interaction (Kav et al., 2007).

The specific objectives of this study are as follow:

1-To understand the cellular signals of the host during infection through the study of selected genes.

2-To identify the global changes in gene expression during oil palm-*Ganoderma* interaction.

3-To identify the proteins that are specifically or predominantly expressed in infected and/or healthy palms (differentially expressed proteins) with the potential for use as biomarkers.

Materials and Methods

2.1   Materials (General)

All chemicals and solvents used were of the highest analytical grade available. The names of all chemicals and disposable items in this study and their respective sources are as listed in Appendix A.

The names of the instruments and their respective sources that were used in this study are as listed in Appendix B1.

The list of stock solutions/reagents used in this study is as listed in Appendix B2. The sterilization of materials is given in details in Appendix C.

2.2   Methods

2.2.1   Research methodologies

The research methodology used in this study was divided into two phases. The first phase of the research encompassed methodologies related to genomic analyses, which included the identification, isolation, and sequencing of selected genes that are potentially involved in plant defense against fungal infection. The second phase included methodologies for research involving exploration of differential gene expression at the protein level in *Ganoderma* infected samples in contrast to healthy control samples. The schematic flow of the research that was conducted is as illustrated in Figure 2.1.

GENOMICS

PROTEOMICS

Sample collection
Sample collected from Sime darby plantions /banting

Genesselection

Protein extraction

DNA extraction
CTAB extraction method
For DNA

RNA extraction
CTAB extraction method
For RNA

BradfordAssay

Degenerate primer design

cDNA synthesis

2-Dimensional electrophoresis

PCR

Gene specific primer design

Staining
Silver staining

Gelextraction

Real-Time PCR

2D-Analysis

Sequencing

Statistical analysis & interpretation

Figure 2.1: The flowchart of the research

All the seedlings used in this study were from normal uninfected seedlings D x P (*Dura x Pisifera* seedlings) of *UR 706/532 x UR 1679/147* crosses supplied by Sime Darby Seeds and Agricultural Services (SDSAS)-Planting Materials Unit (PMU) Layang-Layang, Johor, following the normal nursery practices and certified as *Ganoderma* free. The root tissue from the plant were briefly washed soil-free under running tap water, dried and then frozen dipped in liquid nitrogen ready for direct DNA and RNA extraction.

2.2.3 *Ganoderma* culture

Cultures of *G. boninense* PER71 were obtained from *Ganoderma*& Diseases Research Unit of the Oil Palm Unit Laboratory, MPOB. The culture was maintained at Plant Protection Unit, Sime Darby Research Centre, Banting, and Selangor. To fulfill Koch's Postulates, re-isolation of fungi were carried out from the inoculated tissues by cutting the discolored roots and stems into 1cm sections and plated onto *Ganoderma* Selective Medium (GSM) (Ariffin and Idris, 1992). The plates were incubated at 25°C for 2-3 days and observed for formation of brown halos and growth of mycelia from the root and stem sections. Colonies of *Ganoderma* were white in colour on the surface and the reverse was darkened (pigmented). The culture of *Ganoderma* isolate had an undulating surface in the darkened regions that buckled the agar (Idris et al., 2000).

2.2.4 Preparation of wood blocks for artificial inoculation

The rubber wood block (RWB) method for pathogenicity test used in this study was modified from the method used by Idris et al., (2006). RWBs measuring 6x6x6cm ($216cm^3$) were used as substrates to cultivate the inocula. All RWBs are washed and dried in an oven at 80°C for overnight before autoclave at 121°C for 1 hour. Each RWB was

20ml of Malt Extract (ME) Broth was added into each bag. Bags are sealed and autoclaved at 121°C for 15 min and then left to solidify overnight. The 7 day old dikaryon inocula on the PDA plate were cut into 4 parts and a half of blocks agar was inoculated onto the RWB. The inoculated RWBs were incubated at 25-28°C at 60-70% relative humidity (RH) for about 150 days during which time the *G. boninense* mycelia completely covered the wood blocks.

2.2.5 Plant challenge experiments

The seedlings used in this study were germinated for 1 month then artificially inoculated according to the standardized protocol for *Ganoderma* aritificail infection (Nur Ain Izati and Abdullah, 2008) used by the Plant Protection Unit (Sime Darby Research Sdn. Bhd). The poly-bags were cut and the seedlings removed. A colonised block was placed in direct contact with each of the seedling in a garden pot to avoid root contact with any external sources and then covered with soil and the inoculated mulch.

2.2.6 Experimental layout

The oil palm seedlings were divided into two groups controls (non-inoculated plants) and inoculated plants. From each group destructive sampling was carried out at every two-week intervals to obtain samples at 0, 2, 4, 6 and 8 after inoculation respectively to access the *G. boninense* infection. All oil palm seedlings were placed and arranged in a randomized block design under plant house conditions for 8 weeks. Using tap water, the seedlings were watered twice daily. Similar experimental layout was used for the protemoics study to obtain the samples at 2, 4, 6, and 8 weeks after inoculation.

Genomic DNA extraction was performed using modified CTAB extraction method as described by Doyle and Doyle (1990) with modifications using CTAB extraction buffer (2% CTAB,100mM tris-HCl, pH 8, 2 mM NaCl, 20 mM EDTA,2% PVP 40,2%ß-mercaptoethanol). About 200-250 mg of grounded tissue was added to 1.5 ml eppendorf tube containing 1ml of extraction buffer preheated to 65°C. The mixture was then washed twice with (24:1 chloroform: isoamylalcohol (C: I)), centrifuged at 1000 g for 5 min, and the upper layer was collected and washed with C: I again. The pellet was collected by centrifuging 8000 g for 20 min at room temperature, the pellet washed with 70% ethanol and dissolved after drying and resuspended in 50 µl pH 8.0 TE, or ultra pure d H2O. The RNA was removed using RNase A (20 µg/µl) followed by 30 min incubation at 37°C. The DNA was then stored at -20°C until use.

2.2.8 PCR of targeted genes

2.2.8.1 Primer design

Primers used in this work were designed based on the known sequences of *PGIP*, *LTP* and *PR10* genomic DNA from other plants available at the GenBank (http://www.ncbi.nlm.nih.gov/). The published sequences for the desired genes were retrieved from the GenBank database. DNA and amino acid sequence alignment was performed using ClustalW (http://abs.cit.nih.gov/clustalw/). Their amino acid sequences were aligned to allow the identification of possible conserved regions within the gene. The degenerate primers were then designed based on the sequence of the conserved regions. The specificity of each primer was verified by sequences manipulation suite (http://www.bioinformatics.org/sms2/) using the DNA sequences. Degenerate primers

only using the sequences that were very unlikely to bind to genes other than the genes of interest.

The sequences from four plants obtained from the Genbank were used to design the degenerate primers which were for *PGIP*, *Oryza sativa* (Accession # AM180653), *Triticum monococcum* (Accession # AM180658), *Stephanandra chinensis* (Accession # AF196951) *and Hordeum vulgare* (Accession # DQ995511)(full sequence and DNA alignment see Apendix D)pgip-f (5′ GTTGAACGG GCC TCT TCTC 3′) and pgip-r (5′-ACT TTATGC TTCCGG CTCGT -3′).For *LTPOryza sativa* (Accession # U77295), *Zea mays* (Accession # NM-001112535), *Hordeum vulgare* (Accession # X96979), *Triticum aestivum* (Accession # EF432573)(full sequence and DNA alignment see Apendix E) used to design the primer LTPf(5′ GCCCGTGACAGTTGGTGTTG 3′) and LTPr (5′ TGCGAGCGACTGCCATAGTAG3′).For *PR10Oryza sativa* (Accession # AB127580),*Triticum aestivum* (Accession # EU908212), *Rheum austral* (Accession # EU931221) and *Lycopersicon chilense* (Accession # AY899198) (full sequence and DNA aligenment DNA alignment see Apendix F) were used to designPR10f (5′ ACCTCAGCCATGCCCTTCAGCC 3′)andPR10r(5′TGGCCGTGACAGACTCCTTGGC 3′).

2.2.8.2 PCR reaction

A PCR reaction was used to confirm the presence of the three genes in the oil palm genome using that degenerate primers. PCR reactions were done using MJ Bio-Rade, THERMAL CYCLER) PCR conditions were: 1 cycle at 95°C for 2 min, 30 cycles at 95°C for 30 seconds, (60°C for 1 min for *PGIP* gene, 62°C for PR10 and 48 °C for *LTP* gene as annealing temperatures) 72°C for 1 min, and a final step at 72°C for 5 min.

nic DNA using Qiagen kit was done according to the manufacturer and cloning was carried out using pGEMT® easy Promega cloning vector system I (cat # A1360) as described in the protocol. Plasmid was purified from the recombinant bacterial clones (*E. coli* JM109) using the protocol (Sambrook et al., 1989). Sequencing reactions were performed using the "ABI PRISM dye terminator cycle sequencing ready reaction" kit and DNA sequences were determined with the 3130 *xI* genetic analyzer (Applied Biosystems). Sequence analyses were performed using Chromas software (http://www.flu.org.cn/en/download-49.html) and ClustalW algorithm for multiple alignments.Basic local alignment search tools (BLAST) at the International Nucleotide Sequence Databases (http://www.ncbi.nlm.nih.gov/) databases were performed with programs BLASTx and BLASTn with some changes in the default parameters.

2.2.9 PCR Purification

The purification was done using QIAquick® PCR Purification protocol and QIAquick® Gel Extraction Protocol (Qiagen, Germany). The purpose of these methods was to purify the PCR product in order to obtain only the desired amplicon. Five volumes of Buffer PBI (Phosphorus Buffer Index) were added to 1 volume of the PCR sample and mixed. The mixture was transferred to the QIAquick spin column in a 2ml collection tube and was centrifuged for 1 min to bind the DNA. The flow through was discarded and the tube was placed back to the column and was centrifuged for another 1 min. After that, 750µl of 35% guanidine hydrochloride was added and was centrifuged for 1 min to removed primer dimmer. Then, 750µl of Buffer PE (1 PE: 4 of absolute ethanol) was added to wash the DNA and centrifuged for another 1 min. Then, the column was placed in a clean 1.5ml tube, 30µl Buffer EB was added to the center of the QIAquick column to elute the DNA

after incubation for 1 minute at room temperature. Gel electrophoresis was carried out to verify the results. Purification of PCR sample was also carried out. For each PCR sample, the sample was loaded premixed with loading dye in a ration 5:1 in a big large well and electrophoresed. The gel was then viewed under UV light and the desired fragment was excised using a clean scalpel. The fragment was put in a clean 1.5ml tube and the weight was recorded. Then, 3 volumes of Buffer QG were added to 1 volume of the gel.

The sample was incubated at 50°C for 10 minutes (the sample was inverted for every 2 minutes to ensure the excised gel dissolved completely) before adding 1 volume of isopropanol and vortexing. The sample was applied to QIAquick column (with 2ml collection tube) and centrifuged for 1 mintue to bind the DNA. The flow through was discarded. Then, 500µl of Buffer QG was added and centrifuged for 1 mintue using the same collection tubes to remove all traces of agarose gel. After that, 750µl of Buffer PE (1 PE: 4 of absolute ethanol) was added in order to wash the DNA and was centrifuged for 1 minute. Before centrifugation, the sample was incubated at room temperature for 5 minutes. The flow through was discarded and the collection tube was returned to the column, then it was centrifuged again for another 1 minute to remove the ethanol residue completely. The column was then placed in a clean 1.5ml tube and 30µl of Buffer EB was added DNA elution. The column was incubated at room temperature for 1 minute before centrifugation for 1 minute. Gel electrophoresis was carried out to verify the results.

2.2.10.1 Preparation of LB/ampicilin/IPTG/X-Gal agar plates

Agar plates containing LB/Ampicilin/IPTG/X-Gal were prepared in order to culture the transformed host cell. For preparation of 500ml agar medium, 19.5g of LB agar powder was added in a Scott bottle. Then, 300ml of distilled water was added and the mixture was stirred using magnetic stirrer to dissolve the agar powder. Before the mixture was autoclaved at 121°C, 100kPa for 15 minutes, distilled water was topped up to final volume of 500ml. After that, the autoclaved agar was cooled down (around 60°C), 500µl of 100mg/ml ampicillin, 2500µl of 0.1 M isoprophyl β-D-thiogalactopyranoside (IPTG) and 800µl of 50mg/ml X-gal were added and mixed. Next, the mixture was poured into culture plates. Lastly, the agar plates were left to cool down and solidified before kept in 4°C for further use.

2.2.10.2 Preparation of competent cells

About 2 µl of *Escherichia coli*, JM109 was cultured in sterile 10ml LB Broth overnight at 37°C, 220 rpm in a shaking water bath. Next, 1ml of the overnight culture was then added into another 10ml LB broth. The subculture was incubated at 37°C, at 220 rpm in a shaking water bath until the Optical Density (OD_{600}) reading was 0.5 where the OD_{600} reading was determined using spectrophotometer (Biophotometer, Eppendorf). Before the sample was read, 500µl of sterile LB Broth was used as blank and for the sample, a dilution of 5µl of *E.coli* subculture with 495µl sterile LB Broth was made. After that, the subculture was then transferred into a sterile 15ml Falcon tube and incubated in ice for 30 minutes before it was centrifuged at 4°C, 3000 rpm for 5 minutes. The supernatant was discarded and the pellet was resuspended with 5ml RF1 solution (Table 2.1), pH 5.8 by

he tube. The resuspended solution was placed in ice for 20 minutes before being centrifuged again at 4°C, 3000 rpm for 15 minutes. The supernatant was discarded and 400µl of RF2 solution (Table 2.2), pH 5.8 was added to resuspend the pellet again. The resuspend solution (competent cells) was aliquoted into cold and sterile 1.5 microcentrifuge tubes (500 µl for each tube) and immersed in liquid nitrogen and kept at -80°C for future use (Sambrook and Russell, 2001).

Table 2.1: Recipe for 100ml of RF1 solution

Ingredient	Amount/Volume
$RbCl_2$	1.2g
$MnCl_2.4H_2O$	0.99g
KoAc	0.3 g
$CaCl_2.4H_2O$	0.15g
Glycerol	15ml
Distilled water	Top up until final volume is 100ml

Table 2.2: Recipe for 100 ml of RF2 Solution

Ingredient	Amount/Volume
MOPS	0.21g
$RbCl_2$	0.12g
$CaCl_2.4H_2O$	1.10g
Glycerol	15ml
Distilled water	Top up until final volume is 100ml

Gene cloning for this project was carried out using the pGEM®-T Easy vector systems (Promega, USA). The purified PCR products regions were ligated into the pGEM®-T Easy Ligation Vector using the recipe shown in Table 2.3.

Table 2.3: Recipe for Ligation

Reagent	Volume (µl)
2× Rapid Ligation Buffer, T4 DNA Ligase	5
pGEM®-T Easy Vector	1
ITS regions PCR product	3
T4 DNA Ligase (3 Weiss units/µl)	1

All the reagents were thawed in ice and only 2× Rapid Ligation Buffer can be vortex (vigorously before each use). After brief centrifugation, the reagents were transferred into 0.5ml microcentrifuge tube proportionally to its volume showed in Table 2.3. The ligation reaction was mixed by pipetting the solution and then incubated at 4°C overnight.

2.2.10.4 Transformation

Competent cells were first taken out from -80°C and thawed on ice while the overnight incubated ligation products were centrifuged to collect the content at bottom of tube. Then, 3 µl of ligation product with 100 µl of competent cell was transferred into a clean 1.5ml microcentrifuge tube. The competent cells were pipetted up and down before transferring to the tube. The tube was then gently flicked and inverted before incubating in ice for 20 minutes. The cells were heat shocked for 45 seconds at 42°C in preheated water bath and were immediately and gently transferred into ice for 2 minutes. Later, 900µl of sterile LB

bation at 37°C for 3 hours. After that, the sample was centrifuged at 1000rpm for 10 minutes. Then, 700µl of the supernatant was discarded and the pellet was resuspended with the remaining 300µl (by pipetting up and down) of LB broth. After that, 100µl was used to plate onto the LB/Ampicilin/IPTG/X-Gal agar plate while the remaining in the tube was kept at -20°C. The agar plate was incubated overnight at 37°C in incubator oven.

2.2.10.5 Colony PCR

After overnight incubation, the growth of the *E.coli* colonies could be observed with the presence of white and blue colonies. Competent cell that had been successfully transformed with a vector contained the right insertion will form white colonies while the one with no insertion formed the blue colonies. White colonies were picked, touch inoculated on duplicate plate and mixed into 50µl of distilled water (in 0.5ml microcentrifuge tube) using inoculating loop. Each time a colony was picked, the loop was sterilized with flamed. The samples in the tube were then boiled at 95°C for 5 minutes. The samples were used as the DNA sample for colony PCR amplification. PCR was carried out using the M13 forward and M13 reverse primers (Table 2.4). Recipe for colony PCR and the PCR condition are as listed in Tables 2.5 and 2.6. The PCR results were determined by agarose gel electrophoresis and viewed under the UV. The colonies with the correct insertion were selected.

for colony PCR

	Sequence (5'-3')
M13 F	GTAAAACGACGGCCAGT
M13 R	GGAAACAGCTATGACCATG

Table 2.5: Recipe for colony PCR

Reagents	Final Concentration
10× AmpliBuffer A (EURx, Poland)	1×
MgCl$_2$ (EURx, Poland)	1.5mM
dNTPs	200µM
M13 Forward Primer	10pM
M13 Reverse Primer	10pM
DNA sample	100ng
Taq DNA Polymerase (EURx, Poland)	1 unit

Table 2.6: Amplification program for temperature gradient PCR

Steps	Temperature (°C)	Time (minutes)	Cycles
Initial Denaturation	95.0	5:00	1
Denaturation	95.0	1:00	35
Annealing	60.0	2:00	
Elongation	72.0	2:30	
Final Elongation	72.0	5:00	1

The colonies that had the correct insertion were picked and cultured in 10ml of sterile 1×

LB broth medium using the inoculating loop overnight (12 – 16 hours) at 37°C, 220 rpm in

water bath. The 850 µl of the overnight culture was decanted into 1.5ml microcentrifuge

tube containing 150 µl of 100% glycerol for storage. This tube was then stored in -80°C

for future use. The remaining was transferred into a 15ml Falcon tube and centrifuged at

room temperature, 6000 rpm for 15 minutes. The supernatant was discarded and the pellet

was resuspended using 200 µl of ice cold Solution I (Table 2.7) by vortexing. The

suspension was then transferred into a new 1.5ml microcentrifuge tube and 200 µl of

freshly prepared Solution II (Table 2.8) was added, gently mixed and incubated at room

temperature for 4 minutes. Next, 200 µl of ice cold Solution III (Table 2.9) was added,

gently mixed and was incubated in ice for 15 minutes before centrifuged at 13 000 rpm for

15 minutes. The supernatant was transferred into the clean 1.5ml microcentrifuge tube and

3 µl of 50mg/ml RNAse A (Sigma-Aldrich, USA) was added. After that, the sample was

incubated in water bath at 37°C for 3 hours. Then, 600 µl of phenol (Pierce, USA) was

added, vortexed and centrifuged for 3 minutes. The mixture separated into two layers and

the upper layer was transferred into the new clean 1.5ml microcentrifuge tube before the

same procedure was repeated again using chloroform (Merck, Germany). Next, 0.1

volume of 5M NaCl and 2.5 volumes of ice cold isopropanol was added, gently mixed and

incubated in ice for 20 minutes before centrifuging for 15 minutes at 13 000rpm. The

supernatant was discarded and 1ml of ice cold 70% ethanol was added to the pellet and the

sample was centrifuged again at 13 000rpm for 5 minutes. After the supernatant was

discarded, the pellet was dried by speed vacuum and resuspended by 50µl of distilled water

. The spectrophotometer was used to check the quantity and purity of the extracted plasmid before it was digested using *Eco*RI (Sambrook and Rusell 2001).

Table 2.7: Recipe for Solution I (stored at 4°C)

Reagent	Final Concentration (mM)
Tris. HCl pH 8.0	25
EDTA pH 8.0	10
Glucose	50

Table 2.8: Recipe for Solution II (freshly prepared)

Reagent	Final Concentration
Sodium Hydroxide	0.2 N
SDS	1%

Table 2.9: Recipe for Solution III (stored at 4°C)

Reagent	Final Concentration
Potassium Acetate	3 M
Glacial Acetate acid	11.5%

2.2.10.7 Digestion of plasmids with *Eco*RI

Digestion of extracted plasmids with *Eco*RI (Promega, USA) restriction endonuclease was carried out in order to determine and verify the exactness of the size of its regions insertion into the vector. The reagents were mixed in a new clean 1.5ml microcentrifuge tube and were incubated at 37°C in water bath for 3 hours before incubated at 65°C for 10 minutes using the heat block to deactivate the enzyme. The samples were then electrophoreses in 1%

of insertion. Recipe for *Eco*RI plasmids digestion shown in Table 2.10.

Table 2.10: Recipe for *Eco*RI plasmids digestion

Reagent	Final Concentration
Buffer H (Promega, USA)	1×
BSA (Promega, USA)	0.1mg/ml
*Eco*RI (Promega, USA)	5 unit
DNA	5µg

2.2.11 Sequencing

The product of plasmid extraction with high concentration and good quality of purity were selected and diluted (300ng in 50µl) for sequencing. The sequencer machine used was the ABI PRISM 3130xl, Genetic Analyzer (Toshiba, Japan) using the BigDye® Terminator v3.1 Cycle Sequencing Kit. CHROMAS Version 2.31 software was used to edit the ITS regions sequence chromatogram for the forward and reverse sequences.

2.2.12 RNA extraction

The extraction of RNA from infected and non-infected oil palm root samples was carried out using CTAB method for RNA extraction described by (Ying and Tao, 2002) with modifications. 15 mL extraction buffer was pre-warmed to 65°C in a water bath by adding 20 µl β-mercaptoethanol. 0.4-0.7g of tissues was ground in a mortar using liquid nitrogen. The frozen powder was quickly transferred to the pre-warmed extraction buffer and mixed completely by inverting the tube. Then, incubated at 65°C for 10 minutes with vigorous shaking several times. An equal volume of chloroform-isoamylalcohol was added and shaken vigorously. Then, centrifuged at 10, 000 *g* for 10 minutes at 4°C. The very viscous

o a new tube and re-extracted with an equal volume of chloroform-isoamylalcohol. Then, centrifuged again as above. The supernatant was collected very slowly and carefully to avoid taking the cell lysates in chloroform.

Then, the supernatant was centrifuged at 30,000 g for 20 minutes at 4°C to precipitate the pellet and discard the insoluble material. After that, 0.25 vol of 10 M LiCl was added to the supernatant, mixed well, and stored at 4°C for 4 days instead of incubating overnight. The RNA was recovered by means of centrifugation at 30,000 g for 30 minutes at 4°C. The viscous supernatant was completely discarded and washed the pellet with 75% ethanol 3 times to remove the remaining and air dried it for 10 minutes. The RNA was dissolved in DEPC-treated water and stored the RNA at -80°C until use.

2.2.12.1 RNA analysis

The RNA was analyzed using the standard methods for agarose gel electrophoresis (Sambrook et al., 1989). The RNA quantity and quality was further estimated spectrophotometrically by Nanovuy (GE Healthcare, Sweden) at the absorbance ratios of $A_{260/280}$.

2.2.12.2 DNase treatment

DNase treatment was used to remove DNA from total RNA preparation. To the total RNA sample (~ 1 μg diluted in RNAse-free water) 1 μl 10x DNase reaction buffer, 1 μl 1 RNAse-free DNase (DNase 1, Amp Grade: Invitrogen) was added to a final volume of 10 μl. The mix was incubated for 15 minutes at 28°C. After that, enzymatic reaction was deactivated by adding 1 μl of 25mM EDTA (Invtorgen). The sample was heated after that for 10 minutes at 65°C.After that, the RNA sample was subjected to reverse transcription or kept in -80°C for future work.

oresis

Agarose gel (1%) was prepared in 1X TBE. About 350mg of agarose powder was dissolved in 35ml of 1X TBE and boiled using microwave oven about 1 mintue. The agarose mixture flask was cooled down under running tap water. 1 µl ethidium bromide was added and then poured it into the gel rack and the comb which was inserted into the other side of the gel rack. The comb was removed when the gel was solidified and put into the chamber with 1X TBE. The tank and column were rinsed with 70% ethanol and then washed with TBE which was already diluted into 1X TBE buffer from the 10X TBE buffer stock.During electrophoresis, the gel was submerged in a chamber containing a buffer solution and a positive and negative electrode and the current was applied at 120V for 25 minutes. The DNA/RNA to be analyzed was forced through the pores of the gel by the electrical current. Under an electrical field, DNA/RNA will move to the positive electrode (red) and away from the negative electrode (black). Then, the gel was analyzed by using Geldoc AlphaImager^{TM} 2200, (Alpha Innotech, and U.S.A).

2.2.14 cDNA synthesis

The High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, ABI) was used for cDNA synthesis. The kit uses the random primer scheme for initiating cDNA synthesis. Random primers ensure that the first strand synthesis occurs efficiently with all species of RNA molecules present. Reverse transcription of RNA was carried out in a 20 µL final volume using 0.2ml this-walled PCR tubes. Next, 10µl of 2 X RT master mixes was pipette into each individual tube (Table 2.11). About 10 µl of RNA sample was pipetted into each tube. The tubes were briefly centrifuged to spin down the contents and to eliminate the any air bubbles. The tubes were placed on ice until loading to into thermal cycler. The

ut in the thermo cycler at 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and at 4°C on hold as recommended by manufacturer. The cDNA were stored at -20°C, if not immediately used for real time PCR.

Table 2.11 RT-PCR mixture contents.

Component	Volume (µl)/Reaction Kit	
	With Rnase Inhibitor Kit	**Without Rnase Inhibitor**
10X RT Buffer	2.0	2.0
25X dNTP Mix (100mM)	0.8	0.8
10X RT Random Primer	2.0	2.0
MultiScribe™ Reverse Transcriptase	1.0	1.0
RNase Inhibitor	1.0	-
Nuclease-free H_2O	3.2	4.2
Total per Reaction	10.0	10.0

2.2.15 Real -Time PCR

In general PCR is amplification of a specific DNA sequence by using primers which bind to complementary sequences in the target DNA. By real-time PCR it is possible to monitor the progress of the PCR, since data is collected throughout the process. TaqMan® or SYBR® Green chemistry can be used to detect PCR Product (amplicon).

2.2.15.1 Primer design

Two sets of primers for RT-PCR were designed using Primer Express Software for Real Time-PCR for each of the three genes (Table 2.12). The gene-specific primer set (forward, 5'- ctc cac ccg aac gga agt att c -3' and reverse, 5'- ccc ggc aac cct aca tga ctt g -3') for the

80

the nucleotide sequence information for oil palm actin cDNA (GenBank accession no. AY550991.1). RT-PCR analysis was performed for RNA samples from infected roots from samples 0, 2, 4, 6 and 8 weeks and non-infected as control for each treatment.

Table 2.12: Primers used for RT-PCR analyses of *PGIP, LTP* and *PR10* putative genes in oil palm

Primer	Sequence	Length (bp)
Pgiprtf	5´ AACGCGTTGGATGCATAGC 3´	20
Pgiprtr	5´CTCGTATGTTGTGTGGAATTGTGA 3´	20
Pgip2rtf	5´GCTGCAGGCAAGCTCAAAA 3´	19
Pgip2rtr	5´GCGATTGGCATCTGAAGAAC 3´	20
LTPrtf	5´ ACGCCGCCGTGGCCATCACC3´	22
LTPrtr	5´ AAGGCTCCTCACGCCGCTGCAG3´	22
LTP2rtf	5´ AGGTCAACTCCGCCGTCGGGC3´	23
LTP2rtr	5´ GGACCTGGATGACGTGTTG3´	21
PR10rtf	5´ GCACCGCCATCGAGACGACCAC 3´	24
PR10rtr	5´CTCATCGTTTACCTCCACGCCC 3´	28
PR102rtf	5´GTTGTCCATATCTGACCCTTTTGTATAG3´	28
PR102rtr	5´GGCATACTCCCTTCAGTGGAAA3´	23

2.2.15.2 Real-Time PCR condition

Real-time RT-PCR was performed by using the Applied Biosystem 7500 Fast real time system and the Power SYBR® Green PCR Master Mix(Applied Biosystems, Burlington, ON, Canada) (Cat # 4368577) as recommended by the manufacture.

Each reaction consisted of 100 ng cDNA sample, 12.5 µl of Power SYBR® Green PCR Master Mix, 2.5 µl of primer mixture (forward and reverse), for a final volume of 25 µl.

distributed between the MicroAmp™ Optical 8-Tube Strip (Applied Biosystems, Cat # 4358293). The tube strip was sealed with MicroAmp™ Optical 8-Cap Strip (Applied Biosystems, Cat # 4323032). The tubes were briefly centrifuged to spin down the contents and to eliminate the any air bubbles.Amplification cycles were conducted

After initial denaturation for 10 minutes at 95°C, thermal cycling was performed for 40 cycles with steps of 92°C for 15 seconds, and 60°C for 120 seconds, with the fluorescence being read at the end of each cycle.

Dissociation curve was performed immediately after a completed PCR, to evaluate the presence or absence of nonspecific PCR products, including primer-dimer formation. PCR reactions were heated at 95°C for dissociation of all DNA present in the reaction. Then temperature was decreased rapidly to 60°C. Fluorescence data were continuously collected.

2.2.16 Protein extraction

Sample preparation is the initial experimental procedure for proteomic analyses. In order to select a suitable protein extraction method to be used for the proteomics investigations, three different protein extraction methods were compared to determine one that was most suitable for analysis by 2-DE.

2.2.16.1 TCA method

About 200mg ofuninoculated 1 month old oil palm root was homogenized with a pre-chilled mortar in 5 ml of cold acetone (–20° C) containing 10% (W/V) trichloroacetic acid (TCA) and 0.07% (V/V) 2-mercaptoethanol (2-ME) and 0.1 g of water-insoluble polyvinylpyrrolidine (PVPP). The mixture was kept at –20°C for 1 hour, and then

0 minutes at 4°C. The supernatant was discarded and the pellet was washed with 10 ml of cold acetone containing 0.07% (V/V) 2-ME. After 1 hour at –20°C, the mixture was centrifuged at 13,000 g for 20 minutes at 4°C. The pellet was washed twice with 80% cold acetone. Then the pellet was precipitated by adding 4 volumes of cold acetone. Finally, the white pellet was dried and stored at –70°C (Hu et al., 2002).

2.2.16.2 Sucrose method

About 200mg of uninoculated 1 month old oil palm root with the addition of 0.1 g of PVPP was extracted with 5 ml of extraction buffer (5% sucrose, 4% SDS and 5% 2-ME) for 10 minutes at room temperature with gentle stirring, followed by centrifugation at 10,000 g. The clear supernatant was heated at 100°C for 3 minutes and then cooled at room temperature. Proteins were precipitated by 8 volumes of cold acetone. After at least 1 h at –20°C, the mixture was centrifuged at 10,000 g. The pellet was re-suspended in 5 ml of extraction buffer and centrifuged at 10,000 g. After washed once or twice with 80% cold acetone, the pellet was precipitated by adding 4 volumes of cold acetone, and then was lyophilized and stored at –70°C (Ekramoddoullah, 1993).

2.2.16.3 Phenol/Ammonium acetate in methanol extraction method

Approximately 200 mg of uninoculated 1 month old oil palm root was ground into fine powder using mortar and pestle. The fine powder was dissolved in 5 ml Tris-buffered phenol (pH 8.8) and 5 ml extraction buffer consists of 1 M Tris-HCl pH 8.8, 10 mM EDTA, 0.4% 2β-mercaptoethanol and 0.9% Sucrose. The mixture was vortexed and spun at 5000g for 10 minutes. Phenol phase was removed and aqueous phase was back-extracted with 4 ml phenol/ 4 ml extraction buffer. Total proteins in the phenol phase were

f ice-cold 0.1 M ammonium acetate in 100% methanol. The mixture was kept at –20°C for 16 hours and spun at 5000g for 10 minutes at 4°C. Pellet was washed twice in 20 ml mixture of 0.1 M ammonium acetate in 100% methanol, rinsed twice with ice-cold 80% acetone and 70% ethanol. The protein pellet was dried for 5 minutes and dissolved in 1 ml solubilization buffer (7M Urea, 2M Thiourea, 4% CHAPS, 0.002% of 1% bromophenol blue stock) (Carpentier et al., 2005).

2.2.17 Screening and selection of suitable method of protein isolation

Protein samples isolated from different isolation methods were first screened by SDS-PAGE electrophoresis to determine the quality of the samples. Good quality protein produce a ladder-banding patter instead of protein smears when subjected to SDS-PAGE electrophoresis. The screening was performed with 12% SDS-PAGE gel using GEL PROTEIN System (Bio-Rad laboratories).

2.2.17.1 Preparation of 12% SDS-PAGE gel (PROTEAN System)

Gel plates were assembled as recommended by the manufacturer before the gel mixes were prepared. Reagents for preparation of 12 SDS-PAGE Gel (mini-PROTEAN Tetra handcast System: Bio-Rad) is given in table Appendix D. In these experement two types of gels were prepared, the stacking gel and resolving gels (separating gel). The preparation of both is described below:

Resolving gel (separating gel)-preparation for 2 gels

30:8% Acry: Bis (monomer)	3.33 ml
1.5 MTris –HCl, pH 8.8	2.00 ml
10% SDS	80 µl
sdH2O	2.5196 ml

64 µl

TEMED   6.4 µl

Total volume 8.00 ml

Monomer solution was mixed gently with 1.5 M Tris-HCl buffer, SDS and sdH2O, to prevent formation of bubbles. Finally, APS and TEMED were added to the mixture and mixed carefully. The mixture was immediately poured into the casting mould. The gel was overlaid with isopropanol and left to solidify for 15 minutes.

Stacking gel-preparation for 2 gels

30:8% Acry: Bis (monomer)	0.75 ml
0.5 M Tris –HCl, pH 8.8	1.25 ml
10% SDS	50 µl
sdH2O	2.8936 ml
10% APS	50 µl
TEMED	6.4 µl

Total volume 5.00 ml

After the resolving gel had solidified, the top portion of the gel was washed with dH2O. Monomer solution for stacking gel was mixed gently with 0.5 M Tris-HCl buffer, SDS and sH2O, to prevent formation of bubbles. After APS and TEMED were added to the mixture and mixed carefully, the mixture was immediately poured into the casting mould and a well forming comb was put into place. The gel was left to solidify at room temperature for 30-45 minutes or kept overnight in 4 °C if the electrophoresis is not performed immediately.

rotein markers

In a 0.2 ml tube, 2.0 µl of sample was mixed with 1.0 µl 3 X sample loading buffer and centrifuged briefly. For reference, 7.0 µl protein molecular weight markers (7-205 KDa broad range, Bio-Rad, cat # 161-0318) were used for each loading. The sample mixtures were boiled for 3 minutes in 100 ºC, centrifuge briefly and then loaded into gel.

ii. Gel electrophoresis, staining and documentation

To perform the electrophoresis, the wells of the gel were washed with dH2O to remove unpolymerised acrylamide. The gel cassette was immersed in 1X running buffer before the samples and marker were loaded into the wells. The electrophoresis was performed at 180 volts for 60 minutes. The electrophoresis gel was stained with Coomassie blue for 4 hours or overnight at room temperature with slow agitation (75 rpm). After staining the gel was destined with destaining solution for 3-6 hours. This solution was then replaced with destaining solution II and step was repeated for three to four times. The stained gel was kept in dH2O containing 20% glycerol for at least 20 minutes. The stain gel was viewed under white light and photographed. In this system, proteins denatured in the presence of SDS and 2-mercaptoethanol as thiol reducing agent acquired a rod-like shape and a uniform charge-to-mass ratio proportional to their molecular weights. The gels were stained with colloidal Coomassie stain and the protein sizes were determined by comparing the migration of the protein band to a molecular mass standard (PageRuler prestained Marker, Fermentas).

...otein concentration (Bradford Assay)

The Bradford assay was carried out to determine the concentration of solubilisation protein. For this assay, (SEGMA Bradford reagent) were used which was simple and accurate its involve. The standard 3.1 ml Bradford assay consists of mixing 1 part of the protein sample with 30 parts of the Bradford Reagent. The sample may be a blank, a protein standard, or an unknown sample. The blank consists of buffer with no protein. The protein standard consists of a known concentration of protein, and the unknown sample is the solution to be assayed. Bradford assays are routinely performed at room temperature. Color development begins immediately. The absorbance at 595 nm was recorded and the protein concentration was determined by comparison to a standard curve. The standard assay was performed as follows. A protein standard (BSA) was prepared in seven dilutions, representative of the protein solution to be tasted. The liner range of the assay was between 0 -1.4 mg/ml.

Protein solutions normally assayed in duplicate standard 250 μl of the Bradford reagent was added to each well (96 well plate) and contains 5 μl of the standard dilution and unknown samples, the mixture was incubated at room temperature for at least 5 minutes but not more than 1 hour as it may cause increase of absorbance. The absorbance was measured at 595 nm using Tecan GENios Microplate Reader.

2.2.18 2-Dimensional electrophoresis (2-DE)

2-DE was performed as previously reported by Lyngved and co-workers (2008). First dimension separation was carried out using the Multiphor II Electrophoresis System system and immobile dry strip (IPG) 24 cm, pH 3-10 and pH 4-7. For the second dimesion

ETTAN *DALT six* system for separation on 12.5% SDS-PAGE gel. Both systems were obtained from GE healthcare.

2.2.18.1 First dimension electrophoresis

IPG immobiline dry strip 24 cm (pH 3-10 and 4-7), IPG buffer (pH 3-10 and 4-7) and drystrip cover fluid were obtained from GE healthcare, preparation of reagent/solution for first dimension electrophoresis was described in (Appendix G). Sample was first thawed and gently vortex for 30 seconds and short spin before use. Optimised volumes of samples and buffers were used as previously suggested. Protein was loaded using loading by hydration. About 250 µg and 40 µg of protein for silver staining and commassie stain respectively. The total volume of 450 µl of sample plus the rehydration buffer was pipetted slowly to the rehydration tray avoiding the formation of bubbles.

a) *Rehydration of immobiline dry strip.* In the rehydration step, the immobiline drystrip reswelling tray from GE Health Care was used. The apparatus was rinsed with hot water and air-dried before used, once ready, the 450 µl sample with the rehydraton buffer was transferred to the slot of the reswelling tray.

The dry strip was allowed to slide down slowly to avoid trapping of unwanted bubbles and to ensure even contact with simple with sample mixture solution (gel facing down). Cover fluid (1-2 ml) was pipette on to the gel strip to minimize evaporation and to prevent urea crystallization. The strip was left to rehydrate overnight or up to 18 hours at rooms temperature to complete the rehydration process.

rehydration strip was transferred to the multiphore II flatbed electrophoresis system for isoelectrical focusing. The equipments were set earlier before the run was performed and was carried out according to the supplied by the manufacturer. To set up the equipment, the temperature of IEF apparatus was set to 20°C using a thermostatic circulator. About 10 ml of cover fluid was pipetted onto the ceramic (cooling) plate and the plate was covered with 5 ml cover fluid before placing the drystrip aligner (plastic) try. The dry strip tray was then covered with 15 ml fluid before placing the dry strip aligner carefully avoids bubbles.Once the temperature reaches 20°C, the rehydrated or reswelled IPG strip was positioned inside the aligner with the basic ends (pH 10 or 7) at the bottom of the tray (gel facing up).Electrode strips were placed across the cathodic and anodic ends of the aligned IPG strips. Electrodes were positioned on the electrode strips and before starting the electrophoresis, each strip and before starting the electrophoresis, each strip was checked again to ensure that it was fully covered by the cover fluid for good thermal contact. The parameter for the electrophoresis are stated below and carried out according to recommendation by the manufacturer.

IPG strip pH 3-10/4-7

Steps		Voltage		Time		Time-voltage Kvh
S1		500 v		0.01 h		0.001kVh
S2		500v		5.0 h		2.5 kVh
S3		3500v		6.0 h		3.0kVh
		3500v		1.30 h		39.5kVh
				11:20h		
Total		998		17:50 h		45

completed, the focused strips were transferred to screw cap tubes and then used for the second dimension electrophoresis. Alternatively, the tubes were kept in – 80 C until needed.

2.2.18.2 Second dimension electrophoresis

The list of reagent and preparation for second dimension electrophoresis are given and described in (Appendix I). The preparation of 12.5% SDS-PAGE is described below. The gel plates for the system were assembled as recommended by the manufacturer (Appendix J) before the gel mixes were prepared.

a) *Preparation of 12.5% SDS –PAGE Gel*. The recipes protocols for gels preparation are described below:

*this amount is to prepare 6 gels for the *ETTAN DALT SIX System*

30:8% Acry: Bis (monomer)	125 ml
1.5 M Tris –HCl, pH 8.8	100 ml
10% SDS	4 ml
sdH2O	169 ml
10% APS	2 ml
TEMED	132 µl

Total volume 400 ml

Monomer solution was mixed with 1.5 Tris –HCl buffer, SDS and sdH2O gently, to prevent the formation of bubbles. APS and TEMED were added to the mixture and mixed carefully, and the mixture was immediately poured into the casting mould and overlaid

The gel was then left to solidify for at least one hour. After polymerization, the gel was removed from the caster and stored in humid airtight container in 4°C if the electrophoresis is not carried out immediately. Gels can be kept up to 14 days in this manner.

b) *Equilibration of IPG strip.* The IPG strip needed to be equilibrated before proceeding for the IEF. Firstly, the IPG strips were incubated in 10 ml equilibration buffer with DTT, for 15 minutes with slow rotation (60 rpm) at room temperature. Once completed the solution was replaced with 10 ml of equilibration buffer with iodoacetaide plus bromophenol blue for another 15 minutes at room temperature. The strips were rinsed with cathode buffer before being loaded into the second dimension gel.

c) *Gel electrophoresis.* The second dimension gel electrophoresis was performed according to the procedure recommended by the manufacturer. The thermostatic circulator was set at 16°C. Once the SDS-PAGE gel had solidified, the overlay was described and replaced with the cathode buffer. The strips were slid slowly in between the plate forceps to avoid bubbles, pressed lightly with thin spatula. The cathode buffer that overlaid the gel was replaced with 0.5% warm agarose to seal the strip. The bottom was filled with 3 litres of anode buffer while the upper reservoir was filled with 1 L off the 1X running buffer.

The parameters for electrophoresis are stated below and are carries out as recommended by the manufacture.

Steps	W/GEL	Time
S1	2	0.30h
S2	17(MAX 100)	5.00h

pped at S2 after 5 hours or when the dye front was approximately 1 cm for the bottom of the gel. The gel was removed from the cassette and was stained with Coomassie blue or silver.

2.2.18.3 Staining

1) *Coomassie Blue staining.*Coomassie brilliant blue is an aminotriaylmethan dye that form strong but not covalent complexes with the protein. The uptake of the dye us approximately proportion to the amount of the protein, following the Beer-Lambert law. Basically the electrophoresis SDS-PAGE gel was immersed for several hours in concentrated methanol: acetic acid solution of the dye and excess dye was then allowed to diffuse from the gel during the destaining process (Sambrook et al., 2001).

The reagent for Coomassie Blue staining is given in (Appendix K1). Once the gel was removed from the cassette, the gel was immediately immersed in at least 5 volumes of staining solution and placed on a slow agitation for a minimum of 4 hours at room temperature. The staining solution was discarded and replaced with staining Solution and the procedure repeated until satisfactory results are obtained. The stained gel was kept in dH2O containing 20% glycerol. The stained gel was viewed under white-light and photographs were taken for documentation.

2) *Silver staining* .Silver staining is more difficult to perform in comparison toCoomassie staining but its 100 X more sensitive. The identification of protein by silver staining based on the differential reduction of silver ions that rebound to the side chain of amino acid this staining method is capable of detecting as little as 0.1-1.0 ng of polypeptide in a single band, the reagent for silver staining given in (Appendix K2).

used in this study was modified from that described by Heukeshoven and Dernick in 1988. In each step, gel was placed on slow agitation at room temperature. The PAGE gels were first fixed in fixer solution for 4-12 hr with changing the solution once in between. After fixing, the gel was washed once with 20% ethanol and once with distilled water for 10 minutes each. The gel was then sensitized with 0.02% sodium thiosulphate for 1 minutes. Following sensitisation the gel was washed three times with distilled water for 20 seconds and then stained with staining solution for 30 minutes. Before developing, the gel was washed two times with distilled water for 20seconds. The spots were developed with developing solution. The development was stopped with stop solution once the spots were clearly visible.

2.2.18.4 Gel scanning

The stained gel, either by Coomassie staining or silver staining was scanned using Image scanner ImageScanner™ III from GE Healthcare. The scanning was done according to the procedure proposed by manufacturer. The scanned photos were saved in JEPG and MEL format files and open in ImageMaster 2D Plantinum software for further analysis.

2.2.18.5 Spot analysis

Spot analysis was performed with ImageMaster 2D Plantinum software (GE Healthcare Life Science). Spot detection was conducted without spot editing and the spot were quantified using the % volume criterion. Scattered plots were used to analyze experimental variation and gel similarities.

...d database query

Protein spots with fold changes of >1.5 for at least two timeframes were collected from 2D gels. The protein spots were handpicked from all three gel replicates to increase the protein amount for digestion. Proteins were destained by gently shaking in 20 mM NH4HCO3, 50% acetonitrile (ACN) for 30 minutes at 37°C. This step was repeated until the spots become clear. The gel was first dehydrated in absolute ACN for 5 mintue followed by drying in speed vacuum. The dried gel was treated with (12 μg/ml) trypsin. The remaining trypsin solution was carried out at 37°C overnight by adding in 20 mM NH4HCO3 in 10% ACN. The supernatant containing peptides was collected and the gel pieces were re-extracted using 0.2% TFA. All peptides containing solutions of each spot were combined, dried and stored at 4°C until further MS analysis (Shevchenko et al., 1996; Bringans et al., 2008).

A 5800 Proteomics Analyzer (AB Sciex, USA) mass spectrometer in positive ion reflector was used to analyze the trypsin-digested peptides. Spectra were calibrated using trypsin autolysis products as internal standard. Data analysis was performed using Mascot sequence matching software (Matrix Science, UK). Search was performed using the following parammetrs:Peptide tolerance (Peptide tol) was ± 0.4, MS/MS tol was ± 0.4, with peptide charge +1. Trypsin was set as protyolytic enzyme with 1 allowed missed cleavge (Dahal et al., 2010). The non-redundant protein set from NCBI (NCBInr), Medicago sequencing database and UniPort considering Mascot score with higher than 55 (MASCOT probability $p<0.05$) (Bai et al., 2010).

Results

3.1 DNA isolation and PCR amplification of targeted gene sequences

The first experiments were focused on the amplification and verification of three oil palm genes that are thought to be involved in disease response particularly in response to fungal infection. The genes were polygalacturonase inhibiting protein (*PGIP*), lipid transfer protein (*LTP*) and pathogen related protein 10 (*PR10*) (see sections 1.5.1, 2 and 3). Genomic DNA was initially isolated from about 400 mg of the roots of uninfected oil palm using the CTAB extraction method (Figure 3.1). The oil palm from normal uninfected seedlings D x P (*Dura x Pisifera* seedlings) of *UR 706/532 x UR 1679/147* crosses. Four replicates samples were obtained from Sime Darby Seeds and Agricultural Services (SDSAS)-Planting Materials Unit (PMU) Layang-Layang, Johor. The DNA obtained had a concentration of 1.97 mg/ml and a purity of 1.83 $A_{260/280}$. The DNA was then subjected to PCR reactions using degenerate primers which were designed based on the sequence of the conserved regions from published sequence of other monocots (see appendices D, E and F) to obtain the putative bands for the targeted *PGIP*, *LTP* and *PR10* genes from oil palm (Figures 3.2a, b and c, respectively). The three sequences, which were identified based on their expected amplification sizes, were gel purified and cloned into the pGEMT$^{®}$ easy vector in *E. coli* (JM109). The samples fragments were then sequenced (partial sequence in appendix P1, P2 and P3) and the sequence and the sequence data compared with that of published sequences. The oil palm sequences were labeled as *EgPGIP*, *EgLTP* and *EgPR10* for *PGIP*, *LTP* and *PR10* respectively.

95
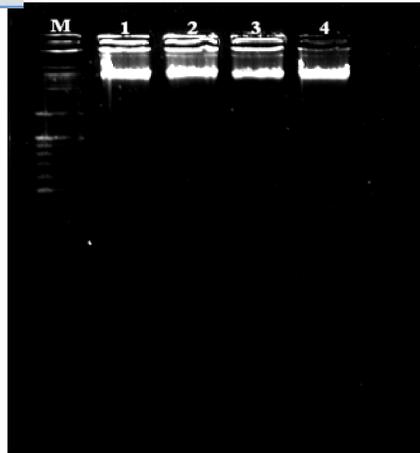
Figure 3.1: Agarose gel analysis of DNA from roots of oil palm (*Elaeis guineensis*, *Dura x Pisifera* seedlings). Oil palm DNA was extracted using CTAB method. Lane M: 1 kb Promega DNA ladder, Lanes 1, 2, 3 and 4 represent biological replicates of DNA from non-inoculated roots of oil palm samples. The gel was run at 120 volts until the dye front reached the bottom of the gel (approximately 30 minutes).

A

Continued on next page

B

M ltp1 ltp2 ltp3 ltp4 ltp5 ltp6

⇐ 500bp

C

⇐ 500bp
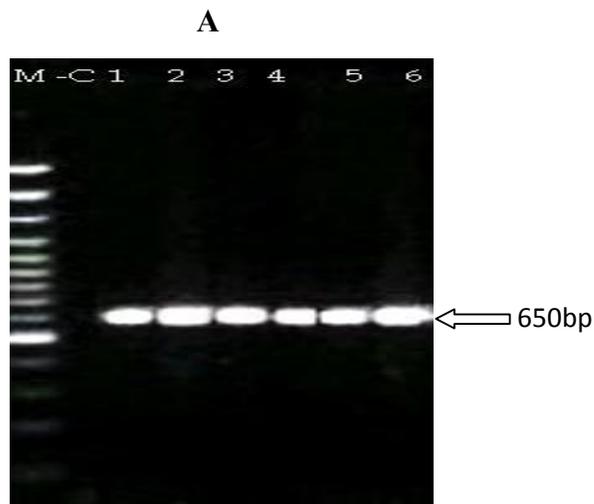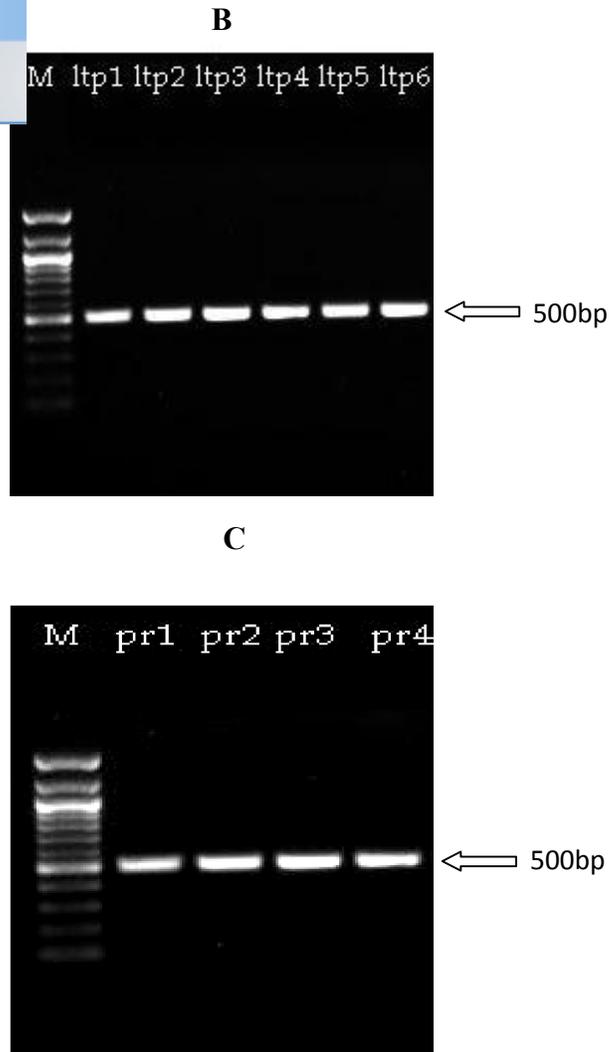
Figure 3.2: Agarose gel of PCR colony product fragments, **A**: wells labeled 1-6 represent PCR colony for *EgPGIP* fragment ,650bp (400bp *EgPGIP* fragment plus ~250bp DNA between the M13 forward and reverse priming sites) , **B**: wells labeled ltp1-ltp6 represent PCR colony for *EgLTP* fragment, 500bp (250bp of *EgLTP* fragment plus ~250bp DNA between the M13 forward and reverse priming sites) , **C:** wells labeled pr1-p4 represent PCR colony for *EgPR10*,500bp (250bp of *EgPR10* fragment plus ~250bp DNA between the M13 forward and reverse priming sites), lane labeled M: 1 kb DNA ladder (Promega). The gel was run at 120 volts until the dye front reached the bottom of the gel (approximately 30 minutes).

97

ndidate genes sequence with other monocot genes

When the putative oil palm *EgPGIP*, *EgLTP* and *EgPR10* gene fragments were aligned to those available in the plant database at NCBI (http://www.ncbi.nlm.nih. gov/blast/Blast.cgi) using BLASTx, high similarities to gene sequence form other monocots were obtained. Tables 3.1, 3.2 and 3.3 show the results of the comparative analyses performed on the *EgPGIP*, *EgLTP* and *EgPR10* genes fragments, respectively.

Table 3.1: Comparison of the oil palm *EgPGIP* gene fragments with other monocots, (Actual deduced amino acid alignment showed in Figure 3.3)

Monocots	Positives%[*]	Bit[§]	Gaps	Similarity%
Oryza sativa	100	231	0	100
Zea mays	70	102	0	70
Hordeum vulgare	79	90	0	79
Brachypodium sylvaticum	85	75	0	85

Table 3.2: Comparison of the oil palm *EgLTP* gene fragments with other monocots, (Actual deduced amino acid alignment showed in Figure 3.4

Monocots	Positives%[*]	Bits[§]	Gaps	Similarity%
Oryza sativa	100	132	0	100
Sorghum bicolor	82	92	1	68
Zea diploperennis	77	84	1	64
Zea mays	82	84	1	66

oil palm *EgPR10* gene fragments with other monocots, (Actual owed in Figure 3.5

Monocots	Positives%[*]	Bits[§]	Gaps	Similarity%
Oryza sativa	83	493	0	93
Triticum aestivum	100	563	0	100
Brachypodium distachyon	94	489	0	90
Zea mays	94	454	0	82

[*]Positives represent fractions of residues that are either identical or similar
[§]Bits represent measurement of the similarity between the two sequences: the higher the better (matches below 50 bits are very unreliable) (Claverie and Notredame, 2007).

All three putative partial gene sequences of *EgPGIP, EgLTP* and *EgPR10* from oil palm showed up to 100% Identity with the other monocots. The bits were high and few gaps were apparently observed. Comparison with other monocots showed similarities ranging from 70-100% for *PGIP,* 64-100% for *LTP* and 82-100% for *PR10* sequences.

Sequence alignment of the deduced amino acid of the putative oil palm *EgPGIP, EgLTP* and *EgPR10* gene sequences also indicated similarities to PGIP, LTP and PR10 amino acid sequences of other monocotyledons. The sequences amplified were highly conserved in the three clones and showed very high similarities (Figures 3.3, 3.4 and 3.5) that probably further confirm the presence of these genes in the oil palm genome.

Figure 3.3: Sequence organization and variability of the query represent *EgPGIP*. (A), (B), (C) and (D) show similarities with *Oryza sativa*, *Zea mays*, *Hordeum vulgare*, and *Brachypodium sylvaticum*, respectively.

```
            ITCGQV+SA+ PCL+Y     G+GPSA+CCSGVR+L +AAST A R
 Sbjct  30  ITCGQVSSAIAPCLSYARGTGSGPSASCCSGVRNLKSAASTAADR  74
```

Figure 3.4: Sequence organization and variability of the query represent *EgLTP*. Regions (A), (B), (C) and (D) show similarities with *Oryza sativa*, *Sorghum bicolor*, *Zea diploperennis*, and *Zea mays*, respectively.

```
Query   224   TSAMPFSLMKERLEFIDADKCEC   292
              TS MPFS+MKERLEF+DADKCEC
Sbjct    61   TSVMPFSFMKERLEFLDADKCEC    83
```

Figure 3.5: Sequence organization and variability of the query represented *EgPR10*. Regions (A), (B), (C) and (D) show similarities with *Oryza sativa*, *Triticum aestivum*, *Brachypodium distachyon,* and *Zea mays,* respectively.

3.3 Relative quantitative analyses of *PGIP, LTP and PR10* genes in oil palm

Real-time quantitative PCR offers a powerful tool for the quantitation of target nucleic acids. In order to compare the level of expression of the candidate pathogen defense related genes, PGIP, LTP and PR10, in *G. boninense* inoculated oil palm roots were investigated using the quantitative RT-PCR at five selected time pointes of post-inoculation.

te for this reaction. Isolation of high quality, intact RNA is the first and often the most critical step in performing many fundamental molecular biology experiments including RT-PCR. High-quality total RNA was obtained using the modified RNA isolation CTAB method(Al-Obaidi et al., 2010)(Comparison between the RNA concentration extarcetd in different incubation timesee Appendix L3). The yields of total RNA (μg/μl) for both control and infected oil palm tissues are listed in Table 3.4. The concentrations of biological replicates of RNA concentration for the infected and control samples are available in the Appendix section (Appendix L1). The total RNA from all oil palm root tissues for the stages tested was found to be of high quality and of sufficient quantity. The oil palm seedlings root tissues for the 0, 2, 4, 6, and 8 weeks for both control and *G. boninense*infected tissues produced RNA with a purity of >1.8 $A_{260/280}$, which met the minimal requirement needed for the construction of the cDNA.

Table 3.4: Purity and concentration of RNA from oil palm roots

Time of infection	Purity A_{260}/A_{280}	Concentration μg/μl	Control/non -inoculated	Purity A_{260}/A_{280}	Concentration μg/μl
0 week	1.96	1.26	0 week	1.87	1.827
2 weeks	1.87	0.847	2 weeks	1.82	0.744
4 weeks	1.84	1.48	4 weeks	1.80	0.853
6 weeks	1.80	0.667	6 weeks	1.81	0.392
8 weeks	1.85	0.43	8 weeks	1.88	0.139

In order to determine the optimal amounts of oil palm root tissues that are required for the construction of the cDNA, different amounts of the tissues were used to extract RNA. Our results demonstrate that 400-700 mg of root tissues were generally required to construct

than 700 mg of root tissues apparently did not generate significantly higher quantities of RNA with the protocol used.

The quantity of the RNA extracted was expressed as µg/µl of each sample preparation this was determined using Nanovuy (GE Healthcare, Sweden). The absorbance at 260 and 280 nm wavelengths in the ratio of OD $A_{260/280}$ were measured against a 'blank' containing water only for each sample. Results were automatically analyzed which ranged from 1.7-2.15, indicating that the RNA was highly pure, lack of protein contamination and without polyphenol and polysaccharide contamination.

3.4 Assessment of the integrity of RNA

The integrity of RNA was assessed by confirming the presence of two ribosomal RNA (rRNA) bands, 28S and 18S when RNA was electrophoresed on 1% agarose gel with a loading buffer. The RNA profiles (Figure 3.6A) and (Figure 3.6B) were obtained with no apparent degradation in the RNA preparation and they can be easily visualized on an agarose gel. The integrity of RNA was assessed by the sharpness of the RNA bands visualized on the gel.

A

B
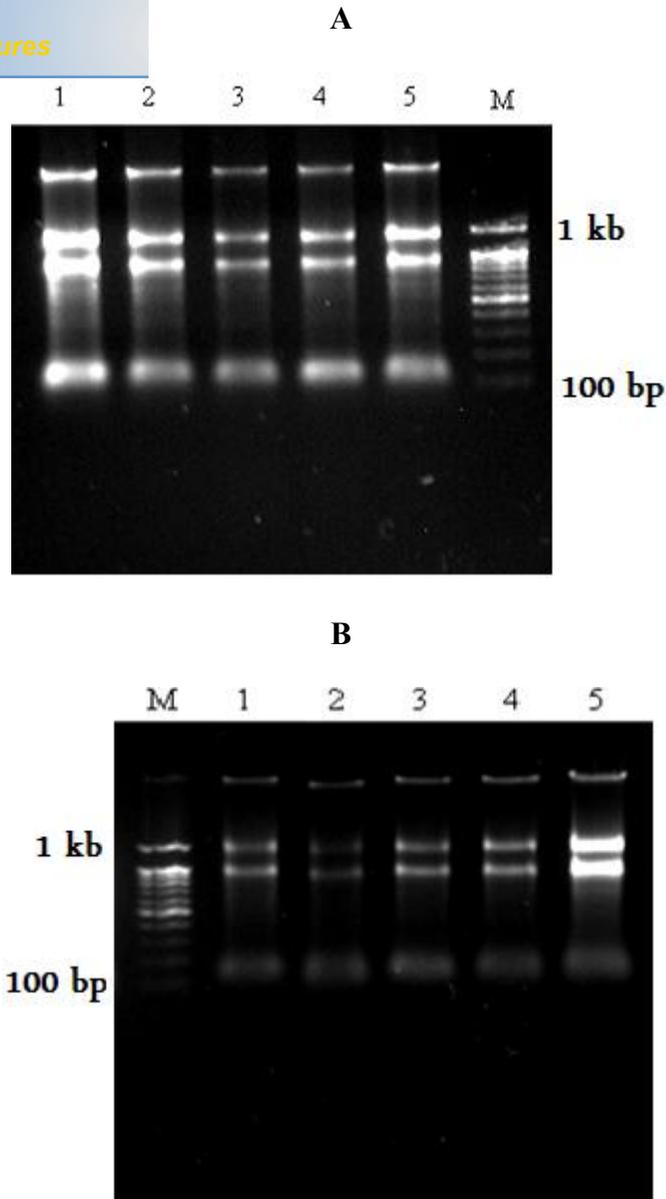
Figure 3.6: Visualization ofRNA extracted from control and infected roots of oil palm. Agarose gel (1.0% w/v) was used to separate the extracted RNA and staining was performed using ethidium bromide. (**A**) Refers to the non-infected (control) sample and (**B**) refers to the *G. boninense*-infected sample, before DNase I Treatment. Wells 1-5 show the RNA samples with 28s and 18s bands. Lane M is the low Range RNA marker (Fermentas).

The extracted RNA was further purified before it was used for cDNA construction and RT-PCR. To obtain pure RNA from the initial total nucleic acid extracts, DNA was selectively removed by the use of Deoxyribonuclease 1, Amplification Grade (DNase 1 Amp Grade) which proved to be efficient. Figure 4.7A and B demonstrate the results of the DNase digestion. Both the control and infected oil palm roots RNA extracts were apparently free of DNA upon visual inspection after gel electrophoresis. RNA that was shown to be free of DNA was subsequently used for RT-PCR from both infected and non-infected oil palm.

A

B
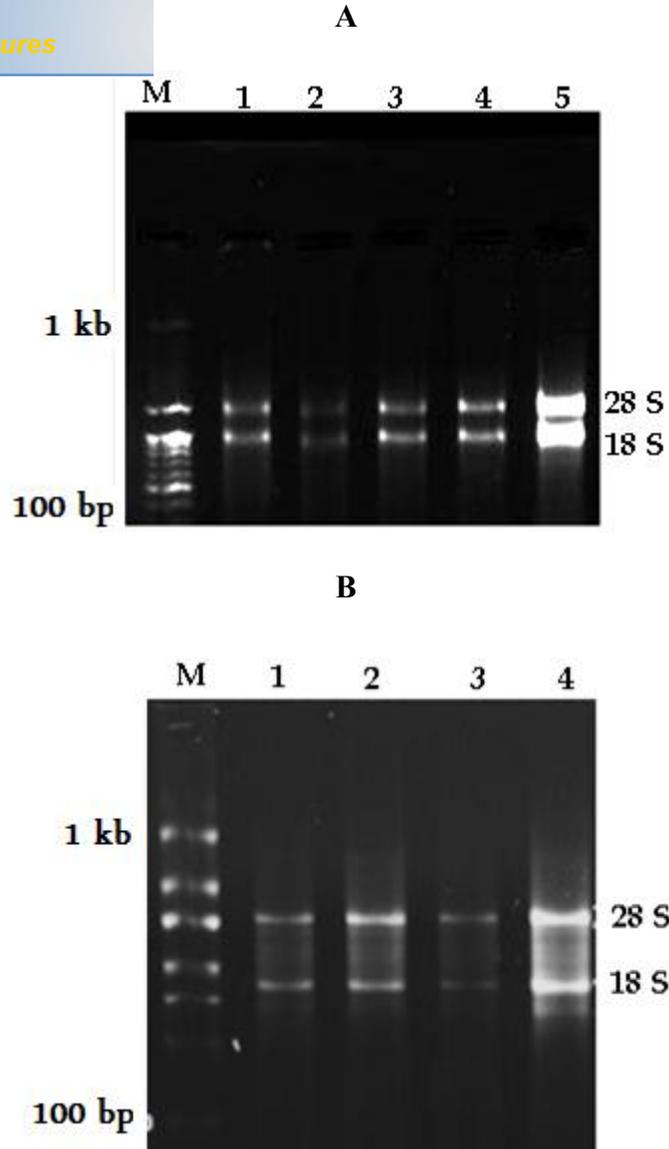
Figure 3.7: Ethidium bromide-stained 1.0% (w/v) agarose gel after DNase treatment (**A**) for infected samples and (**B**) for the non-infected root sample. Lane M: Low range RNA marker (Fermentas).

The use of DNA-free RNA samples to construct a cDNA using the High Capacity cDNA Reverse Transcription Kits from Applied Biosystems (Table 3.5) generated high concentration of constructs, which also of high purity. The quantity of cDNA generated from each reaction ranged from1562-1899 ng and the purity ($A_{260/280}$ ratios) ranged from 1.70-1.92. Results of the RT-PCR experiments also provide evidence on the good quality of the RNA that was earlier obtained. Concentrations of the biological replicates of the constructed cDNA for the infected and control samples are available in the Appendix section (Appendix L2).

The constructed cDNA was subsequently used as template to proceed to real-time quantitative PCR for further analysis of gene expression.

Table 3.5: Purity and concentrations of cDNA from oil palm roots

Time of infection	Purity A_{260}/A_{280}	Concentration ng/μl	Control	Purity A_{260}/A_{280}	Concentration ng/μl
0 week	1.74	1689	0 week	1.92	1899
2 weeks	1.70	1562	2 weeks	1.79	1782
4 weeks	1.73	1730	4 weeks	1.71	1699
6 weeks	1.72	1627	6 weeks	1.77	1563
8 weeks	1.74	1540	8 weeks	1.75	1834

LTP and *PR10* genes in *G. boninense* infected and uninfected oil palm

The expression of candidate pathogen defence related genes, PGIP, LTP and PR10, in *G. boninense* infected oil palm roots were investigated using quantitative RT-PCR at five selected time points of post-inoculation. Similar sampling time points were used with the un-inoculated control palms.

3.7.1 Time point sampling of *G. boninense G. boninense* infected and uninfected oil palm

According to the experimental layout,the oil palm seedlings were divided into two groups controls (non-inoculated plants) and inoculated plants. From each group destructive sampling was carried out at every two-week intervals to obtain samples at 0, 2, 4, 6 and 8 after inoculation respectively to access the *G. boninense*infection.

3.7.2 Quantitative PCR analysis

Quantitative real-time PCR analysis revealed an abundance of the three gene transcripts in all samples of both infected and control oil palm root. To amplify a 120bp cDNA fragment of each gene, RT-PCR was carried out done with three sets of primers, i.e., *PGIP*rtf and *PGIP*rtr for the PGIP gene, *LTPrtf*and *LTPrtr* for the LTP gene and PR10rtr and *PR10*rtr for the PR10 gene. In general, for all three genes studied, higher levels of expression were found in the controls (from 0 week), while after infection with *G. boninense*, the expression levels were lower at all treatment time-points (2, 4, 6 and 8 weeks after infection). The real time full set of runs (4 replicates for each gene) are shown in Appendix M.

by RT-PCR with primers, *PGIP*rtf and *PGIP*rtr, resulted in the expected 120bp amplicon (Figure 3.8). The results confirmed that the primers used were effective and the target gene was expressed in plants under the conditions studied as shown by the dissociation curves for amplifications using both primers for endogenous*β-actin* as well as for the target gene *EgPGIP* (Figure 3.9). The amplification plot for the real time analysis of expression of *EgPGIP* post-inoculation is shown in Figure 3.10.
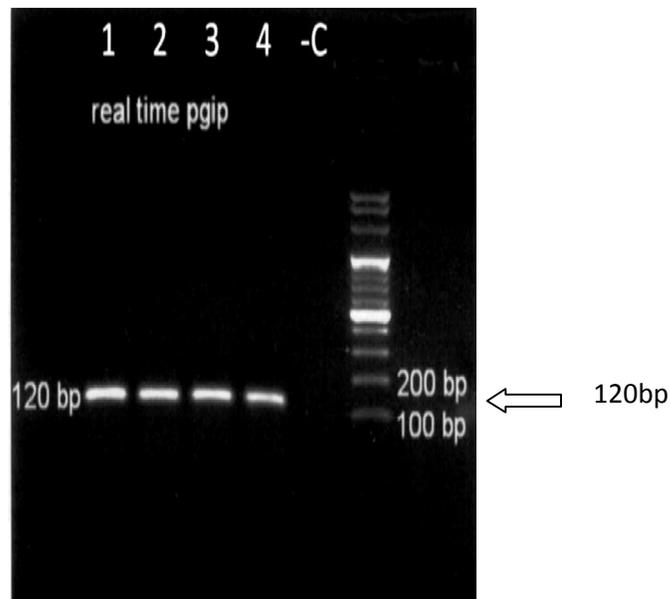
Figure 3.8: Ethidium bromide-stained 1.0% (w/v) agarose gel of PCR (real time condition) products, using oil palm cDNA as a template and real time specific primers (*PGIP*rtf and *PGIP*rtr). With the exception of the negative control (well –C), positive amplification showing presence of PGIP cDNA fragments (~120bp) was obtained for all wells. Lane M: 100bp DNA ladder (Promega).

A

PGIP
TM=60°C

B
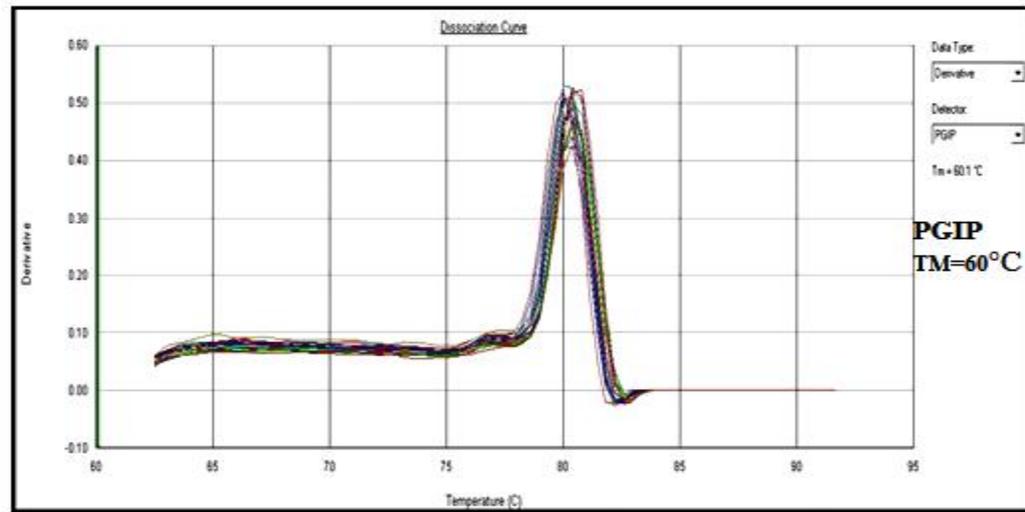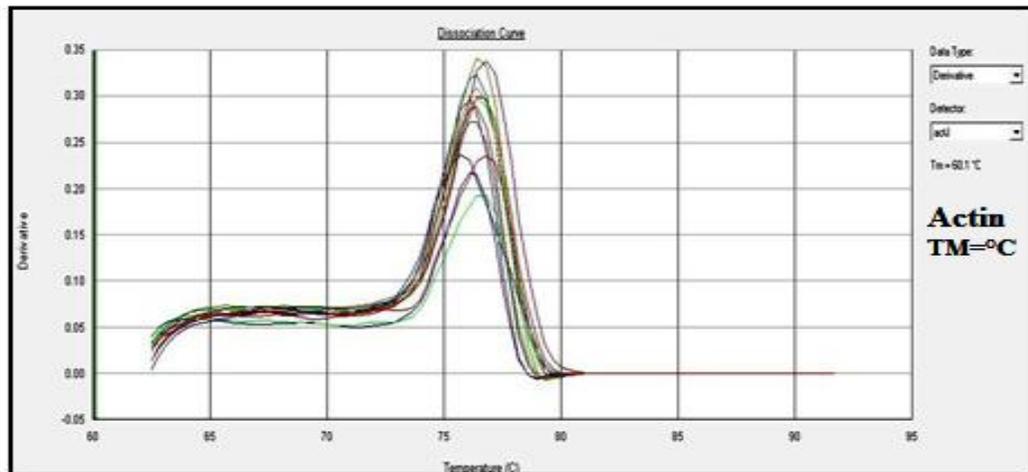
Actin
TM=°C

Figure 3.9: Dissociation curve for target gene *EgPGIP* and endogenous control *β-actin*. (**A**) Dissociation curve for target gene *EgPGIP*, showing the primer efficacy for a specific product and amplicon size of 120bp, and (**B**) Dissociation curve for endogenous control *β-actin*, showing the primer efficacy for a specific product and amplicon size of 80bp. Single peaks at 80°C for the target gene and 78°C for *β-actin* gene verify that the primers used were efficient and effective for both infected and non-infected sample.

Cycle Number

Analysis Settings
Auto Ct
Manual Ct   Automatic Baseline
Threshold: 0.1663145   Manual Baseline: Start (cycle): 3   End (cycle): 15   Help

Line Color: Well Color
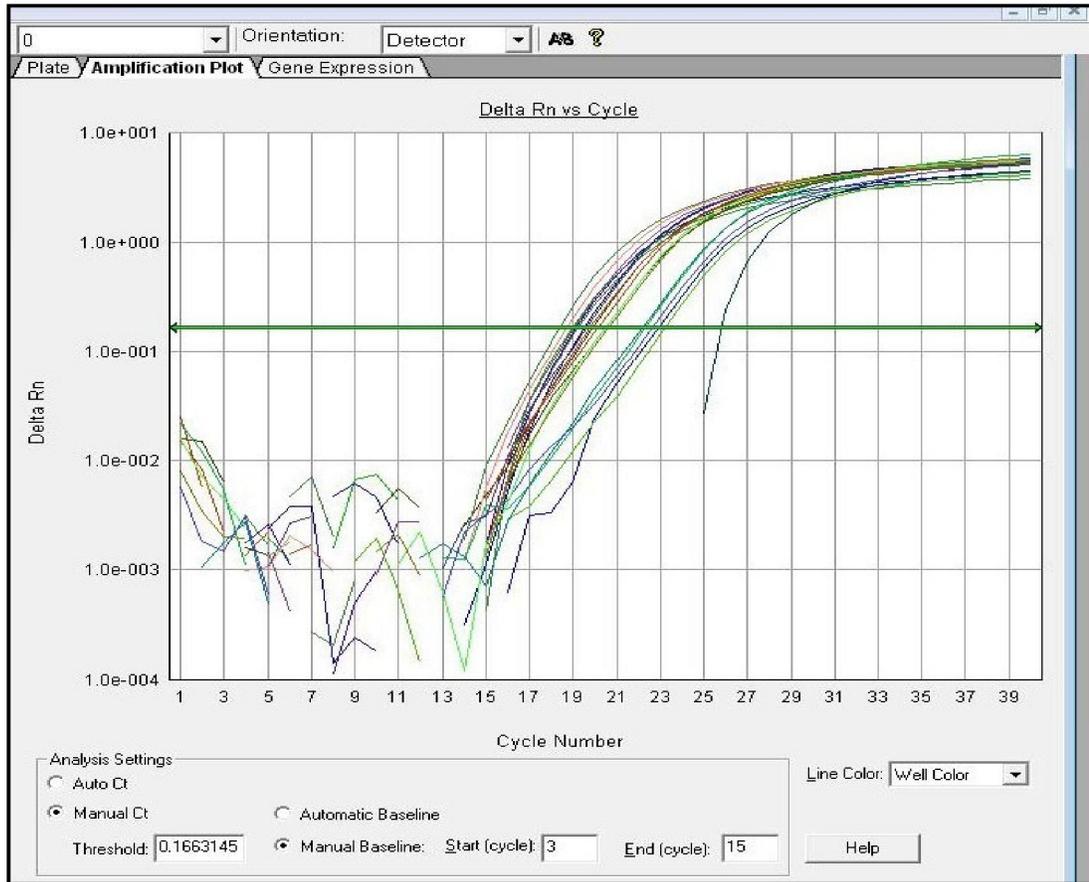
Figure 3.10: Amplification plot for the Real Time-PCR reaction. The figure shows the fluorescence signal versus cycle number for both target *EgPGIP* and endogenous control *β-actin* genes using oil palm cDNA as a template at 0, 2, 4, 6 and 8 weeks for both infected and non-infected sample.

112

s (Figure 3.11) shows that the highest expression level of PGIP was found in the uninfected oil palm at all timepoints studied (0, 2, 4, 6 and 8 weeks). The expression level then appeared to be significantly down-regulated when measured at 2 weeks after infection with *G. boninense* and reached its lowest level at 4 weeks post-inoculation, in comparison with the control. The levels remained suppressed when measured up to 8 weeks post-inoculation. The control plants (uninfected) showed no significant changes of the *EgPGIP* during the same time-points (p value > 0.5). Amplification of the *EgLTP* with the primers *LTP*rtf and *LTP*rtr, and *EgPR10* by RT-PCR with primers *PR10*rtf and *PR10*rtr resulted in the expected 120bp amplicon for each of the genes (Figure 3.12 and Figure 3.13). The results confirmed that the primers used were effective and the target gene was expressed in plants under the conditions studied, as shown by the dissociation curves for amplifications using both primers for *β-actin* as well as for the target genes *EgLTP and PR10* (Figure 3.14 A and B) and (Figure 3.15 A and B, respectively). Figures 3.16 and 3.17 show the amplification plot for the real time analysis of expression for *EgLTP* for *EgPR10* genes, respectively, after inoculation with fungus.
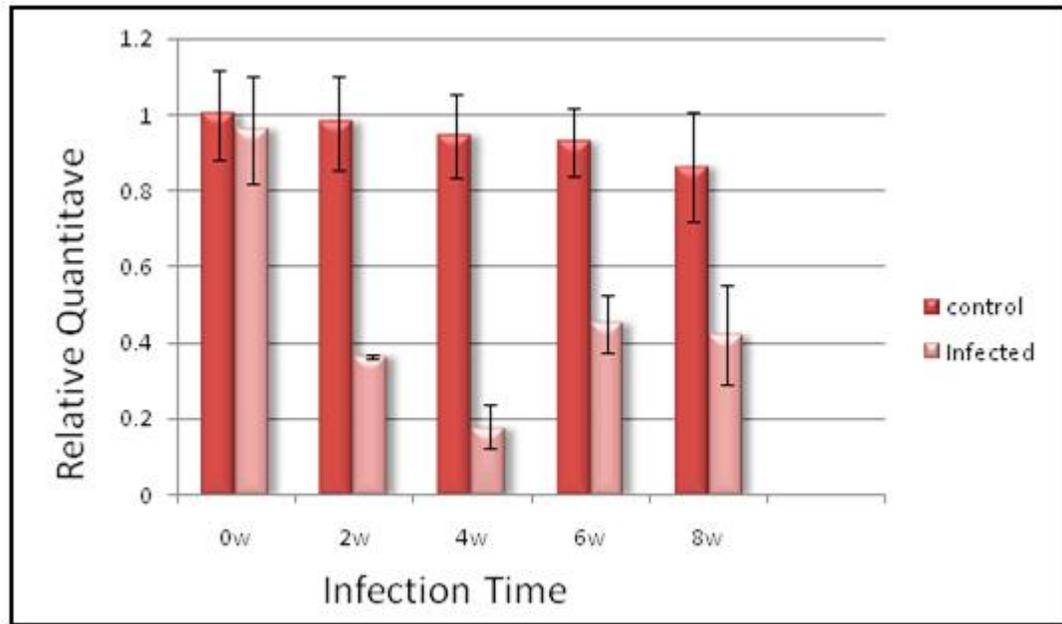
Figure 3.11: RT-PCR analysis of *PGIP* gene expression in oil palm inoculated with *G. boninense*. Size of the amplicon of *EgPGIP* gene transcripts and *β-actin* gene transcript were 120bp and 80bp, respectively. The latter was used as the constitutive control. Bars in the graph shows RQ values for expression of the gene at 0, 2, 4, 6 and 8 weeks of post-inoculation and the error bar shows the standard deviation of the mean of four biological replicates. All p values for the five post-inoculation time points stages were less than 0.05 (statistically significant).

⇐ 120bp
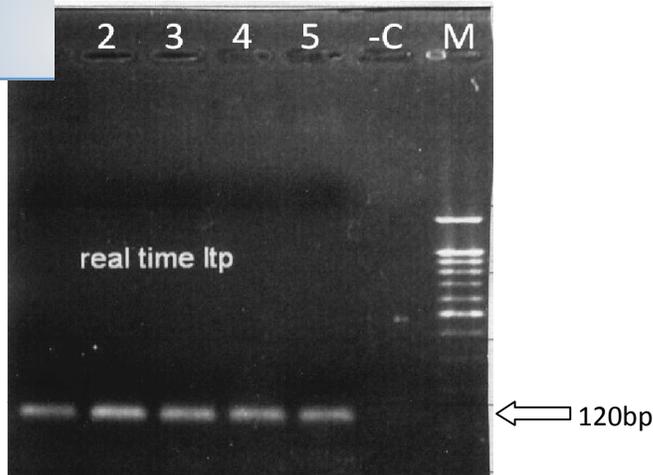
Figure 3.12: Ethidium bromide-stained 1.0% (w/v) agarose gel of PCR (real time condition) products, using oil palm cDNA as template and real time specific primers (*LTP*rtf and *LTP*rtr).   With exception of the negative control (well –C), positive amplification showing presence of LTP cDNA fragments (~120bp) was obtained for all wells. Lane M: 100bp DNA ladder (Promega).

⇐ 120bp
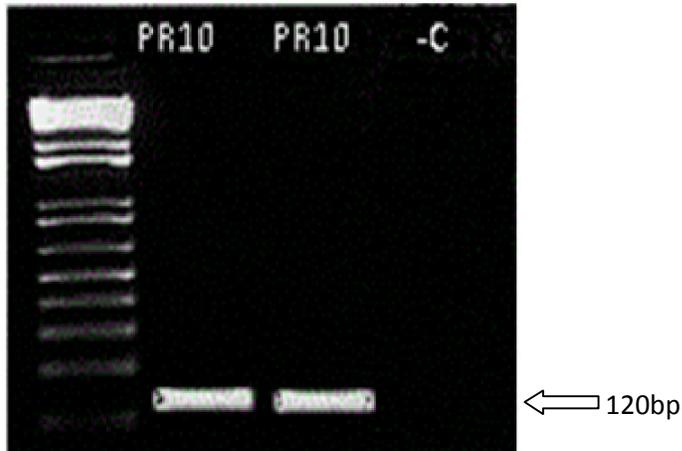
Figure 3.13: Ethidium bromide-stained 1.0% (w/v) agarose gel of PCR (real time condition) products, using oil palm cDNA as template and real time specific primers (*PR10*rtf and *PR10*rtr).   With exception of the negative control (well –C), positive amplification showing presence of PGIP cDNA fragments (~120bp) was obtained for all wells. Lane M: 100bp DNA ladder (Promega).

A
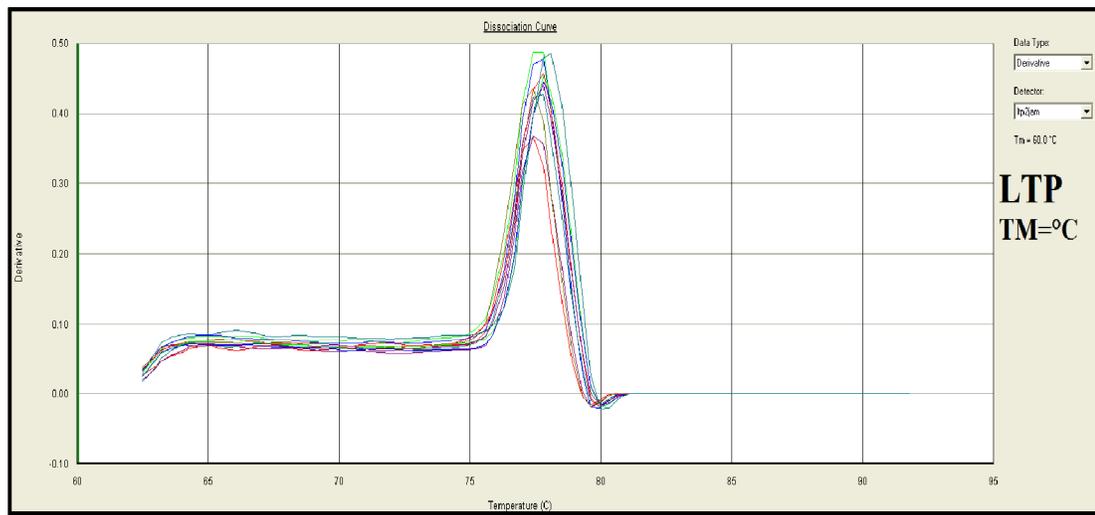
B
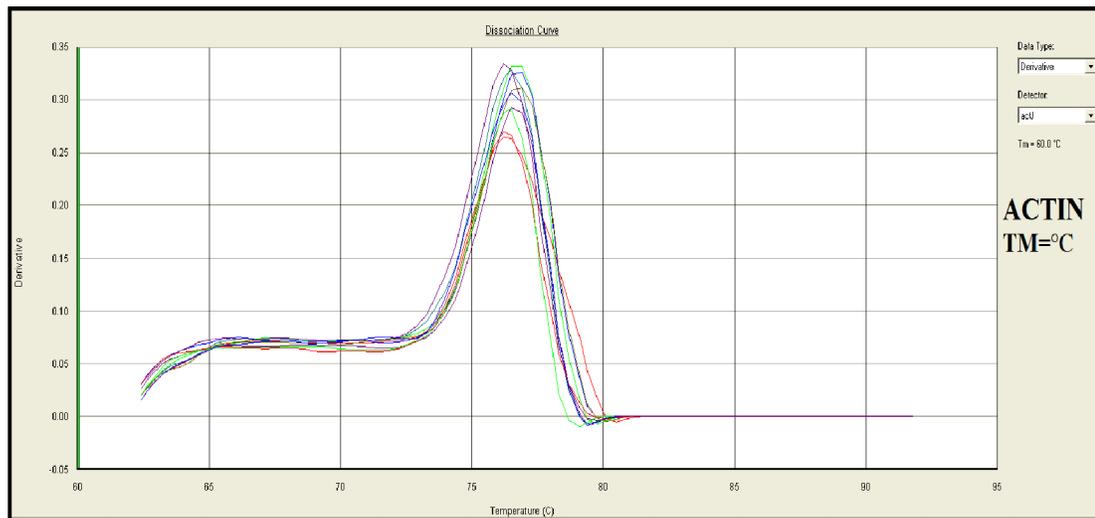
Figure 3.14: Dissociation curve for target gene *EgLTP* and endogenous control *β-actin*. (A) The dissociation curve for target gene *EgLTP*, showing the primer efficacy for specific product, amplicon size 120bp. (B) The dissociation curve for endogenous control *β-actin*, showing the primer efficacy for specific product, amplicon size 80bp. The single peak at 77.5°C for target gene and 77°C for *β-actin* gene verify that the primers used were efficient and effective for both infected and non-infected sample.

A
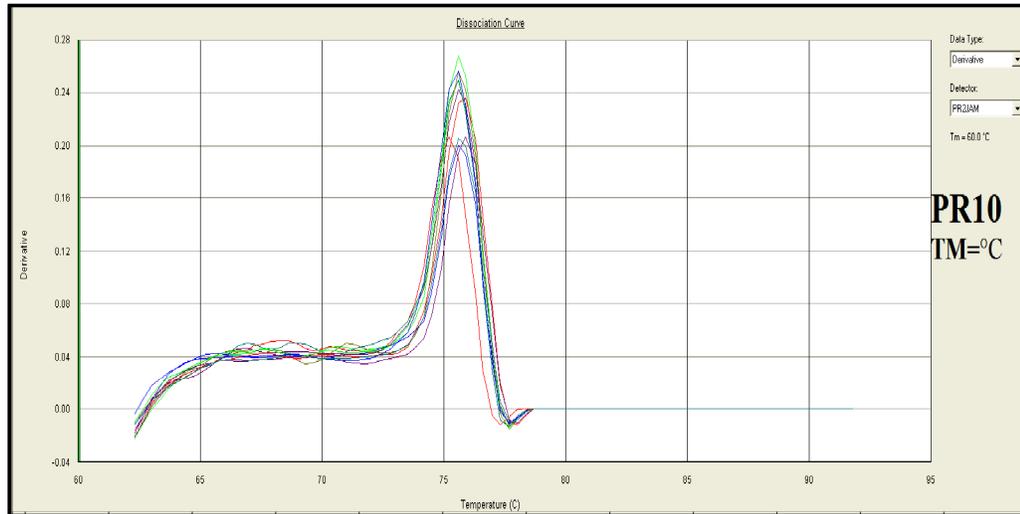
B
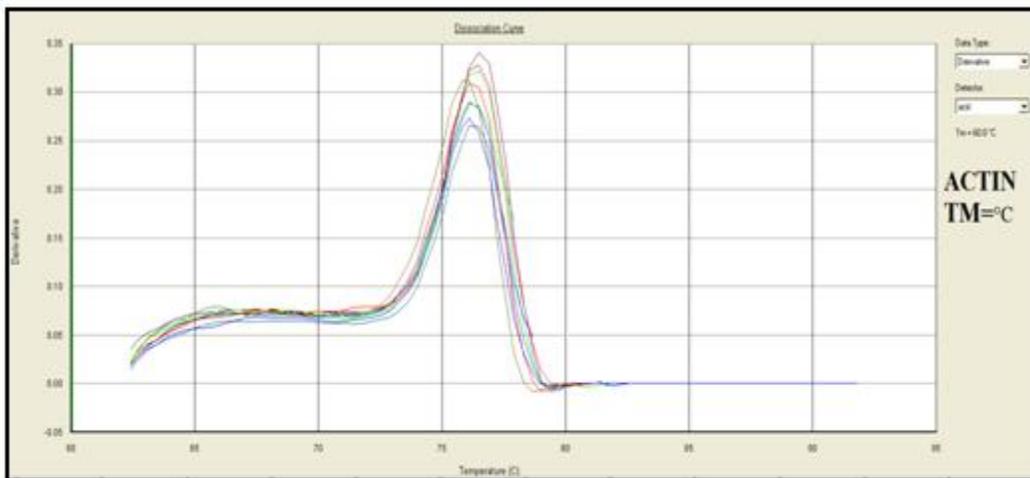
Figure 3.15: Dissociation curve for target gene *EgPR10* and endogenous control *β-actin*. (A) The dissociation curve for target gene *EgPR10*, showing the primer efficacy for specific product, amplicon size 120bp. (B) The dissociation curve for endogenous control *β-actin*, showing the primer efficacy for specific product, amplicon size 80bp. The single peak at 77.5°C for target gene and 77°C for *β-actin* gene verify that the primers used were efficient and effective for both infected and non-infected sample.

117
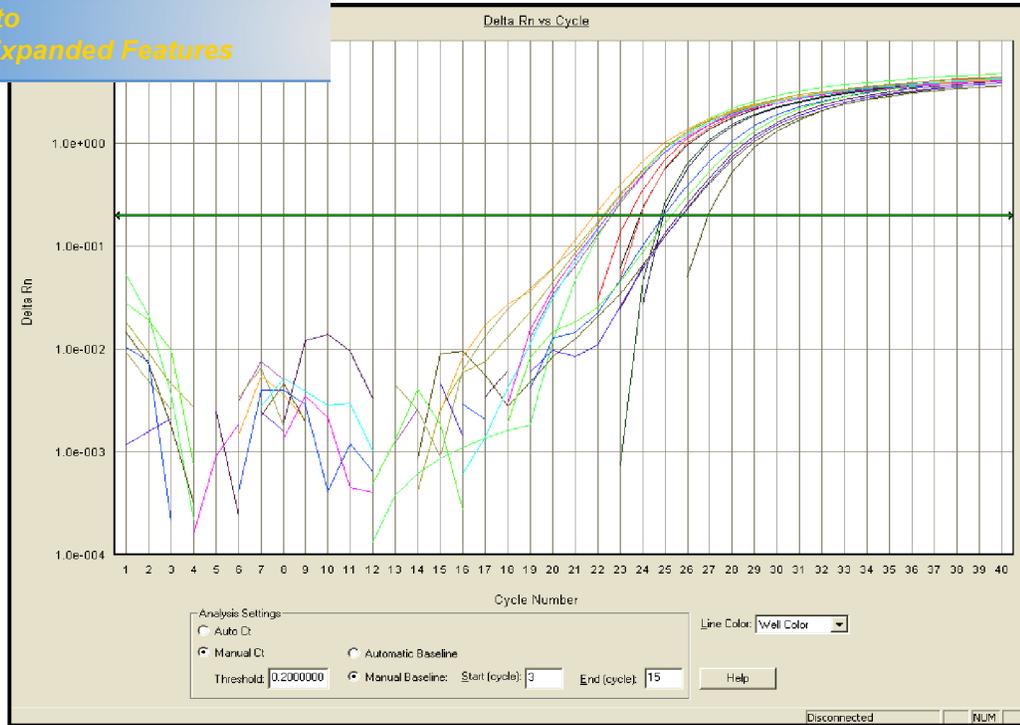
Figure 3.16: Amplification plot for the Real Time-PCR reaction. The figure shows the fluorescence signal versus cycle number for both target *EgLTP* and endogenous control *β-actin* genes using oil palm cDNA as a template at 0, 2, 4, 6 and 8 weeks for both infected and non-infected sample.

1.0e-004 1   3   5   7   9   11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
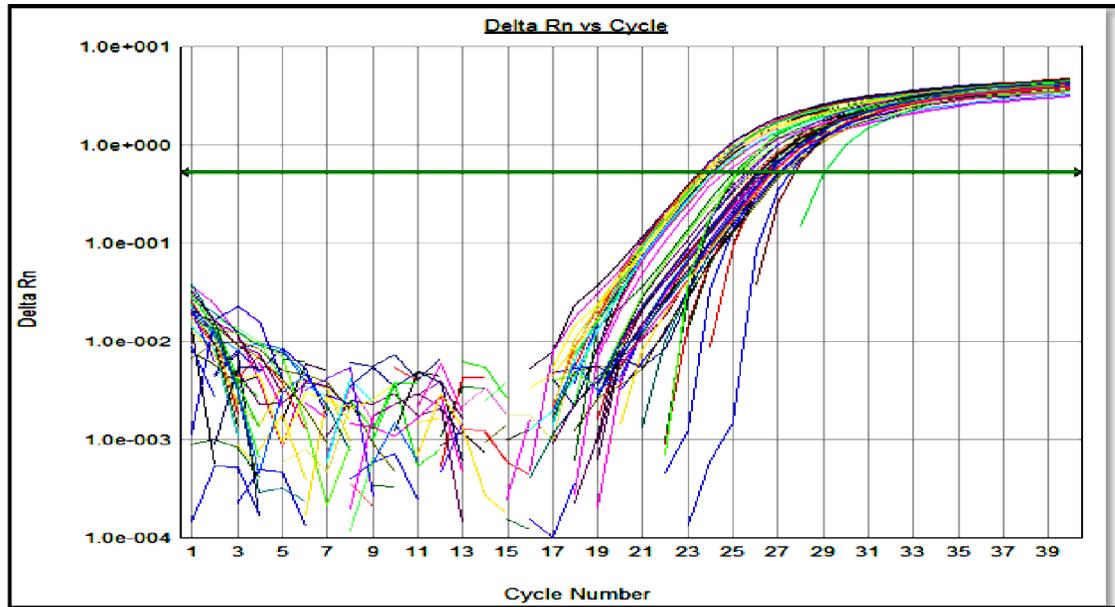
Cycle Number

Figure 3.17: Amplification plot for the Real Time-PCR reaction. The figure shows the fluorescence signal versus cycle number for both target *EgPR10* and endogenous control *β-actin* genes using oil palm cDNA as a template at 0, 2, 4, 6 and 8 weeks for both infected and non-infected sample.

The Quantitative-PCR proved to be highly sensitive. The expression levels of the LTP and PR10 genes (Figure 3.18 and Figure 3.19) were highest in the uninfected oil palm in all the selected time points (0, 2, 4, 6 and 8 weeks). The expression level appeared to be drastically down-regulated when measured 2 weeks after inoculation with *G. boninense*, and reached its lowest level at 8 weeks post-inoculation in comparison with the uninfected control for both the genes tested. Control plants (uninfected) showed no significant changes of the *EgLTP* and *EgPR10* genes during the same period. The RT-PCR reaction of biological replicates for the infected and control samples for the three candidate genes are available in the Appendix section (Appendix M).
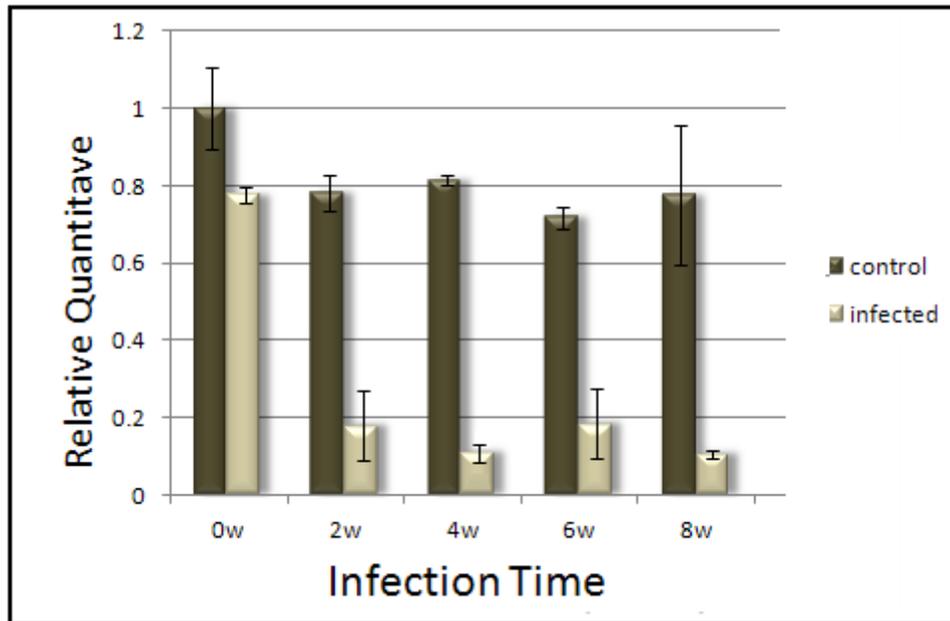
Figure 3.18: RT-PCR analysis of *LTP* gene expression in oil palm inoculated with *G. boninense*. Size of the amplicon of *EgLTP* gene transcripts and *β-actin* gene transcript were 120bp and 80bp, respectively. The latter was used as the constitutive control. Bars in the graph shows RQ values for expression of the gene at 0, 2, 4, 6 and 8 weeks of post-inoculation and the error bar shows the standard deviation of the mean of four biological replicates. All p values for the five post-inoculation time points stages were less than 0.05 (statistically significant).

Ow   2w   4w   6w   8w
Infection Time
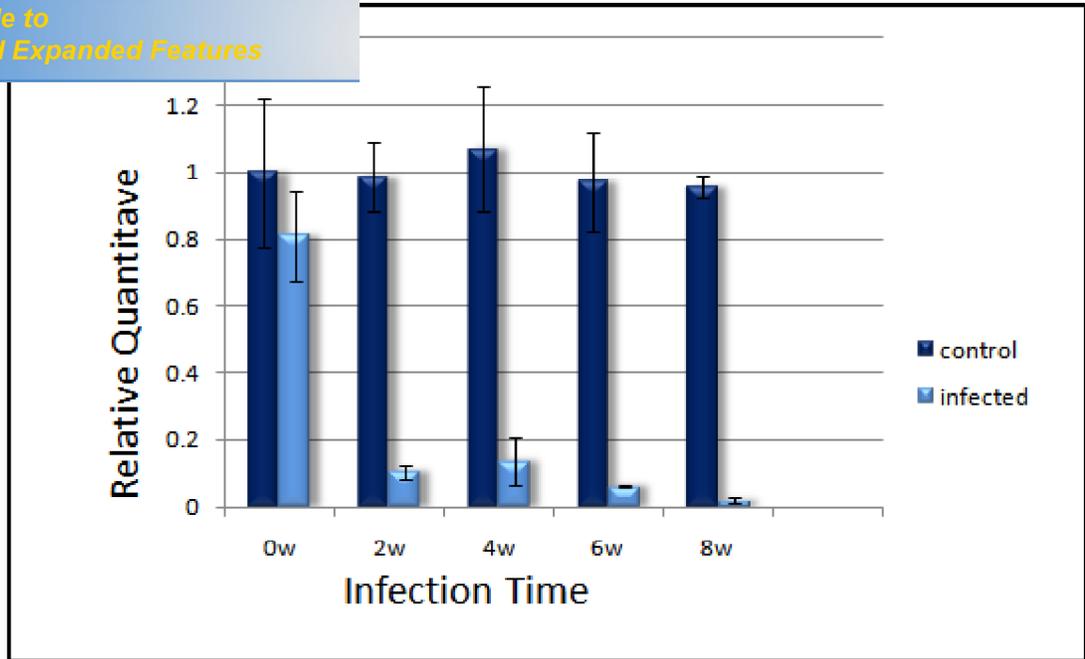
Figure 3.19: RT-PCR analysis of *PR10* gene expression in oil palm inoculated with *G. boninense*. Size of the amplicon of *PR10* gene transcripts and *β-actin* gene transcript were 120bp and 80bp, respectively. The latter was used as the constitutive control. Bars in the graph shows RQ values for expression of the gene at 0, 2, 4, 6 and 8 weeks of post-inoculation and the error bar shows the standard deviation of the mean of four biological replicates. All *p* values for the five post-inoculation time points stages were less than 0.05 (statistically significant).

3.8   Proteomics investigation

This study was performed to investigate differences in the expression profiles of proteins from the roots of oil palm infected with *G. boninense*,compared to their healthy counterparts.The oil palm seedlings were divided into two groups controls (non-inoculated plants) and inoculated plants. From each group destructive sampling was carried out at every two-week intervals to obtain samples at 2, 4, 6 and 8 after inoculation respectively to access the *G. boninense*infection.Protein was isolated in triplicates fromabout 200mg of root material from both groups using Phenol/Ammonium acetate in methanol extraction

differentially expressed have the potential to be used as biomarkers for early diagnosis of BSR infection.

3.8.1 Determination of protein concentration (Bradford Assay)

Estimation of protein concentrations was carried out using the Bradford method of protein assay as described in section 2.2.17.2 (see Materials and Methods). Bovine serum albumin (BSA) at a concentration ranging between 0 to 1.4 µg/ml was used as the standard protein (Figure 3.20). Estimation of proteins was conducted on samples that were prepared by different extractions protocols using sucrose, TCA, and phenol/ammonium acetate (Table 3.6).
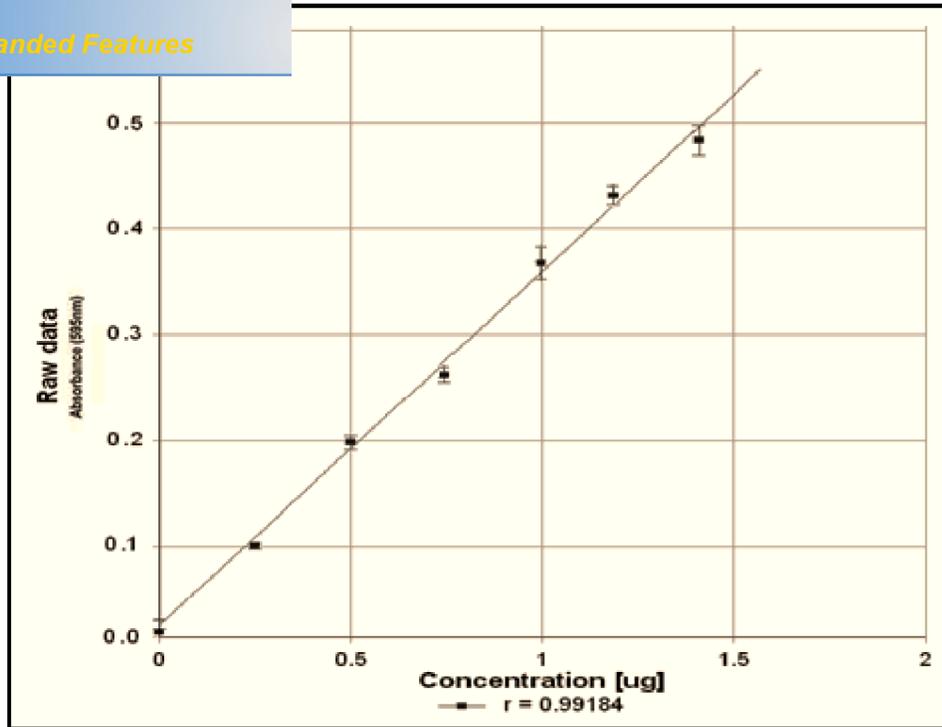
Figure 3.20: A typical standard curve, which was constructed based on a standard solution of BSA. The BSA was diluted with lyses buffer to obtain protein concentrations ranging from 0 to 1.4 mg/ml. The estimation of root oil palm proteins was performed by referring to a typical standard curve such as this. Absorbance of the protein-dye complex was read at 595 nm.

using different methods of protein extraction protocols.

Method of protein extraction	Oil palm root sample (200mg unifected 1 month old tissue)	Final concentration (mg/ml)
Sucrose	sample 1	0.012
	sample 2	0.047
	sample 3	0.014
TCA	sample 1	0.12
	sample 2	0.147
	sample 3	0.14
Phenol/Ammonium acetate in methanol	sample 1	1.98
	sample 2	2.54
	sample 3	2.35

The mean protein concentrations were 0.02, 0.13 and 2.29 mg/ml for the methods using sucrose, TCA and phenol/ammonium acetate, respectively. The highest concentration obtained was by extracting proteins from the oil palm root using phenol/ammonium acetate. This experiment was repeated three times for each of the three methods used. Similar results were obtained for each of the extraction method used.

n extraction methods using SDS PAGE

To analyse the proteins that were extracted using different methods involving use of different solvents, the oil palm root tissue extracts were subjected to profiling using SDS-PAGE. Figure 3.21 demonstrates the results of the experiments. All three methods appeared to generate high resolution protein profiles, although their protein concentrations were not exactly the same.
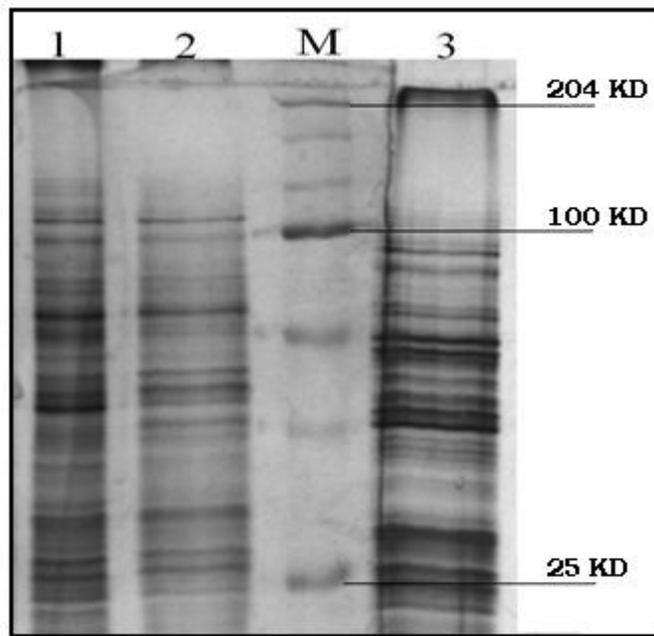
Figure 3.21: SDS-PAGE profile of proteins extracted from the root of *Elaeis guineensis*. Lane 1: Proteins extracted using TCA. Lane 2: Protein extracted using sucrose. Lane 3: Protein extracted using phenol/ammonium acetate in methanol. Lane M: pre-stained protein marker (Bio-Rad, cat # 161-0318).

The phenol/ammonium acetate in methanol method was used in all subsequent studies because it generated the highest quantity of proteins that were also well resolved by SDS-PAGE.

...ctrophoresis

3.9.1   Optimization of 2-DE gel electrophoresis

Separation of the crude extract of proteins from the oil palm root tissues by 2-DE generated protein profiles that were highly-resolved.   These proteins were generally separated on the basis of their pI and molecular weight.   When the root tissue extract was subjected by 2-DE using immobilin dry-strips pH 3-10, most of the proteins were apparently focused at the pH range of 4 to 7 (Figure 3.22).   Hence, the 2-DE experiment was repeated using immobilin dry-strips with pH values of 4 and 7, and this resulted in a well-separated and highly-resolved profile of the majority of the proteins that are present in the root tissues of the oil palm (Figure 3.23).   Therefore, IPG strips of pH 4-7 were used in all 2-DE experiments throughout the rest of the study.
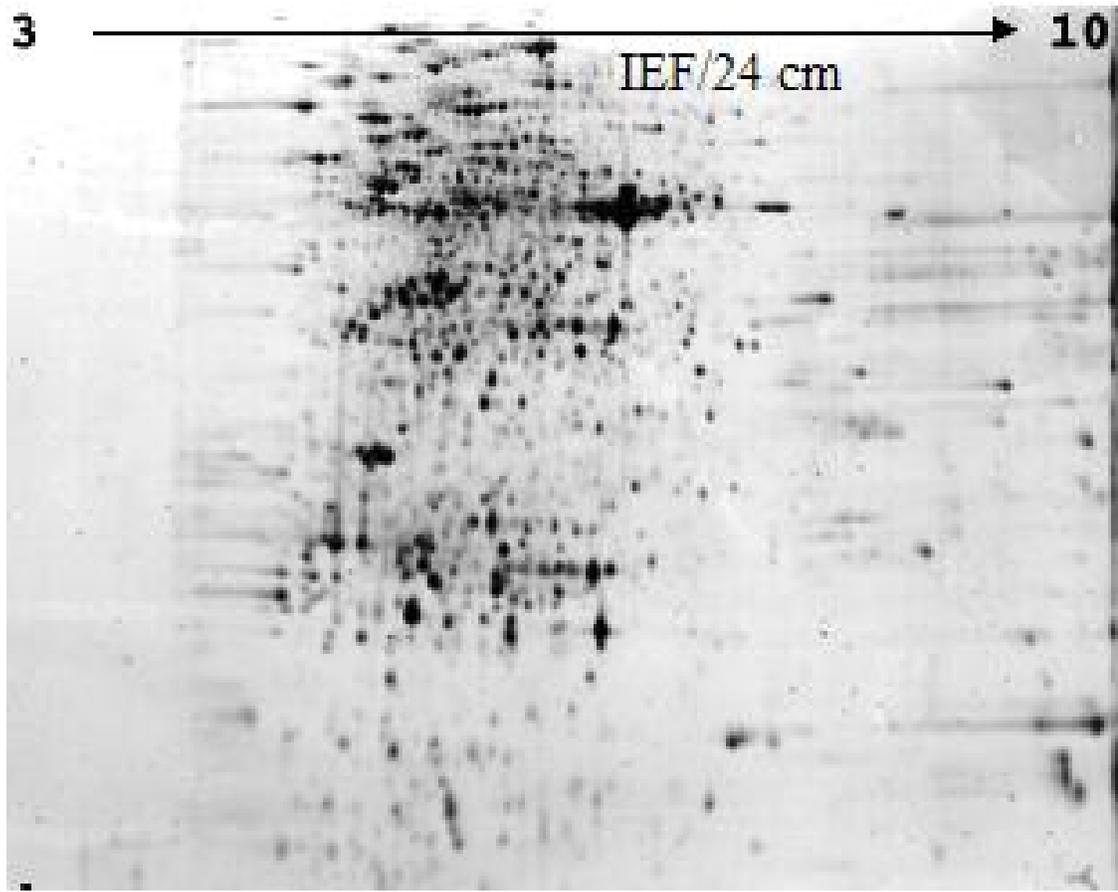
Figure 3.22: A representative silver stained 2-DE protein profile of oil palm root tissue performed using IPG strip with pH 3-10.
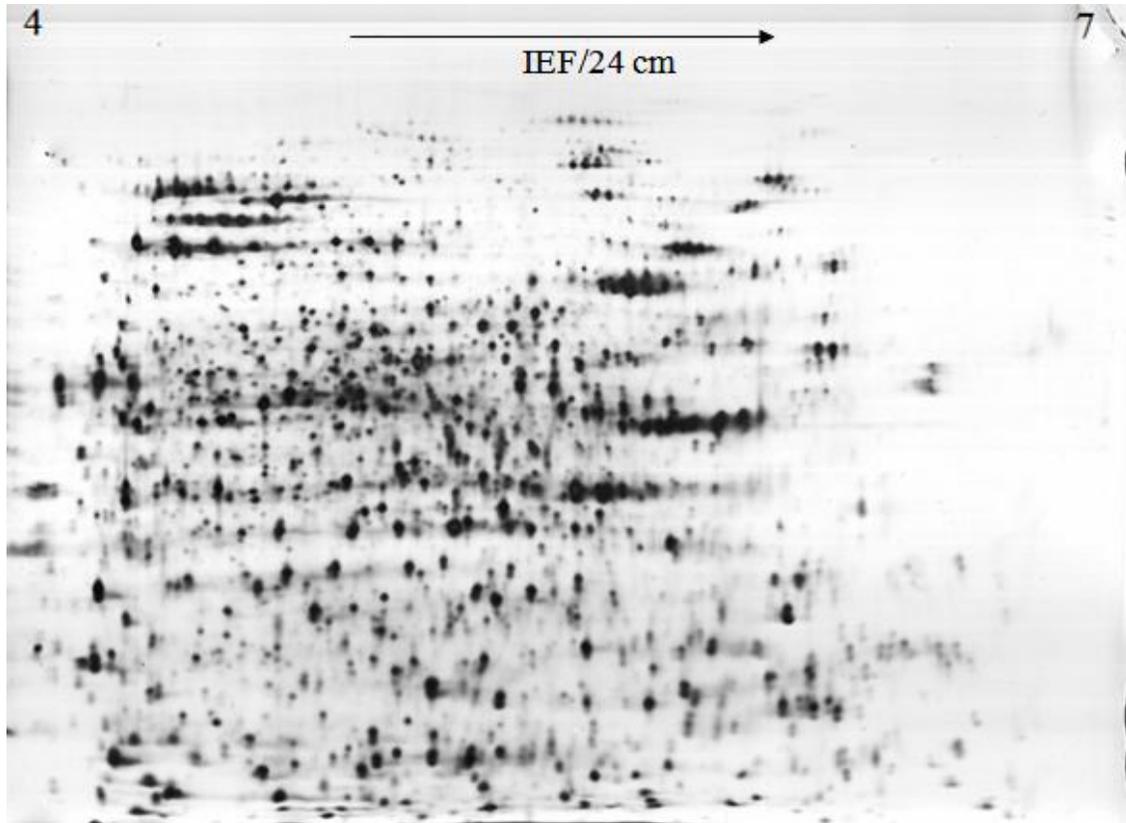
Figure 3.23: A representative silver stained 2-DE protein profile of oil palm root tissue performed using IPG strip with pH 4-7.

128

sment of the 2-DE profiles

Assessment of the reproducibility of the 2-DE profiles was performed to determine the reliability of 2-DE results and to eliminate the possibility of errors caused by sample handling. All samples from *G. boninense* infected and control oil palm roots were analysed in triplicate to minimize variation between samples. These analyses involved spot matching of the triplicate gels before subjecting them to a scatter plot analysis. In the scatter plot analysis, the percentage of matched protein spots and the correlation coefficient (*corr*) of percentage of volume of protein pairs were determined using the Image Master 2-DE platinum software. Figure 4.24 shows the scattered plot generated from the spots detection and matching. A fitting equation in the range of 0.833-1.39 with correlation coefficient (*corr*) in a range of 0.727-0.865 was obtained (Table 3.7).

Table 3.7: Reproducibility assessment of triplicate of 2-DE gels from scatter plot analysis

	Fitting equation	Correlation Coefficient (corr)	No. of matched Protein spots in triplicate gels
Control			
2 weeks	0.991*x + 0.0071	0.7393	2411
4 weeks	1.01*x + 0.008	0.8203	2342
6 weeks	0.999*x + 0.0031	0.774	2289
8 weeks	1.13*x + 0.00271	0.844	2044
Infected			
2 weeks	0.999*x + 0.0031	0.774	2289
4 weeks	0.833*x + 0.0073	0.7318	2716
6 weeks	1.19*x + 0.00139	0.727	2294
8 weeks	1.39*x + 0.00304	0.865	2418
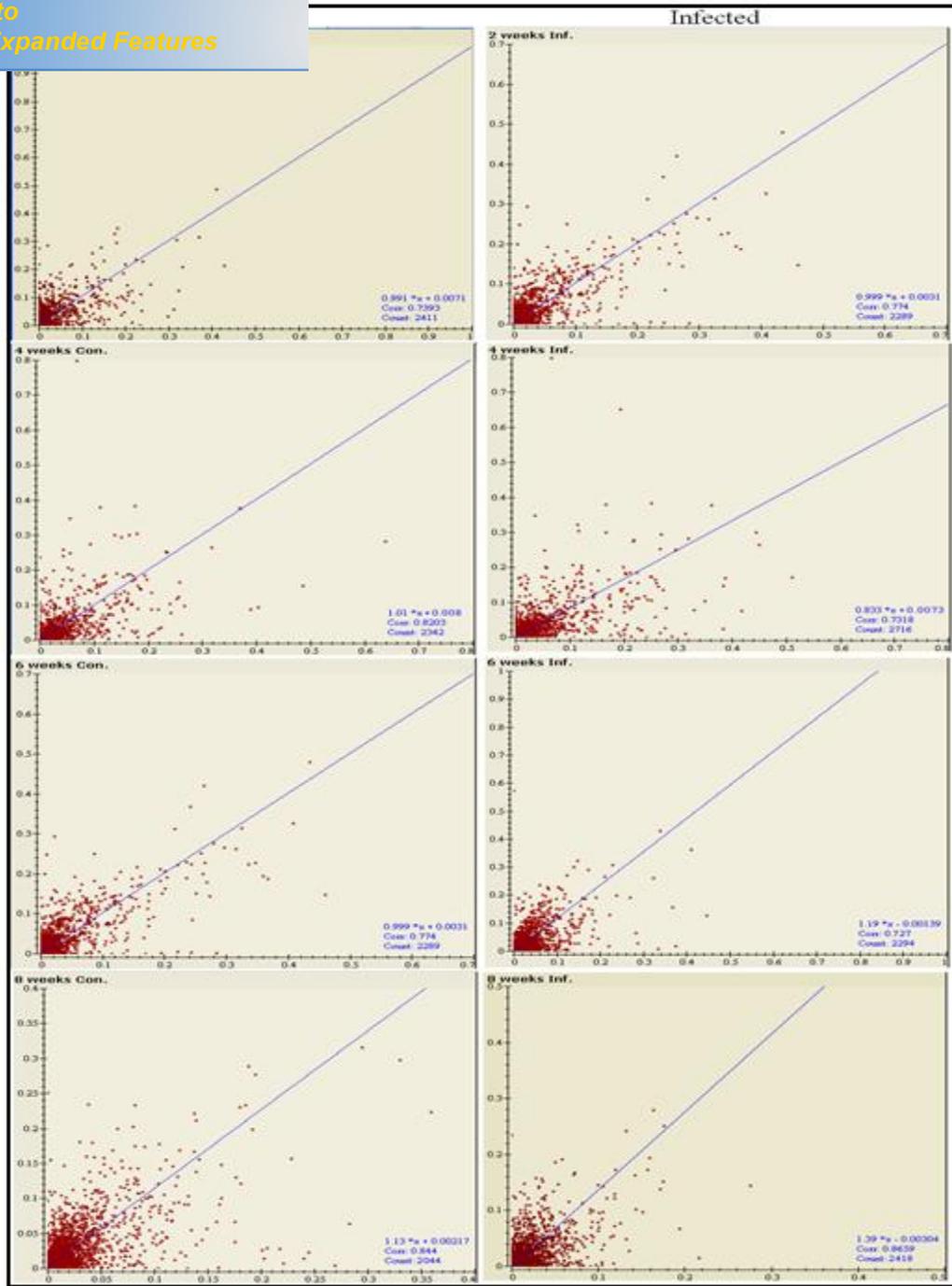
Figure 3.24: Spot matching analysis of silver stained 2-DE SDS-PAGE gels that were generated from control and infected root samples at 2, 4, 6 and 8 weeks. The Image master 2 D platinum v7.0 was utilised in performing the spot detection and matching of the triplicates.

protein profiles of control and infected oil palm root

To investigate the root protein expression patterns in response to progressive basal stem rot infection caused by *G. boninense*, the 2-DE root protein profiles of *Elaeis guineensis* at different timeframes of harvest were examined. Triplicate gels were obtained from three independent experiments and the representative gels for each of the infected sample s as well as their respective controls are shown in Figure 3.25 (One set of gels). The protein spot profiles of the oil palm root extracts that were harvested at the same time-frames were compared and a virtual master profile image was constructed using the images and also from the subsequent cross comparison (Figure 3.26). To avoid generating excessive information that is typical to the proteomic studies presented in the literature, a very stringent comparison was opted. For this analysis, the relative protein spot intensities of different gels were averaged. Any spots that showed inconsistent intensity among extracts of the same harvest timeframe, i.e., not present in all gel images compared, were ignored.
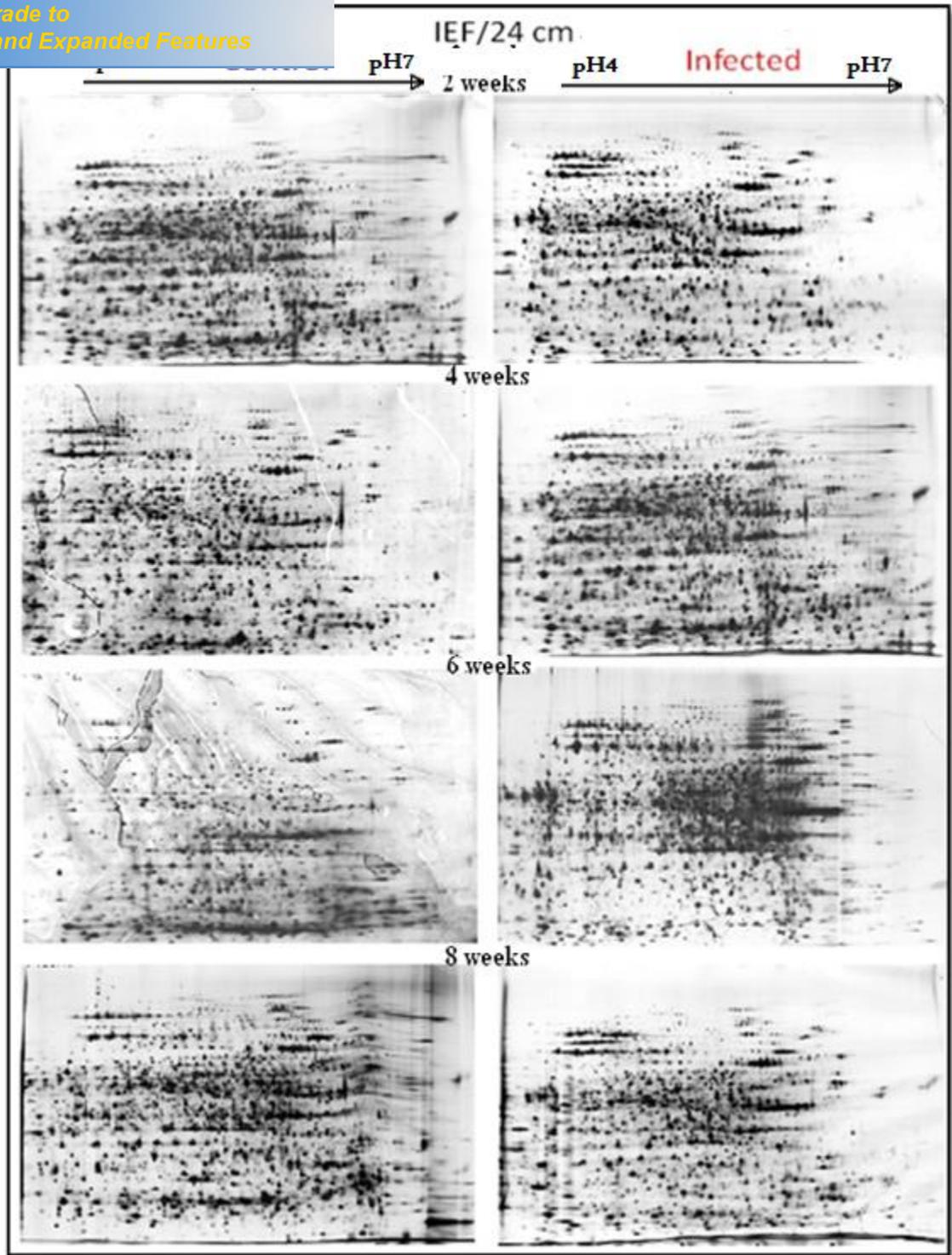
Figure 3.25: Typical 2-DE profiles of control and infected oil palm root proteins. Root proteins from control and infected oil palm tissues were harvested at 2, 4, 6 and 8 weeks before being subjected to the 2-DE profiling.

A

B
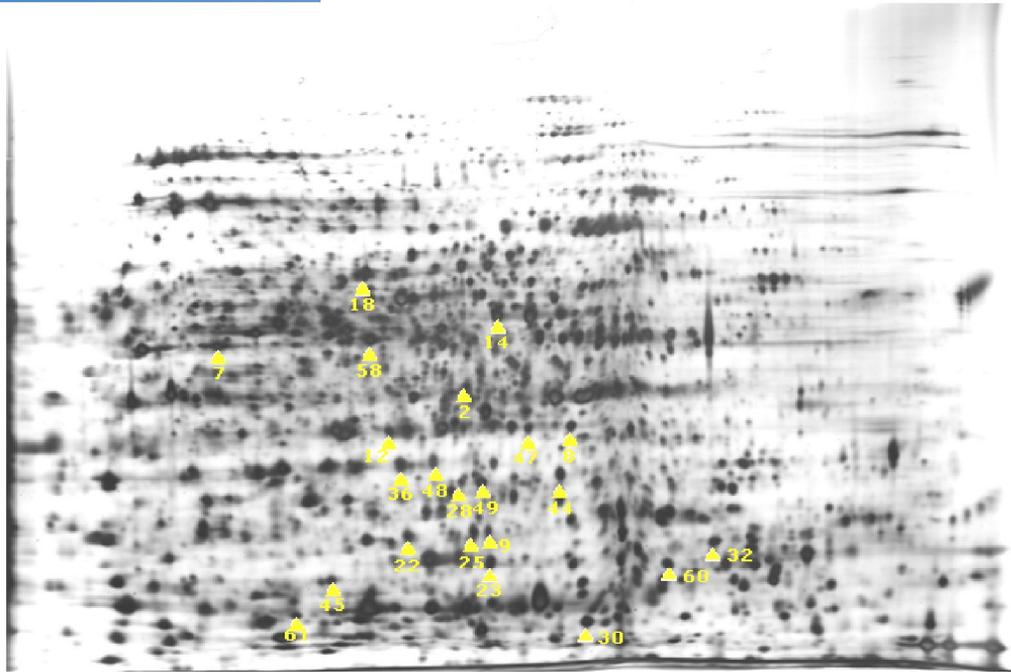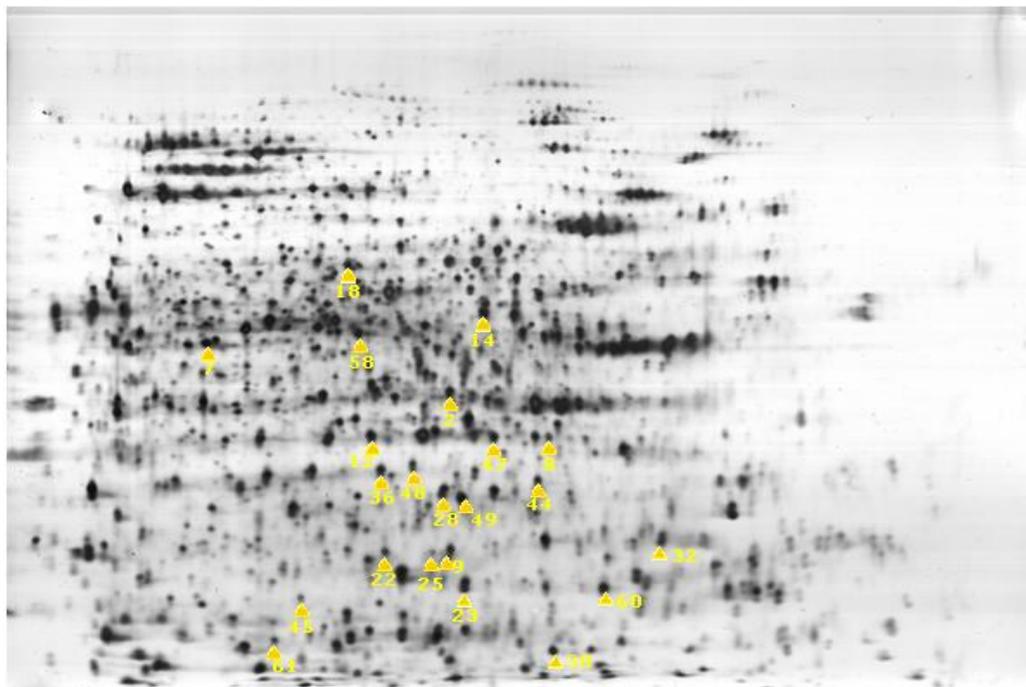
C
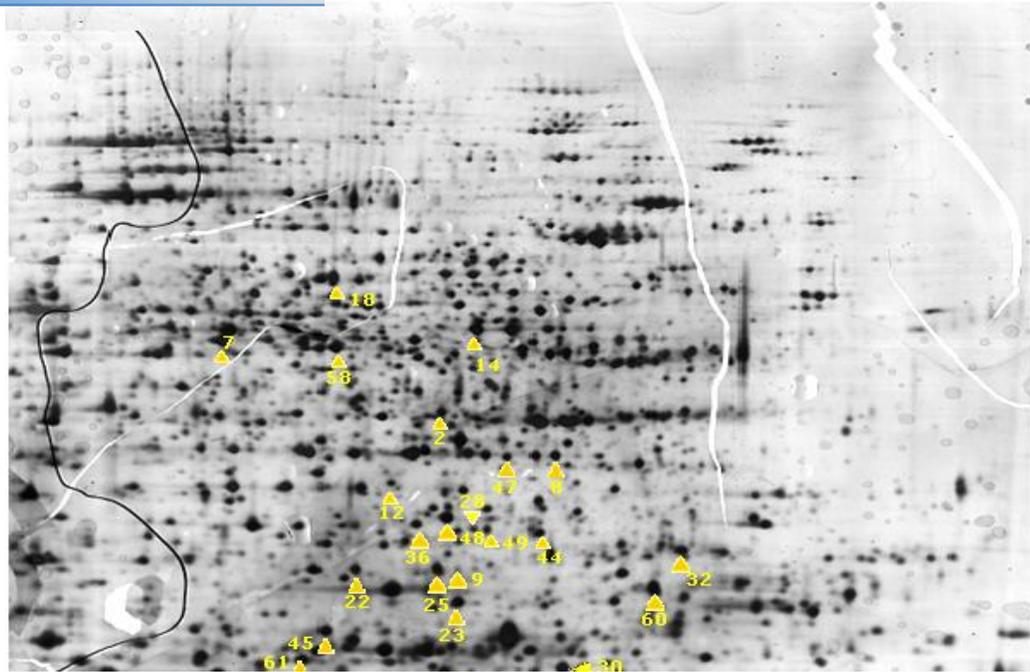
D
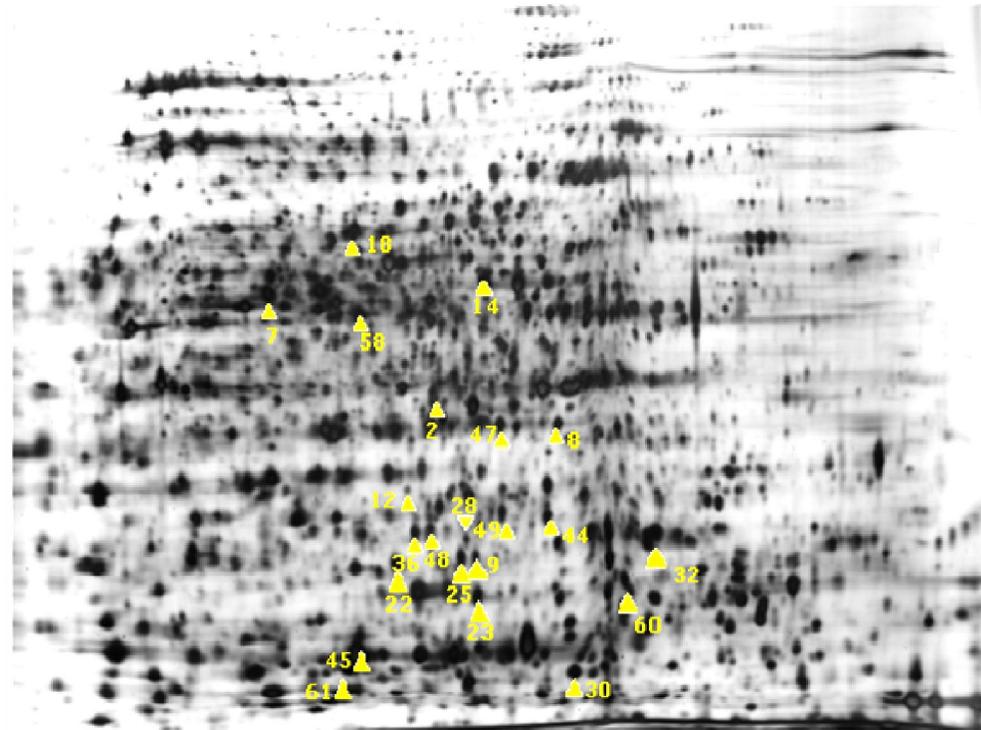
Continued on next page

E
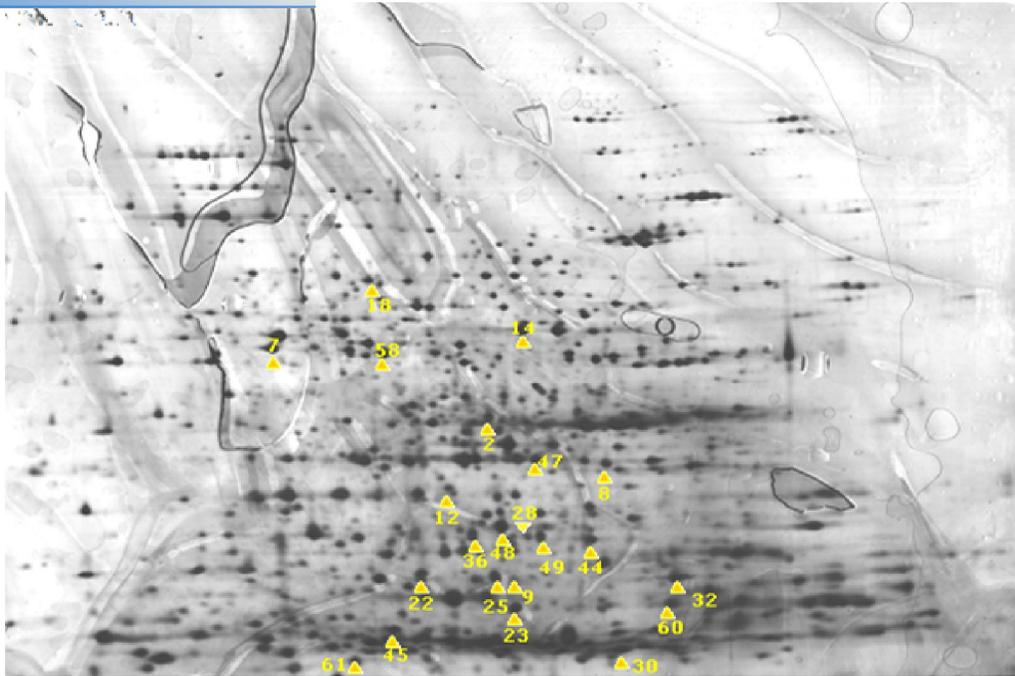
F
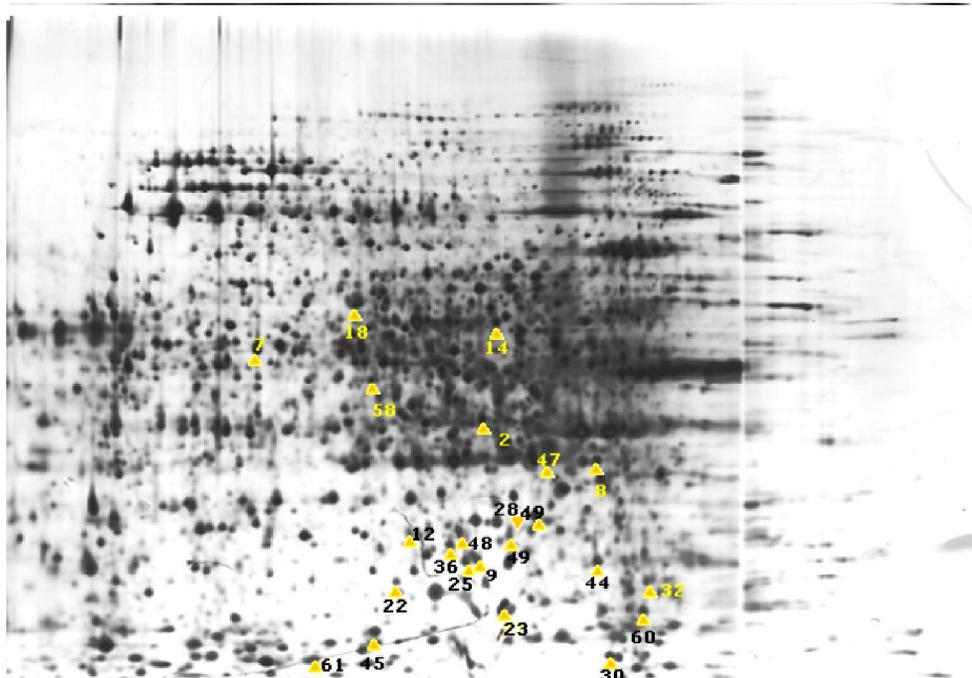
Continued on next page

G
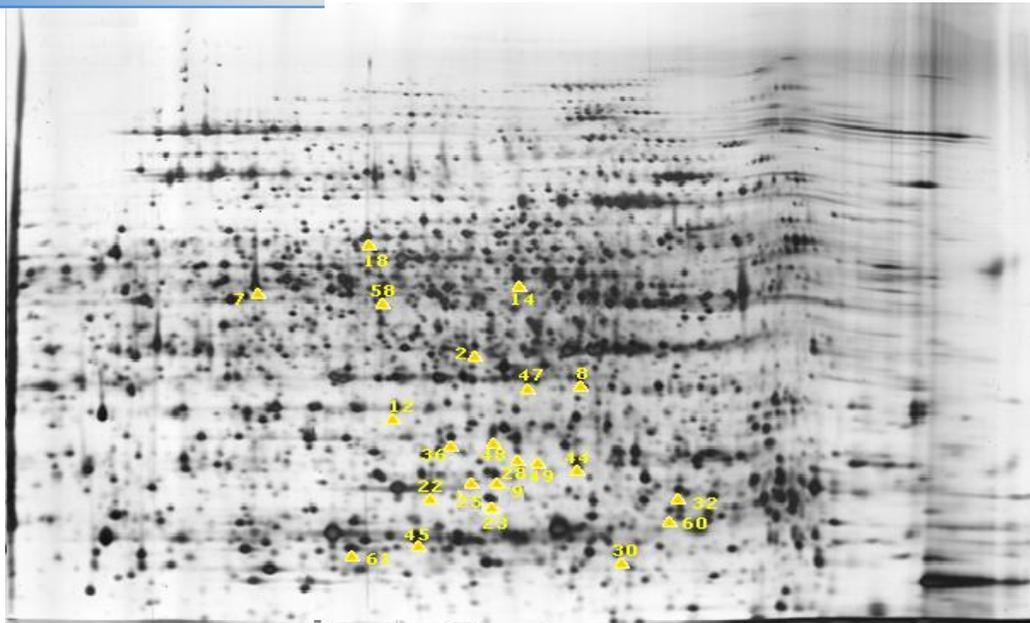
H
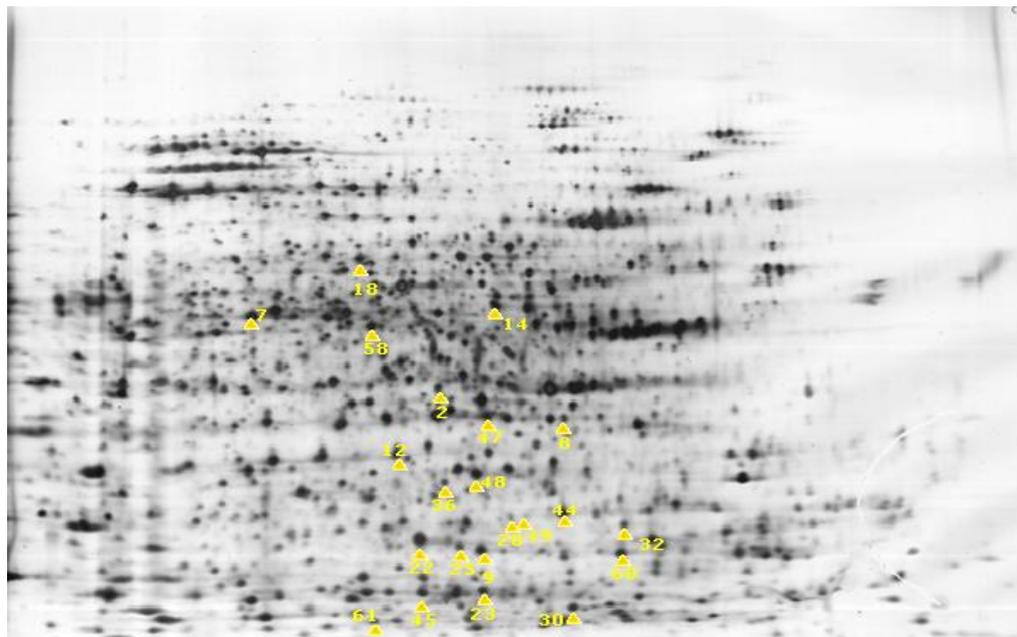
Figure 3.26: Analysis of oil palm root protein sample, 2 weeks sample control (**A**), 2 weeks sample infected (**B**), 4 weeks sample control (**C**), 4 weeks sample infected (**D**), 6 weeks sample control (**E**), 6 weeks sample infected (**F**), 8 weeks sample control (**G**) 8 weeks sample infected (**H**) were subjected to 2-DE.

DE oil palm root protein profiles of control and infected sample

When image analysis was performed on the proteins that were detected in the 2-DE gels, 61 spots were found to be differentially expressed between control and infected samples throughout the selected timeframes of study. Figure 3.27 demonstrates a typical 2-DE gel of the oil palm root tissues that showed differential expression of proteins. The mean percentage of volume of the protein spots were analysed using the Image master 2-DE-platinum software version 7.0 and the Student's *t-test* was employed to determine the protein spots that show significant change in their abundance (p value <0.05). The principle of the statistical analysis used in the study is explained in the Appendices (see Appendix N). Among the 61 protein spots that were identified to be differentially expressed, 22 were further subjected to MS/MS analysis. Selection of the 22 protein spots was performed on the basis of their higher difference of protein expression. The protein spots with fold changes of >1.5 for at least two timeframes were chosen for this analysis (Figure 3.28). The percentage volumes of contribution of all the oil palm root proteins are available in Appendix section (see Appendix O).
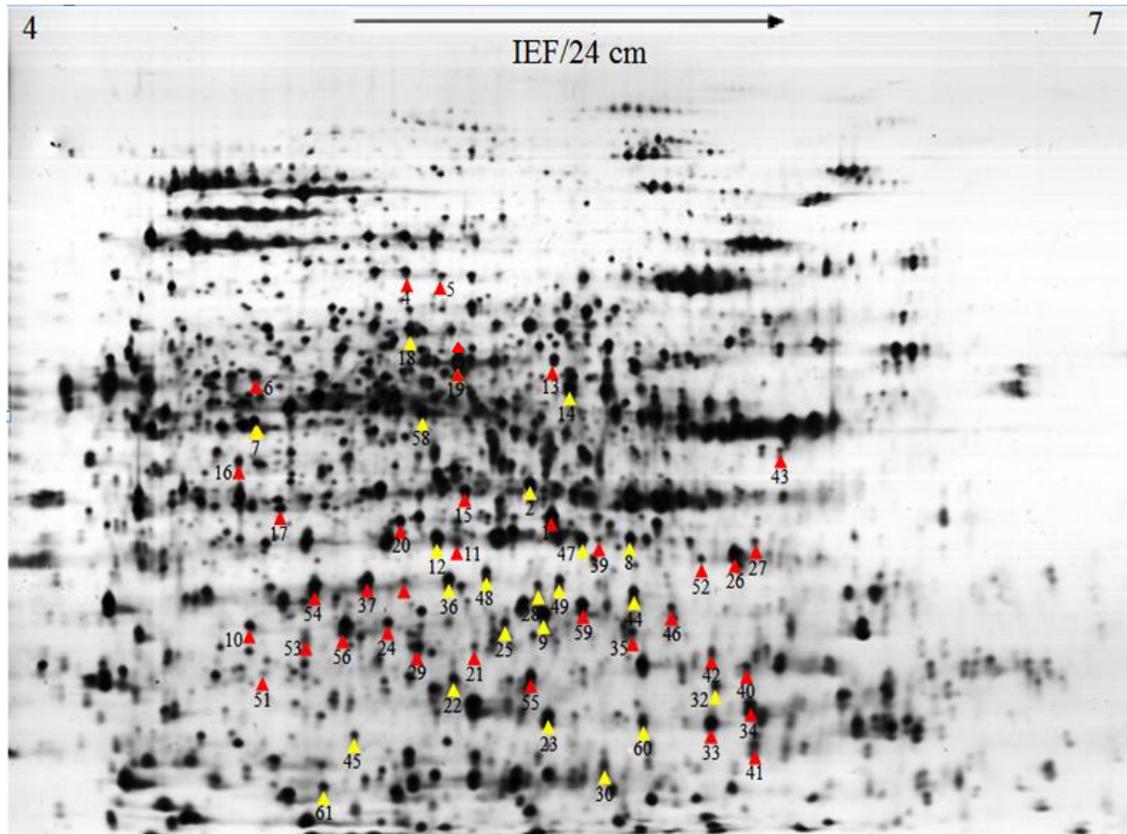
Figure 3.27: Typical 2-DE profile for control oil palm root proteins harvested at 2 weeks. Coloured triangles represent proteins that were differentially expressed, while the yellow triangles represent the proteins that were selected for analysis by MS/MS.
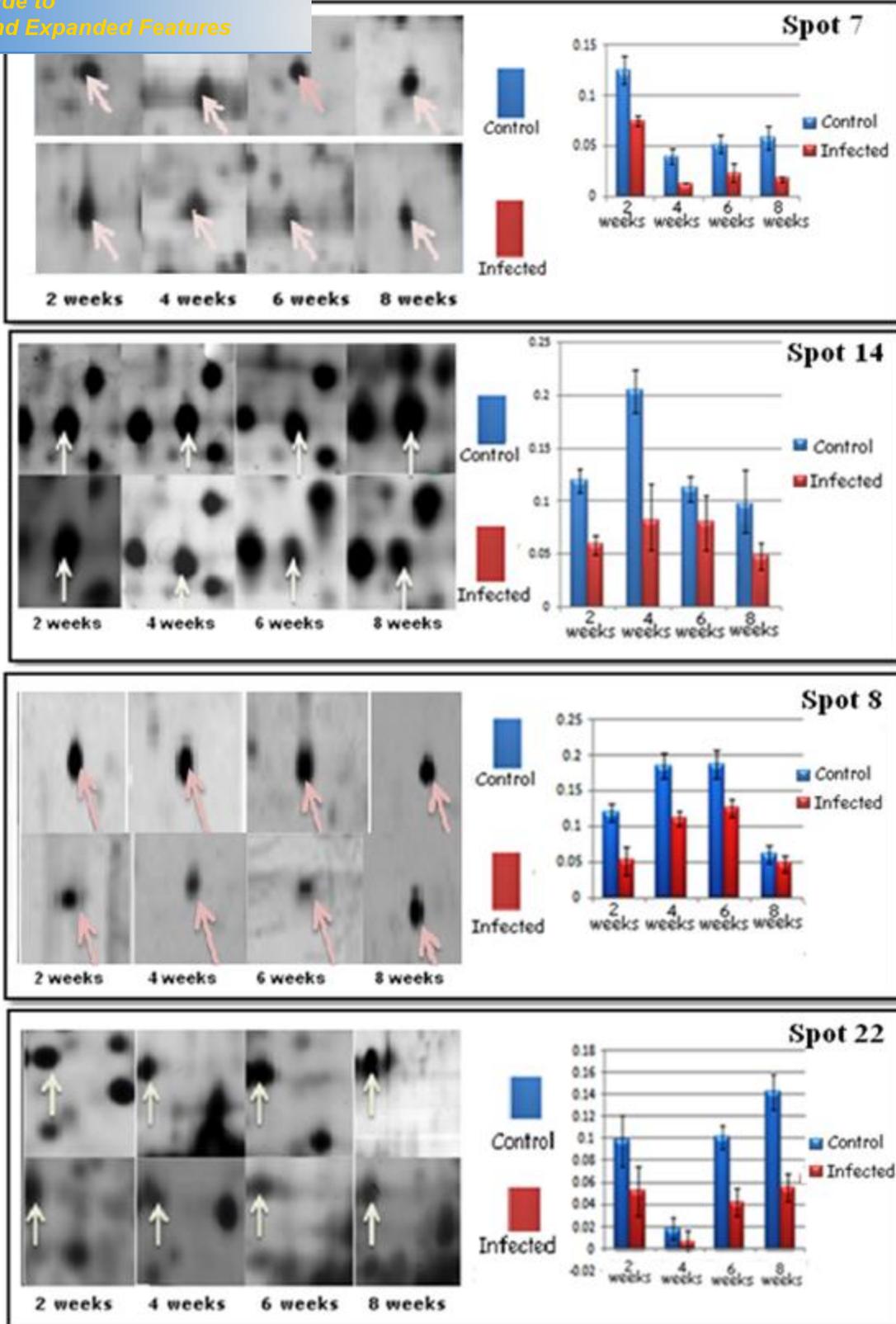
Continued on next page…
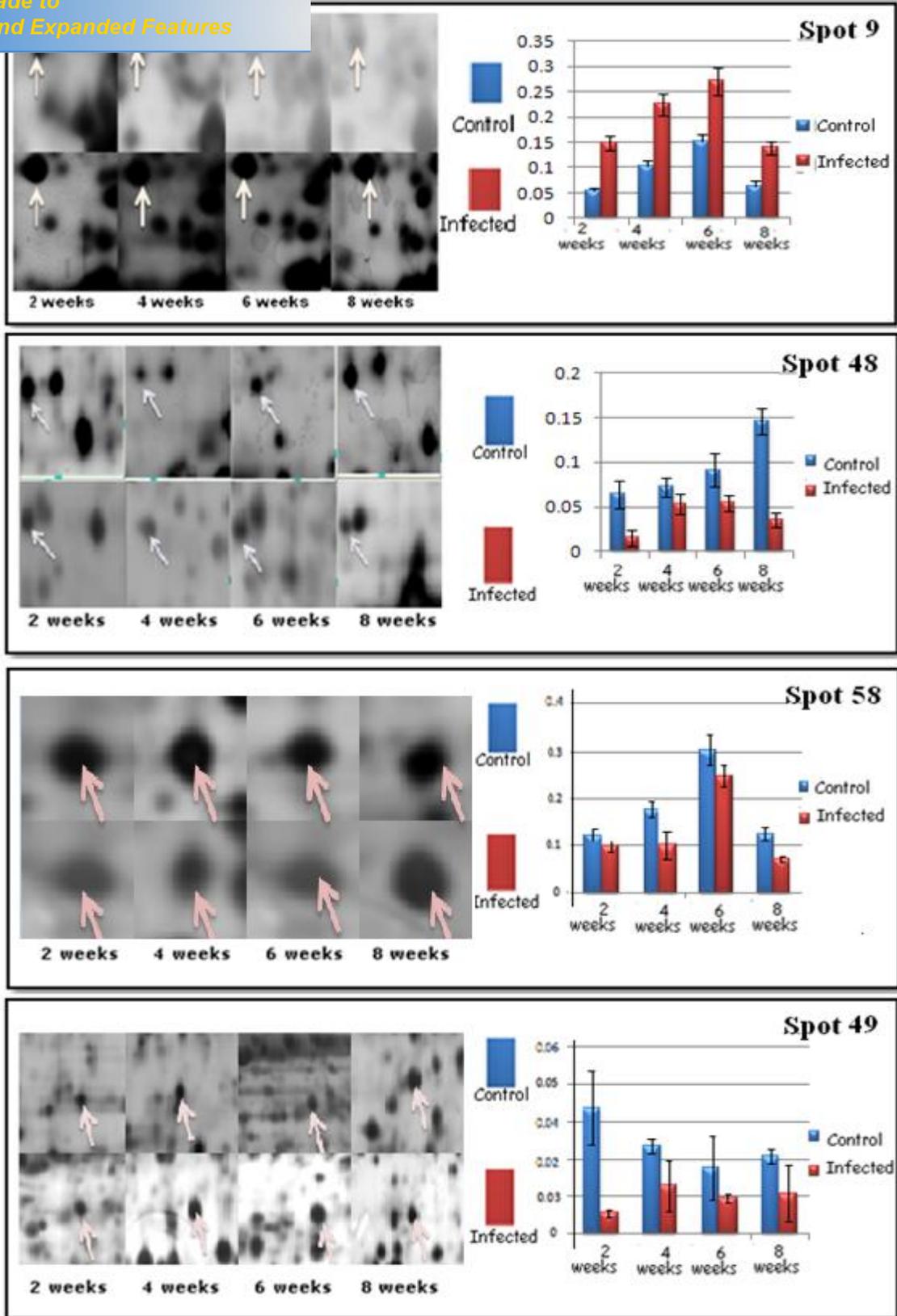
Continued on next page…

Continued on next page…

Spot 32

Spot 44

Spot 60

Spot 2
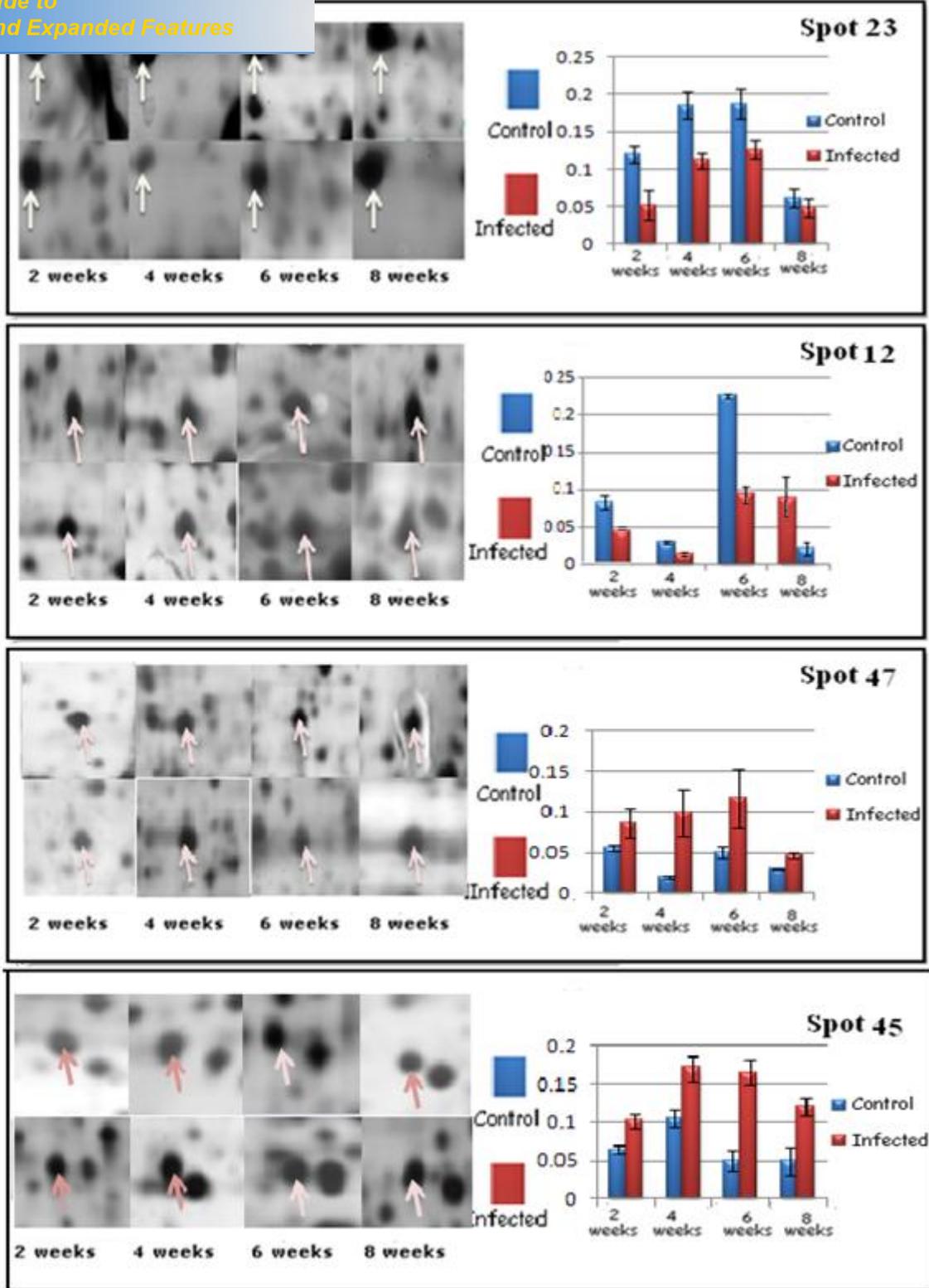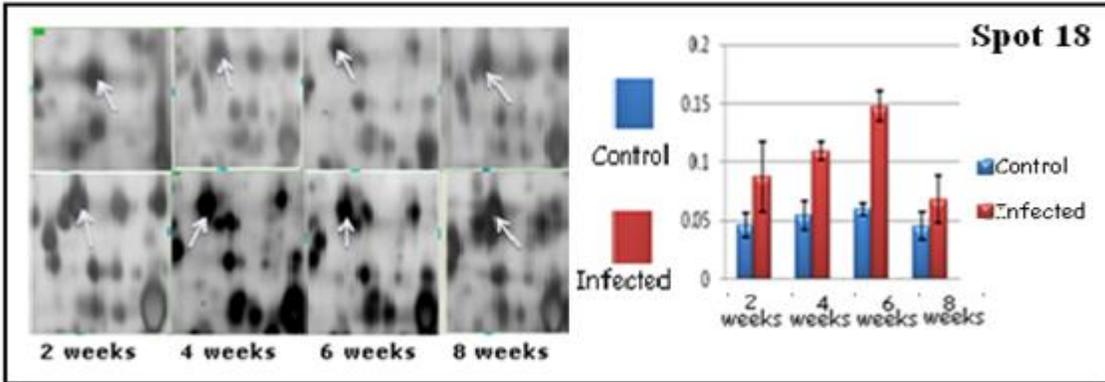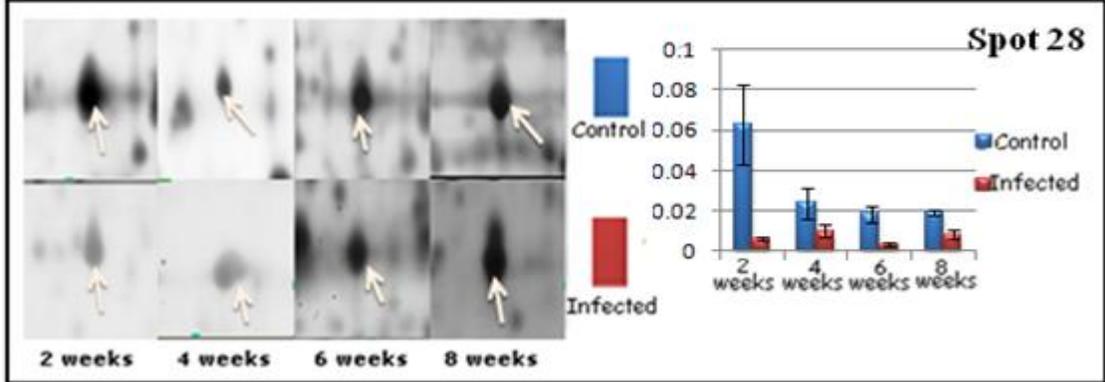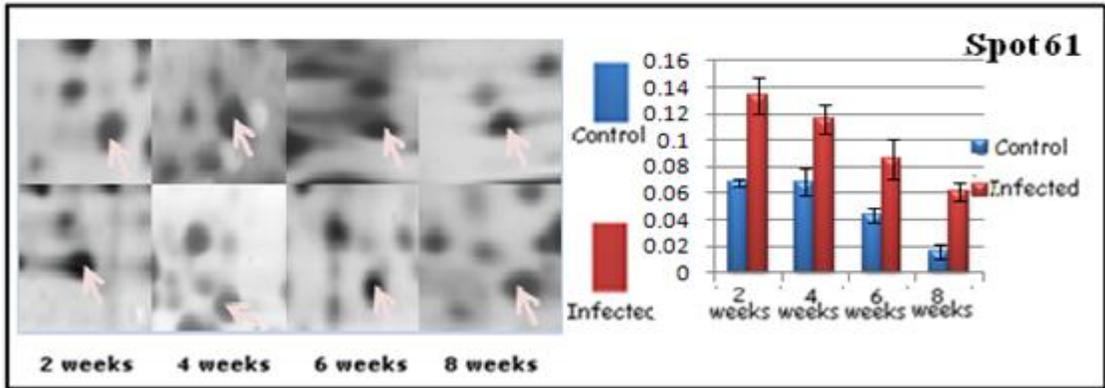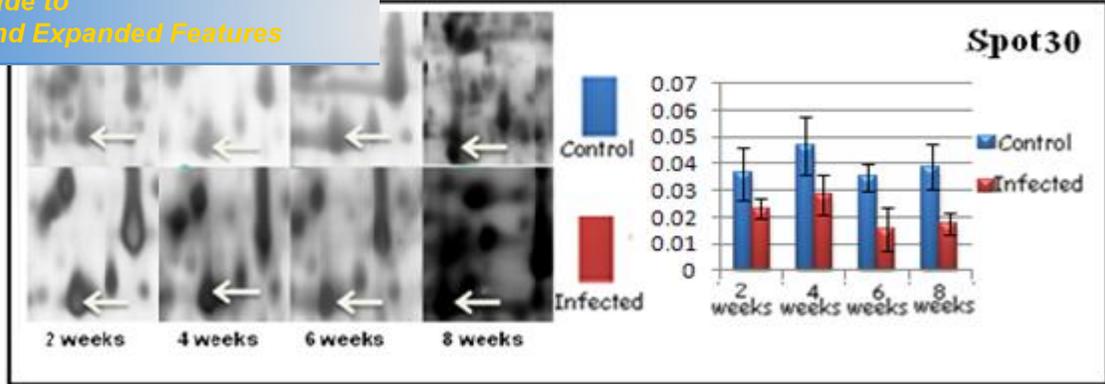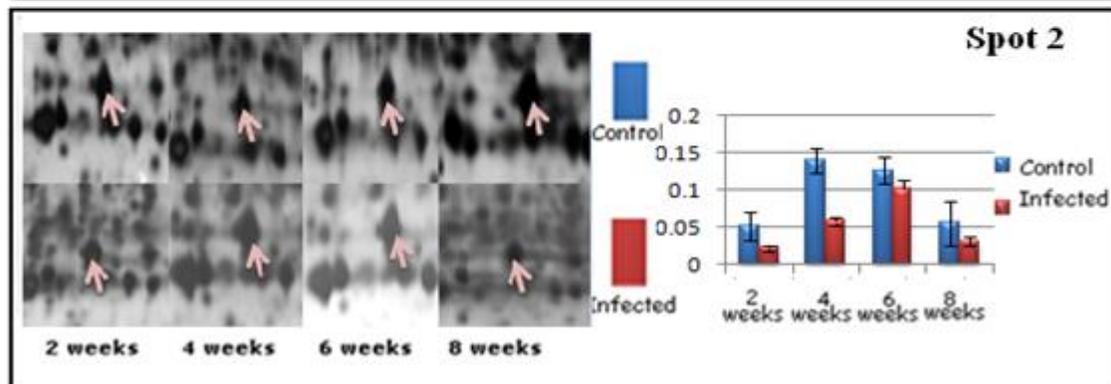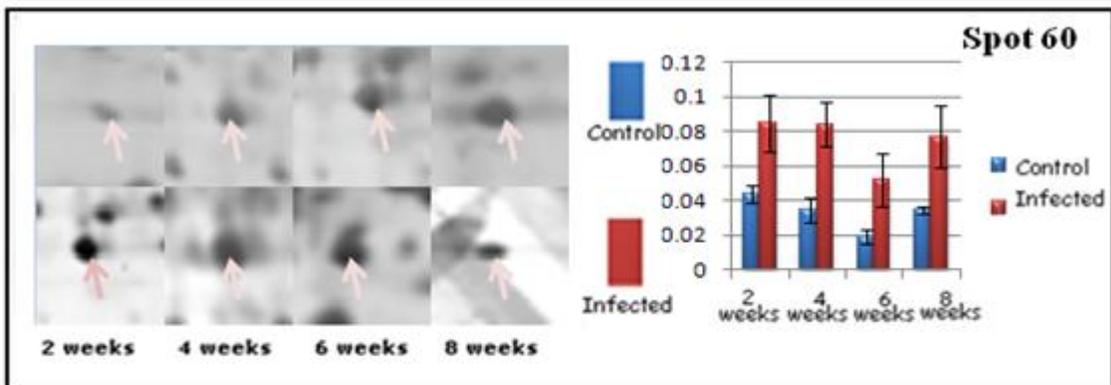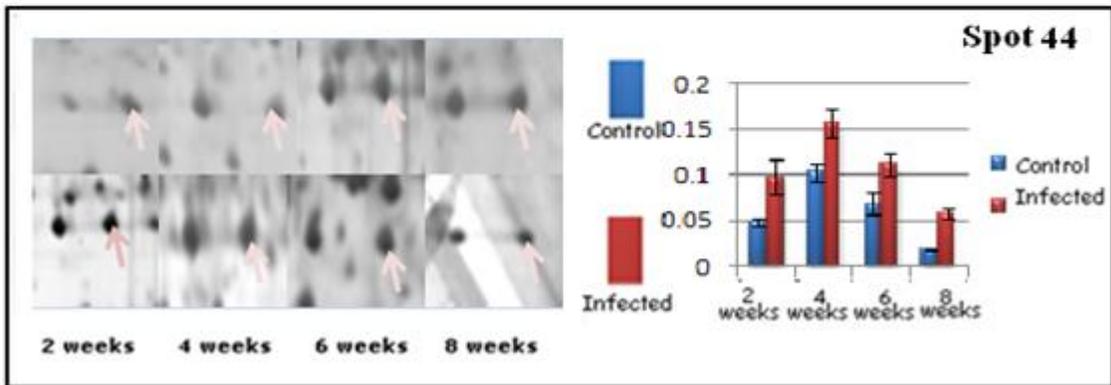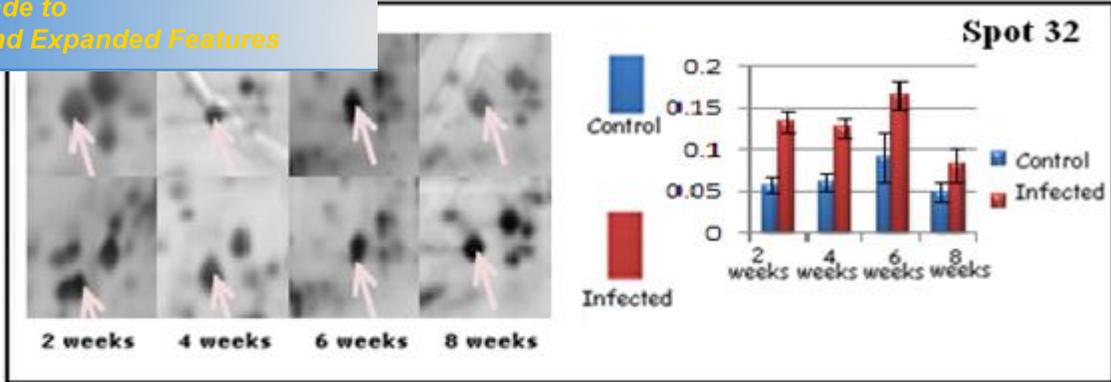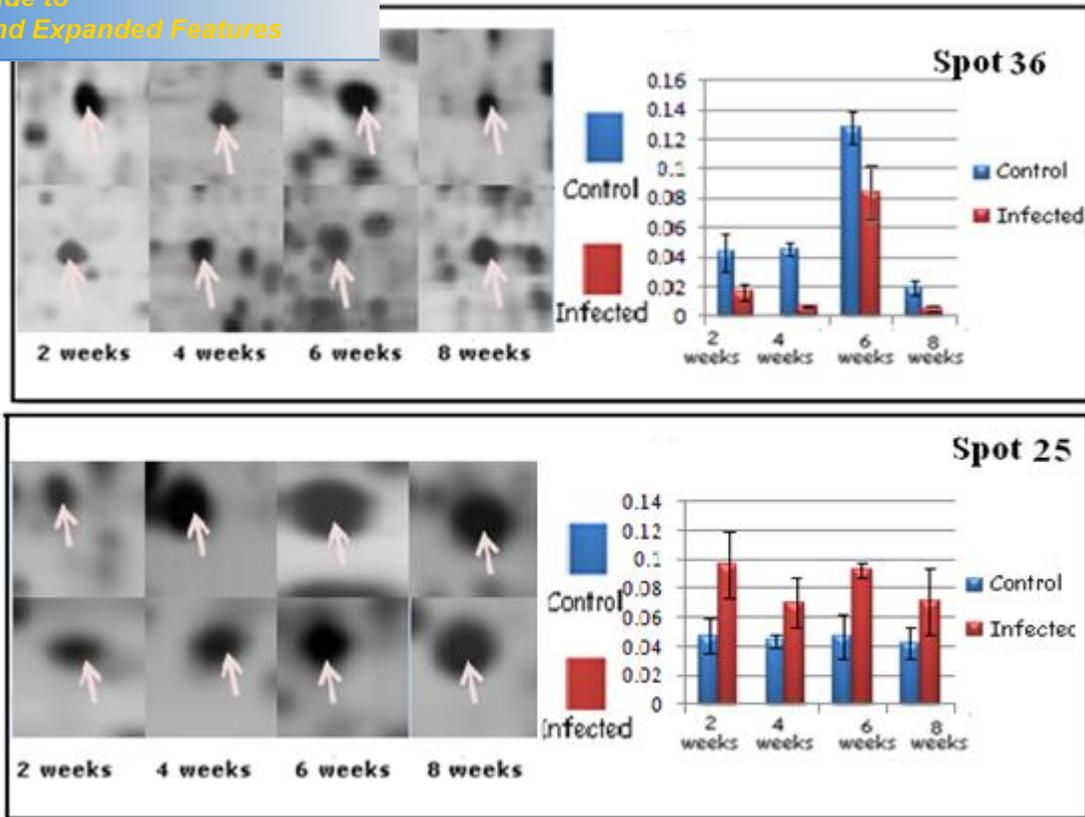
Figure 3.28: Changes in level of oil palm proteins expression at different time in response to *G. boninense* infection. Root tissues from each plant were harvested at 2, 4, 6, 8 after infection, the bars in the graph showing the quantification of the up-and down-regulated protein spots between the infected and control tissue at 2, 4, 6, 8 after infection. Error bars are standard deviation of the average spot densities.

mass spectrometry

MS/MS was performed on 22 of the aberrantly expressed protein that showed significant statistical difference after image analysis. The selected spots were subjected to *de novo* MS/MS protein partial sequencing. Most search queries included 20 to 60 amino acids, resulting from 2 to 6 peptide sequences. Using these queries, all 3 homology-based search algorithms yielded identifications with a score significantly better than the threshold score. Only when the top results (first hits) from the searches yielded the same protein, the identification was considered as positive. Using this approach, we were able to obtain 21 hits out of the 22 protein spots that were subjected to analysis by MS/MS (Table 3.8). However, only eleven of the proteins had MASCOT scores of more than 55 and therefore considered to be positively identified. Among these 11 proteins, two were without any known function.

rentially expressed proteins in oil palm (*Elaeis guineensis*) during

Spot ID	Up/down regulation	Protein	Accession No.	Organism	Theoretical/ observed MW kDa	Theoretical PI	MASCOT score	% coverage
7	↓	ATP synthase alpha subunit	D2XT90‡	*Allium cepe*	55.3/42	6.1	341	31
36	↓	Enoyl-Acyl carreir protein reductase	C1K4Q1‡	*Elaeis oleifera*	19.4/28	8.01	184	19
14	↓	Amino peptidase	D7MBK*	*Arabidopsis lyrata*	48/24	6.12	177	12
25	↑	Malate dehydrogenase	D7FBC0*	*Vitis vinifera*	35.8/23	6.18	121	20
8	↓	Caffeic acid O-methyltransferase	Q9SRY8*	*Thalictrum tubersum*	40.1/27	5.5	92	6
22	↓	Enolase	B3TLU4‡	*Elaeis guineensis*	48.1/18	5.6	82	11
48	↓	Fructokinase	Q0J8G4*	*Oryza sativa*	35/27	5.02	70	10
9	↑	Pyridoxal-5-phosphate-dependent enzyme beta subunit	Medtr5g006410.2†	*Medicago truncatula*	29.9/19	5.4	61	8
58	↓	Unknown Protein	A9SZX7‡	*Physcomitrella patens*	46.1/44	5.58	58	2
49	↓	Unknown Protein	A9RYJ1‡	*Physcomitrella patens*	39.08/27	5.3	56	4
23	↓	Caffeoyl Coa O-methyl-transferase	Q9SWC0*	*Eucalyptus globules*	19/17	4.6	55	25
12	↓	Unknown Protein	D84T85‡	*Selaginella moellendorffi*	51/30	8.3	41	2
47	↑	Unknown Protein	A5AG57*	*Vitis vinifera*	48.1/32	9	40	2
45	↑	Unknown Protein	F2CWX2‡	*Hordeum vulgare*	29/18	5.66	40	7
32	↑	Unknown Protein	B4FSR1‡	*Zea mays*	28.4/21	9.12	40	7
44	↑	Unknown Protein	B6SKV9‡	*Zea mays*	51.3/27	8.99	37	2
30	↓	Unknown Protein	A5BKQ6*	*Vitis vinifera*	36.7/15	4.7	34	2
61	↑	Unknown Protein	A5AV26*	*Vitis vinifera*	25.2/15	9.4	29	4
28	↓	Proteinase inhibitor	Medtr8g011570.1†	*Medicago truncatula*	19.58/26	5.2	22	3
18	↑	Polyphenol oxidase	Q3YJ63*	*Euterpe oleracea*	26.1/54	9.01	19	9
60	↑	Unknown Protein	Medtr2g091930.1†	*Medicago truncatula*	8.1/19	10	18	14

↑represent the protein showed up-regulation,↓represent the protein showed down-regulation after fungal infection. (‡) Uniprot accession number, (*)NCBI accession number and (†)*Medicago truncatula* sequencing database available at http://www.medicago.org/genome.

Discussion

4.1   Studies on *Ganoderma* interaction with oil palm

Many approaches have been used to attempt to better understand the interactions between pathogens and their hosts to enable thier design of improvd detection or more effective control measures. In this study we chose to look at understanding the cellular signals of the host during infection through the study of selected genes as well as whole protein expression profiling. For gene expression three defence genes (PGIP, PR10, and LTP) were selected to investgate thier expression during a specific time course in early stage *Ganoderma* infection. A more global approach was then taken using a proteomics study to look at the expression of proteins during the same period of infection.

4.2   RNA extraction and cDNA synthesis

Essential to the enable the reproducible and accurate study of interactions within a cell system is the ability obtain good quality starting material to be used in the experiments. In this case the study of the expression profile of the selected genes requires the availability of high quality RNA. Extraction of good quality RNA from root tissues is usually difficult due to the high amounts of polysaccharides, phenols, and/or secondary metabolites that may accumulate (Zamboni et al., 2008). Several reagents and kits are in existence for the isolation of RNA from plant tissues (e.g., Trizol, RNeasy plant kit, QIAGEN). However, whilst they may work very well for most plant tissues, they may not be efficient to use for the extraction of RNA from oil rich plants. A high quality RNA from plant tissues is essential for the construction of a good-quality cDNA and for most molecular-

of many of the RNA extraction protocols which either result in low yields of RNA or impure RNA samplehas encouraged a considerable number of researchers to find new improved protocols for the isolation of RNA from several unruly plant tissues (Yoo et al., 2004; Sangha et al., 2010).

The extraction of RNA from plant tissues is often performed using Cetyltrimethylammonium bromide (CTAB) (Chang et al., 1993). This method is sometimes, but not always, good for the extraction of plant RNA and appered to be very much dependent on the physical attributes of each plant cell type (Kiefer et al., 2000; Iandolino et al., 2004). For example, Meisel et al. (2005) had used the CTAB method for the extraction of RNA from peach resulted in good quality RNA, but unfortunately the yield was very low.

Previouse studies in oil palm described different methods of RNA extraction from mesocarp (Abdullah et al., 2002), leaves (Morcillo et al., 2006), and fruits (Khemvong et al., 2005). Till the date of starting this research there was no sufficient method to extract the RNA from the oil palm root sample.

In the present study, the CTAB method was modified to find the most suitable protocol to generate oil palm root RNA of high quality and quantity. By eliminating the need for lyophilisation, changing the use of phenol with chlorophorm:isoamyalcohol (24:1), reducing the frequency of centrifugation, eliminating the use of ultracentrifugation, and increasing the precipitation time of RNA for up to 4 days, an improved method for the extraction of RNA from the root tissues of oil palm was developed (Al-Obaidi et al., 2010).

ctors are indicative that the newly modified protocol has the capability to produce good yield of intact and pure RNA extract from the roots of oil palm. This method has the advantages of being simple, free of phenol and easily applied to a broad range of plants tissues, when compared to the other protocols that were previously used in the extraction of RNA from recalcitrant plant tissues including oil palm (De Keukeleire et al., 2006; Birtic & Kranner, 2006; Le Provost et al., 2007; Wang et al., 2008; Fort et al., 2008).

To further remove the DNA contaminants from the RNA extracts that were obtained using the newly-modified protocol, DNA was selectively removed by digestion with DNase. Complete removal of DNA is crucial for the highly sensitive RT-PCR assays, the DNase digestion step resulted in RNA extracts that appeared to be free of DNA after gel electrophoreses.

4.3 Understanding the response profile of defence related genes in oil palm

Plant cells have inherent defence systems to avoid probable infection by a wide range of phytopathogens, including fungi, bacteria, oomycetes, viruses, nematodes and insects. The plant cell does not possess antibody-dependent immune system like those of the mammalians. As an alternative, plants generally depend on a variety of defence mechanisms, including strengthening of their cell walls, producing a lot of antimicrobial proteins and coordinating or integrating the defence gene expression (Shibayama et al., 2002; Hyun et al., 2011).

Instead of understanding the functions of individual genes that are expressed in the defence response, most of the previous research had focused on the understanding of the mechanisms plant protection (David et al., 2008). One of the most dominant results

gene expression profiling is the ability to observe the progression of regulated functions, in which different categories of transcripts show regulation at different time points. Identification of the genes that are differentially expressed during a Basal Stem Rot infection will help with the proper understanding for the plant defense mechanism (De Lorenzo et al., 2001).

Expression profile studies, using tools such as real time PCR can help to create a working hypothesis about the response of the plant cells during an infection (David et al., 2008). In this study, the expressions of three plant defense-related genes were studied in the roots of oil palm. Firstly, a partial *EgPGIP* gene sequence was successfully identified in the oil palm, based on the amplification using primers designed on the conserved regions of the gene in *Oryza sativa, Brachypodium sylvaticum and Hordeum vulgare*. The results are supported by the bioinformatic analysis, where the *EgPGIP* gene fragment showed homology with the *PGIP* gene in rice and more than 63% similarity with those in other monocots. In many plant species, the *PGIP* gene family members are differentially regulated and have different functions. The expression of this gene during disease development differs in response to different stimuli. For example, in pear and apple, the levels of gene expression were higher in immature than in mature fruits. The *PGIP* transcripts were less abundant in flowers than in fruit and were not detectable from leaves (Stotz et al., 1994). PGIP activity has also been detected from the scab-inoculated apple leaves. In wheat, PGIPs have been characterized, even though their N-terminal sequences (Lin and Li 2002; Kemp et al., 2003) did not show any similarity with the typical PGIP sequences that were determined from purified proteins or deduced from gene sequences (De Lorenzo et al., 2001). In addition, rice *PGIP* gene has been reported to be involved in

al., 2003). In *Brassica napus*, *Bnpgip1* is more strongly expressed than *Bnpgip2* in the flower buds, but both of them are expressed atsimilar levels in roots, open flowers and stems, and arenot expressed in the leaf blade (Li et al., 2003). The easily detected expression of *EgPGIP* in healthy oil palm roots also suggests their role in the normal development of the plant.

Higher plants have developed a range of systems to protect themselves from damage associated with biotic and abiotic stress. Certain developmental stages are particularly vulnerable and must be afforded extra protection in order to allow successful completion of the reproductive cycle. A good example is the flower, which often contains tissues rich in macromolecules, which may therefore be a target for invading herbivores or microorganisms (Lotan et al., 1989). In the present study of the roots of the oil palm, the Q-PCR showed an obvious down-regulation of the *EgPGIP* gene in response to the *G. boninense* infection through an unknown mechanism (Al-Obaidi et al., 2010).

These findings are compatible to the results obtained from the monocot rice PGIP (*OsPGIP*), which were also down-regulation during the fungal infection (Sella et al., 2004). In dicots, the soy bean *GmPGIP* and arabidopsis *AtPGIP1* and *AtPGIP2* genes were up-regulated in response to *Botrytis cinerea* infection (Ferrari et al., 2003). Similarly, while the soybean *Gmpgip1*, *Gmpgip3*, *Gmpgip4* were expressed in 7-day-old seedlings, the *Gmpgip2* was only expressed following *Sclerotinia sclerotiorum* infection (D'Ovidio et al., 2006). In arabidopsis, *Atpgip1* and *Atpgip2* were up-regulated co-ordinately in response to the *Botrytis cinerea* infection, but through separate signal transduction pathways (Ferrari et al., 2003). These results generally suggest that the response to fungal infection in the oil palm is similar to that in rice. The altered expression of *PGIP* during a fungal infection

produced by the fungal hyphae (Jiang et al., 2008). Possible explanation for the down-regulated PGIP expression during the infection with BSR was that its absence prevents the formation of the PGIP-PG complex, which is necessary for PG deactivation (Di Matteo et al., 2003).

The second defense-related gene, *EgLTP,* was partially identified in the oil palm and a partial sequencessuccessfully isolated based on similarities with other monocots. The results was supported by bioinformatics analysis, where the *EgLTP* gene fragment showed homology with the *LTP* gene of rice and more than 64% similarity with other monocots. The identification of the genes involved in resistance to *G. boninense* infection of oil palm roots could provide the necessary information for the development of strategies to prevent the infection of the fungus, especially at the early stages of the plant growth. Changes in the expression profiles of these genes may give insights into the stage of the infection and the behaviour of the fungus in the root cells.

In this study, the Q-PCR results of *Ganoderma* infected roots showed that the levels of *EgLTP* expression were obviously reduced after two week infection, compared to the uninfected control. These findings are compatible with the previous reports in wheat, where the *LTP* gene was shown to be down-regulated after microbial infection (Gaudet et al., 2010). The expression of the *LTP* gene in barley (*Hordeum vulgare)* was also shown to be down-regulated after infection with the powdery mildew fungus *Blumeria graminis* (Eichmann et al., 2005). In addition, several other studies have reported that the root *LTP* gene was also down-regulated under deferent stress conditions (Fernandez et al., 2008).

On the contrary, there were also considerable studies that showed the increase of the *LTP* gene expression in plants that were infected with fungus (Garcia-Olmedo et al., 1995; Park

2006; Mackintosh et al., 2007). The *LTP* genes express a class of small plant lipid transfer proteins (LTPs) that are soluble and basic in nature. These proteins were so named because of their ability to stimulate the transfer of a broad range of lipids between membranes *in vitro* (Yamada, 1992). The proteins, which are initially synthesized as prepeptides, with putative signal sequences, are targeted to the epidermal cell walls in plants. The LTPs have been suggested to be involved in the secretion and deposition of extracellular lipophilic materials and in the transport of cutin monomers required for the biosynthesis of surface wax (Thoma et al., 1994).

LTPs exhibit broad antimicrobial activity *in vitro* (Garcia-Olmeda et al., 1995), and because of their high isoelectric point, they may act as membrane permeabilizing agents. The lipid signal is essential for the activation of plant defence responses, but the downstream components of the signalling pathway are still poorly defined. The biosynthesis of LTPs is induced in response to an infection by a pathogen (Van Loon and Van Strien, 1999). Purified LTPs have strong antifungal activities although the underlying mechanism of action is still unclear (Ge et al., 2003). One of the suggested mechanisms for LTP antifungal activity is that the LTP could possibly form pores when inserted into the fungal cell membrane, allowing low molecular weight compounds, such as nucleotides and coenzymes, to leak but the results show that these activity was inhibited by an unknown mechanism and caused a reduction of LTP starting from 2 weeks time after inoculation.

LTPs can interact with receptors located on plant plasma membrane, which were then identified as the elicitin receptors (Buhot et al., 2001). The binding of elicitins to their receptors is known to induce disease resistance in some hosts. The LTPs are apparently

e receptor-ligand complex (Maldonado et al., 2002). In this study, the down-regulated expression of LTPs after being infected with *G. boninense* may have affected the elicitin-induced response and hence allowed the infection to occur and helped the pathogen to escape from host recognition (Schlink, 2010). This may have helped the pathogen to complete its life cycle and enter the necrotrophic growth stage, which causes extensive loss of roots. As a consequence, the roots may not be able to support the demand of water for the plant (Flores et al., 2002; Liu et al., 2006).

Alternatively, the LTP antifungal mode of action may have occurred through the formation of pores in the fungal membranes (Wong et al., 2010). This results in an efflux of intracellular ions, which culminates in cell death. The down-regulated expression of LTPs after being infected with *G. boninense* that was shown in the present study may have prevented the formation of the pores at the membrane and therefore allowed the fungus to colonize the host plant.

The third defense related gene that was investigated in the present study, PR10, has also been well associated with pathogen infection, particularly those of the fungus (Hashimoto et al., 2004). However, it is still difficult to assign a causative role of the PR10 proteins in plant resistance to the pathogens (Edreva, 2005). The reason for this is that the numerous data on PRs as disease resistance factor are mostly of correlative character. Apart from being present in the primary and secondary cell walls of infected plants, PRs are also found in cell wall appositions (papillae), deposited at the inner side of the cell wall in response to a fungal attack (Jeun, 2000). This has also been shown using sensitive immunological techniques, which allowed the detection of PRs in roots of tobacco and tomato plants inoculated with the fungal pathogens *Chalaraelegans* and *Fusarium oxysporum*,

al., 1990; Benhamou et al., 1991), as well as in lupine and birch roots exposed to abiotic stress (Utriainen et al., 1998; Przymusinski et al., 2004).

The results of the present study showed the significant down-regulation of *EgPR10* gene after infection with *G. boninense*. This was similarly observed in the European peach (*Fagus sylvatica*; Schlink, 2010) when the plant was infected with the fungal-like pathogen, *Phytophthora citricola*. Similar results have also been shown by Hyun et al. (2011) in their study of *Capsicum. annuum* var. Yeoju after being infected by the fungus, *Colletotrichum acutatum*. Xie and his group compared the expression of *ZmPR10.1* and *ZmPR10* in *Zea mays* and also noticed that the expression of PR10 was down-regulated in response to treatment with Abscisic acid (Xie et al., 2010).

Pathogen recognition evokes a strong signal and coordinated defence reaction in all infected plants during incompatible interactions while the lack of proper recognition in compatible interactions leads to more individualized and less concerted responses among infected plants. The suppression of defence activation during earlier stages of the interaction observed in this study demonstrates how well the pathogen controls the host response and is an important factor for success of this hemibiotrophic pathogen.There are growing indications that some of the effectors are targeted to the host nucleus and perhaps interfere with transcription of defence genes (Schlink, 2010). This opens up further opportunities for studies concerning the genes involved in energy metabolism during infection in order to support this theory.

hogenesis

Collectively, the gene expression investigation that was performed in this study demonstrated the coordinated down-regulated expression of defence related genes *PGIP*, *LTP* and *PR10* in the oil palm roots during the early stages of infection with *G. boninense*. This differential expression may provide some indication to how the fungus actively suppresses the host response and/or escape being recognised by the host system allowing for the establishment of the infection (Al-Obaidi et al., 2010). Upon infection of the host, the fungus would also initiate its biotrophic growth within the roots and therefore utilize the plant nutrients. This would eventually increase the sink effect of the roots. To support the roots energy demand under the circumstances where a pathogen attack was not recognized, the plant leaves may mobilize its resources to the roots, which may further compromise its defence mechanism. This possibly enabled the pathogen to complete its life cycle and to enter the necrotrophic growth phase, which causes an extensive root loss. The root loss will then lead to a decreasing potential of the roots to maintain the water demand of the upper-ground organs. This is especially problematic for young plants with small root systems. The desiccation of the leaves triggers a jasmonic acid (JA) signal to activate drought stress response which co activates JA-responsive pathogen-related genes. Hence, the seedlings quickly die from the infection as a consequence of the root loss (Schlink, 2010).

PDF Complete

Your complimentary use period has ended. Thank you for using PDF Complete.

Click Here to upgrade to Unlimited Pages and Expanded Features

To better understand the global response of the oil palm to the pathogen *Ganoderma* at the early stages of infection a proteomics study was conducted within a time course study as described for the gene expression study. The aim of the experiments was to attempt to identify other perhaps novel genes which are affected during the early stages of invasion of the pathogen. There have been limited publications on proteomic investigations of the oil palm plant and this study represents the first on the root proteome of the oil palm for a time course study of *Ganoderma* infection. The goal of this study was to identify major root proteins that were differentially expressed during the infection of the oil palm with *Ganoderma*. Such proteins may function in the signaling pathway in the root tissue or may also play a role in the physiological changes and/or metabolic switch that is a key for plant defence.

Sample preparation is one of the most crucial, yet a problematic step, in the proteomic studies of plants (Abril et al., 2011). Preparation of samples from plant materials usually yields low quantities of proteins. Plant materials also has the tendency to contain high levels of interfering compounds such as storage polysaccharides, pigments, lipids, phenolic compounds and a wide range of secondary metabolites that may affect the separation of proteins (Wang et al., 2007; Yun et al., 2010). The plant cell wall is hard to breakdown and therefore causes loss of proteins during cells homogenization (Song et al., 2012).

In addition, the plant cell is also rich in protease and contains a cell wall that makes protein extraction a problematic procedure and a big challenge (Jorrin-Novo et al., 2009).

In this study, three different protein extraction methods were compared to determine one that was most suitable for analysis by 2-DE. Because non-protein impurities can critically

...aration, it was necessary to estimate, normalize and choose an efficient method to extract the proteins from roots of oil palm. Among the three methods, phenol extraction, which also involved precipitation by methanol/ammonium acetate, produced highest amounts of proteins that were also well-resolved by SDS-PAGE. This may probably be related to the chemical composition of the research materials, which lacked interfering compounds that may affect resolution of the proteins that were subjected to electrophoresis. This extraction method was thus used in all subsequent studies on the *G. boninense* fungal infection of the roots of oil palm.

Different from the classical proteomic analyses that generally produce large quantity of information, this study screened biological sample replicates of the infected and non-infected roots of oil palm for proteins which exhibit significant but consistent change in their expression levels during the time course of the experimental infection. Therefore, the study chose to focus on 61 spots passed our screening criteria. Since the ultimate aim of the study was to identify proteins with the potential for use as biomarkers, only 21 spots of highest differential expression were chosen for identification by mass spectrometry and database search. Among the 21 proteins of interest, 12 were significantly down-regulated, while 9 were significantly up-regulated after the inoculation with *G. boninense.*

When the 21 oil palm root proteins of interest were subjected to mass spectrometry and plant database search, 11 were considered positively identified based on the generated MASCOT score of more than 55. However, among these 11 proteins, two were of unknown function. The other differentially expressed oil palm root proteins included enolase, fructokinase, caffeoyl-CoA O-methyltransferase, caffeic acid O-methyltransferase,

carrier protein reductase, pyridoxal 5- phosphate (PLP)-dependent enzyme, malate dehydrogenase and ATP synthase.

Enolase (2-phospho-D-glycerate hydrolyase) is an enzyme involved in the glycolytic pathway. The main role of this enzyme is in the conversion of 2-phosphoglycerate to phosphoenolpyruvate (Miernyk and Dennis, 1982). Because its catalytic action is reversible, enolase is also considered part of the gluconeogenesis pathway, which is important in starch accumulating seeds like maize (Ostergaard et al., 2004). Aside from being involved in energy and carbohydrate metabolism, enolase is also essential in the early stages of embryogenesis and seed formation (Mechin et al., 2007).

To the best of our knowledge, this is the first report on the down-regulated expression of enolase in the oil palm roots that were infected with *G. boninense*. This result is compatible to a previous proteomics study performed by Dahal et al. (2010), which also showed the suppression of enolase in tomatoes during infection with *Ralstonia solanacearum*. Enolase is involved in the carbohydrate metabolism that is critical for the generation of energy during root growth (Miles et al., 1991). Therefore, the down-regulation of enolase after a *G. boninense* infection may be due to the limited supply of sugars in the oil palm roots during the infection (Dahal et al., 2010). However, a few other reports have conversely indicated the up-regulated expression of enolase after microbial infection. Enolase activity was apparently induced during *Agrobacterium tumefaciens-Beta vulgaris* interaction (Rode et al., 2011), while its transcripts were induced in tomatoes that were invaded by *Oidium lycopersicum* (Li et al., 2006).

Fructokinase, another enzyme that was detected to be down-regulated in the present study, plays an important role in the metabolism of fructose (Kortstee et al., 2007; Sharma et al.,

...ssion of fructokinase after an infection with *G. boninense* may be due to decreased metabolism of fructose in the oil palm root, just like enolase in glycolysis (Curto et al., 2006). Previously, the wheat fructokinase had also been shown to be down-regulated during a drought stress (Xue et al., 2008). However, the expression of fructokinase was apparently up-regulated in tomatoes during a *Ralstonia solanacearum* infection (Dahal et al., 2010). The rice fructokinase also showed elevated levels of expression during the infection with the fungus, *Magnaporthe oryzae* (Ryu et al., 2009).

In plants, lignins belong to a copious class of plant chemicals that play an important role in a range of defence responses (Zhang and Erickson, 2012). Lignin may act as a physical wall for protection of neighbouring tissues from further damage (Chen et al., 2000). Both caffeoyl CoA 3-O-methyltransferase (CCoAOMT) and caffeic acid O-methyltransferase (COMT), enzymes that was detected to be down-regulated in the present study, are involved in the biosynthesis of lignin (Kim et al., 2006). The plant caffeoyl-CoA O-methyltransferase (CCoAOMT) and the caffeic acid O-methyltransferase (COMT) particularly have an important role in the synthesis of the guaiacyl (G) lignin subunits and in the supply of components for the synthesis of syringyl (S) lignin units (Baucher et al,. 2003; Vanholme et al., 2007; Zhang and Erickson, 2011; Figure 4.1).

PER/LAC

G-lignin

PER/LAC
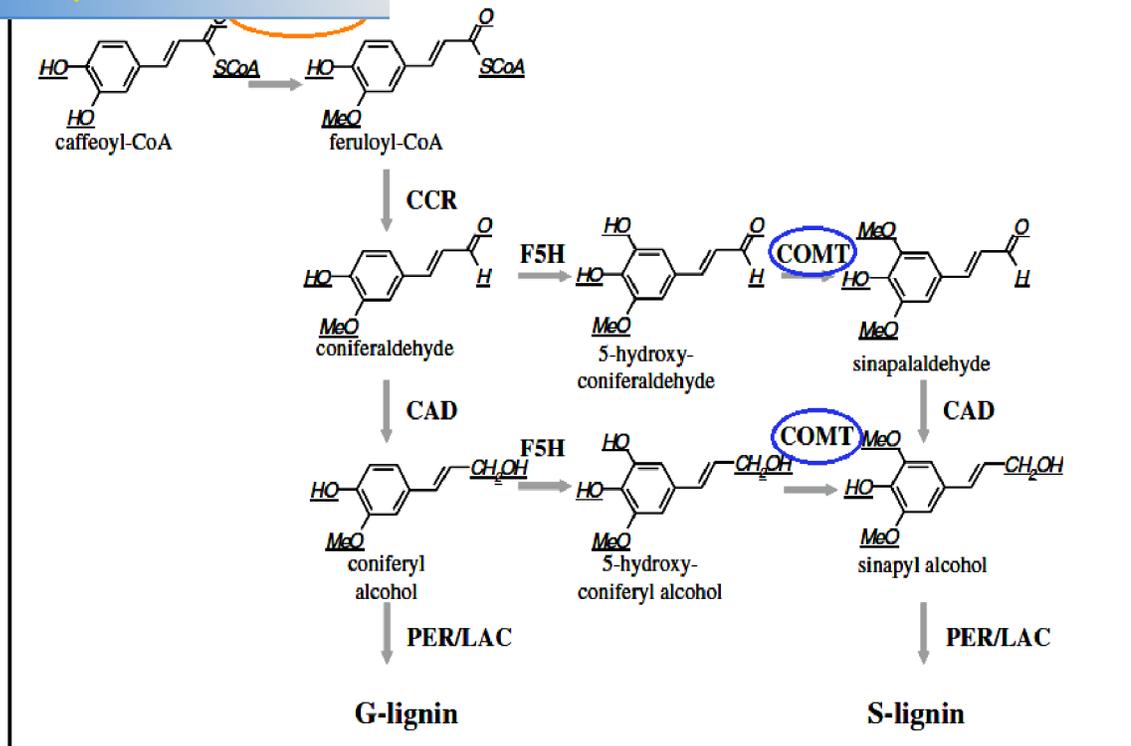
S-lignin

Figure 4.1: A view of lignin biosynthetic pathway (adapted from Hisano et al., 2009).

Previous studies in tobacco have shown that the suppression of the COMT gene caused the altered amounts and composition of lignin (Pincon et al., 2001). The down-regulation of COMT in maize was reported to cause an obvious decrease in the S unit content and consequentially affect the synthesis of lignin (Piquemal et al., 2002). Similarly, the down-regulation of CCoAOMT gene in different plant species such as alfalfa, maize, and poplar, also led to a reduction of lignin content (Boerjan at al., 2003). The proteome study carried out on the roots of *Brassica napus* after an infection with the fungus *Plasmodiophora brassicae* also showed similar significant down-regulation of the CCoAOMT gene (Cao et al., 2008).

ions process in plant cells is considered part of a basic host defence response to fungal infection (Moldenhauer et al., 2006). Hence, the significant down-regulation of oil palm root CCoAOMT and COMT observed in our results was not quite surprising. It suggests a reduction of the biosynthesis of lignin, indicating that the defence mechanism of the oil palm root was being compromised by unknown function .Another possible explanation of CCoAOMT and COMT down-regulation might be due to the fact that *G. boninense* belong to the phylum basidiomycetes, which the only identified microorganisms with the capability of degrading lignin (Bhadauria et al., 2010). Basidiomycetes been proposed to have special metabolic systems which have the ability to degrade a variety of aromatic compounds (Shimizu et al., 2005) which are thought to play an important role in the structure and function of the plant cell wall (Cairns and Esen, 2010).

Aminopeptidase is the fifth enzyme that was detected to be down-regulated in the roots of the oil palm after an infection with *G. boninense*. Aminopeptidase activity is usually detected in all plant tissues and the enzyme has been shown to play a role in plant defence mechanism (Kloetzel and Ossendorp, 2004). Aminopeptidase catalyzes the hydrolysis of N-terminal amino acid residues from proteins and peptides (Walling, 2006). The tomato aminopeptidase has been shown to be involved in the regulation of wound defence response (Walling, 2006). The in expression of aminopeptidase in the oil palm roots infected with *G. boninense* may possibly indicate the severity of the infection since aminopeptidase inactivates proteins that are important for pathogen growth and spread. Alternatively, it may be an indication of the increasing protein change in cells in response to an infection; to supply sufficient amino acid pool for the translation of the abundantly

...oteins (Ru´ız-Rivero and Prat 1998). On the contrary, however, previous investigations had also shown the up-regulation of aminopeptidase under stress conditions – like the rice roots after an infection with the fungus *Magnaporthe grisea* (Ryu et al., 2009)*,* and the tomato roots upon exposure to cadmium (Boulila-Zoghlami et al., 2011).

Enoyl-acyl carrier protein reductase (ENR) is the sixth enzyme that was shown to be down-regulated in the oil palm roots that were infected with *G. boninense*. ENR catalyzes the final step of the elongation cycle during the synthesis of fatty acids (Figure 4.2; Kater et al., 1991). The biosynthesis of fatty acids is also considered a necessary process for the survival of plants (Massengo-Tiass and Cronan, 2009).
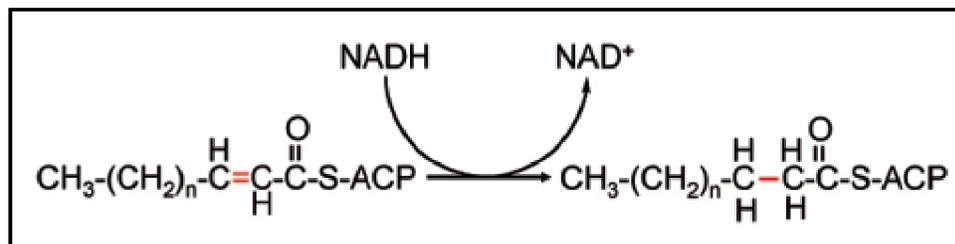
Figure 4.2: The enoyl-acyl carrier protein reductase (ENR) reaction (Massengo-Tiass and. Cronan, 2009).

The result obtained in this study suggests that *G. boninense* causes the inhibition of the ENR expression in the oil palm root, which interrupts the process of fatty acid synthesis. A similar result was also observed in *Arabidopsis,* where the expression of ENR was down-regulated, and that led to a premature cell death (Mou et al., 2000). Aside from these studies, there was apparently little information that was available in the literature on

fungal infection, except the effect of the deficiency of boron that apparently caused the up-regulated expression of the *Brassica napus* root ENR (Wang et al., 2010b).

ATP synthase is an enzyme involved in the synthesis of ATP from ADP via photosynthesis (McCarty et al., 2000). The alpha-subunit of this enzyme was found to be down-regulated in the infected oil palm root in comparison with the control healthy root in the present study. However, this is in contrast to previous published results on rice and chilli. ATP synthase was found to be overexpressed in rice that had fungal infection (Tsunezuka et al., 2005) as well as chilli (*Capsicum annuum*) during its interaction with the phytopathogenics fungi *Fusarium oxysporum* (Wongpia and Lomthaisong, 2010). In view of these contradictory results, it is difficult to make a speculation on why ATP synthase was differentially expressed subsequent to a fungal infection.

Pyridoxal-5-phosphate (vitaminB6)-dependent enzymes play central roles in the metabolism of amino acids.PLP-dependent enzyme catalyses a variety of enzymatic reactions, including decarboxylation, transamination, deamination and trans-sulfuration reactions in the metabolism of amino acids (Mooney et al., 2009). In plants, PLP play important role in ethylene biosynthesis (Capitani et al., 1999) chlorophyll and tetrapyrrole synthesis (Tsang et al., 2003). PLP-dependent enzyme from oil palm roots identified up-regulated up on *G. boninense*in comparison to non-infected oil palm roots. Similar results obtained in *Arabidopsis* PLP-dependent (AtPLPR1) enzyme which show down-regulation in response to both NaCl and manitol (Herrero et al., 2011). Another similar result showed up-regulation level of *Malus xiaojinensis* PLP-dependent enzyme study on root during iron deficiency. However, till now there is no report for PLP-dependent enzyme up-regulation

...zyme in plant in response to fungal infection (Wang et al., 2010a).

The up-regulated expression of oil palm root malate dehydrogenase (MDH) was shown in this study after an infection with *G. boninense*. Earlier proteomics studies by Geddes et al., 2008 had shown that MDH was also elevated in barley during the infection with the phytopathogenic fungus *Fusarium graminearum*. Similar results were obtained from the proteomics analysis for *Arabidopsis thaliana*, where the expression of MDH was up-regulated after an infection *Plutella xylostella* (Collins et al., 2010). The proteome profile analysis of *Medicago truncatula* leaves also showed the up-regulated expression of MDH in response to an infection with the pathogenic fungi *Uromyces striatus* (Castillejo et al., 2010a). When taken together, the expression of MDH appears to be up-regulated when plants were subjected to stress conditions, particularly during a fungal infection, (Scheibe, 2004). MDH is the main enzyme involved in the synthesis of malate in the Kreb's cycle. However, the enzyme has been shown to be present in the plant cell organelles and the plant cell wall and also reported to have an essential role in removing toxic radicals formed during oxidative stress (Lance and Rustin, 1984). Hence, the precise role for the up-regulated expression of MDH in response to fungal infection shown in this study as well as those of others is not clear.

Identification of genes involved in resistance to the *G. boninense* in oil palm roots could provide information for development of strategies to prevent the infection of the fungus especially in the early stages of the plant growth. In the present study, The *PGIP, LTP* and *PR10* genes were shown to be down-regulated in the oil palm roots that were infected with *G. boninense*. Changes in the expression of these genes may give insights into the stage of the infection and the behavior of the fungus in the root cells and that will be helpful to enable the development of successful management practices for disease control.

The proteomics analysis of the oil palm roots infected with *G. boninense* also detected the differential expression of nine proteins with known functions. While the expression of enolase, fructokinase, caffeoyl-CoA O-methyltransferase, caffeic acid O-methyltransferase, aminopeptidase, enoyl-acyl carrier protein reductase, and ATP synthase was shown to be down-regulated in response to the *G. boninense* infection, the expression of pyridoxal 5-phosphate (PLP)-dependent enzyme and malate dehydrogenase appeared to be over-expressed. Biomarker discovery is possibly the most important practical application of proteomics in the last decade (Cilia et al., 2012). While the altered expression of these proteins may have some physiological relevance to the plant, such as the need to change its metabolism or being involved in its defence mechanism, these proteins may also be exploited for their potential use as biomarkers for an oil palm root infection.The analysis of activation and synthesis of infection/stress related proteins identified can potentially generate a set of biomarkers to discriminate between different defence-related strategies,diagnostic tool, therapy guidance and prognosis monitoring of BSR infection.

of biomarkers represents one of the ultimate tools for the improvement of plant infection early diagnostics, monitoring and for the evaluation of the treatment strategies.Applicationsofmassspectral-basedtechnologiesareprovidedsolutionsforthediscoveryofnovel biomarkers (Hale et al., 2003).For the future work, these biomarkercandidatesrequiredto bevalidated andultimatelyassayedinaformatthatlendsitselfintohighthroughputanalysis.A number of open questions also remain about the correlation of activation and expression patterns of stress proteins during fungal infection.

Abril, N., Gion, J. M., Kerner, R., Muller-Starck, G., Cerrillo, R. M. N., Plomion, C., et al. (2011). Proteomics research on forest trees, the most recalcitrant and orphan plant species. *Phytochemistry, 72*(10), 1219-1242.

Abdullah, S. N. A., Cheah, S. C., & Murphy, D. J. (2002). Isolation and characterisation of two divergent type 3 metallothioneins from oil palm, *Elaeis guineensis*. *Plant Physiologyogy and Biochemistry, 40*(3), 255-263.

Al-Obaidi, J.R., Mohd-Yusuf, Y., Chin-Chong, T., Mhd-Noh, N., Othman, R.Y. (2010). Identification of a partial oil palm polygalacturonase-inhibiting protein (EgPGIP) gene and its expression during basal stem rot infection caused by *Ganoderma boninense*. *African Journal of Biotechnology, 9*(46): 7788-7797.

Abubakar, A. (2004). Oil palm (median edition), Report on Survey Of Selected Agricultural Raw Materials In Nigeria, Abudja., 1:1-3.

Abugoukh, A. A., Strand, L. L., & Labavitch, J. M. (1983). Development Related Changes in Decay Susceptibility and Polygalacturonase Inhibitor Content of Bartletter Pear Fruit. *Physiological Plant Pathology, 23*(1), 101-109.

Adaskaveg, J.E., Gilberston, R.L., Blanchette, R.A. (1990). Comparative studies of delignification caused by *Ganoderma* species.*Applied and Environmental Microbiology 56*(6), 1932-1943.

Aebersold, R., and Mann, M. (2003). Mass spectrometry-based proteomics.*Nature, 422*(6928), 198-207.

Agosin,E., Blanchette,R.A., Silva,H., Lapierre,C., Cease,K.R., Ibach,R.E., Abad,A.R., Muga,P. (1990). Characterisation of palo podrigo, a natural process of delignification in wood.*Applied and Environmental Microbiology 56*(1), 65-74.

Agrawal, G. K., & Rakwal, R. (2006). Rice proteomics: a cornerstone for cereal food crop proteomes. *Mass Spectrometry Reviews, 25*(1), 1-53.

nd Agrawal, M. (2009). Ultraviolet-B Induced Changes in dants in Plants.*Advances in Botanical Research: Oxidative Stress and Redox Regulation in Plants, Vol 52, 52*, 47-86.

Agrios, G.N. (1997). *Plant Pathology*.Fourth Edition.Academic Press, adivision of Harcourt Brace and Company, San Diego, California, USA, p. 635.

Aguero, C. B., Uratsu, S. L., Greve, C., Powell, A. L., Labavitch, J. M., Meredith, C. P., et al. (2005). Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene.*Molecular Plant Pathology, 6*(1), 43-51.

Ambak, K., and Melling, L., (1999). *Management practices for sustainable cultivation of crop plants on tropical peatland.* In Proceedings of the International Symposium on tropical peatlands, Bogor, Indonesia, 22-23 November 1999.

Anderson,N. L., . Anderson.N. G., Pearson, et al. (2009).A human proteome detection and quantitation project. *Molecular and Cellular Proteomics, 8*, (5) 883–886.

Apoga, D., Ek, B., and Tunlid, A. (2001). Analysis of proteins in the extracellular matrix of the plant pathogenic fungus Bipolaris sorokiniana using 2-D gel electrophoresis and MS/MS. *FEMS Microbiology Letterers, 197*(2), 145-150.

Archana, P. (2006) Proteome analysis of Pseudomonas putida KT2440 using 2D Gel Electrophoresis and LC/ESI-Q-TOF Mass Spectrometry, Unpublished master's thesis, Rochester Institute of Technology Rochester, NY.

Ariffin, D and Idris, As. (1992).*The* Ganoderma *Selective Media (GSM)*.PORIM Information Series. Palm Oil Research Institute Malaysia, Bangi.

Ariffin, D., Idris, A.S., Singh, G., (2000). Status of Ganoderma in oil palm. In: Flood, J., Bridge, P.D., Holderness, M. (Eds.), *Ganoderma Diseases of Perennial Crops*. CABI Publishing, Wallingford, UK, pp. 49–68.

Arora, D.K., Bridge,P.D., Bhatnagar,D. (2004). *Fungal Biotechnology in Agricultural, Food and Environmental Applications*. New York, NY, USA: Marcel Dekker.

mpson,R.J., Clarke,A.E., Anderson,M.A. (1993). Proteinase stigmas are derived from a precursor protein which is processed into five homologous inhibitors. *The Plant Cell, 5*(2), 203–213.

Azhar, B, Lindenmayer, D, Wood, J et al (2011). 'The conservation value of oil palm plantation estates, smallholdings and logged peat swamp forest for birds. *Forest Ecology and Management*, *262*(12), 2306-2315.

Bae, M. S., Cho, E. J., Choi, E. Y., & Park, O. K. (2003).Analysis of the Arabidopsis nuclear proteome and its response to cold stress.*Plant Journal, 36*(5), 652-663.

Bai, S., Liu, S., Guo, X., Qin, Z., Wang, B., Li, X., et al. (2010).Proteome analysis of haptoglobin in cerebrospinal fluid of neuromyelitis optica.*Molecular Biology Reports, 37*(3), 1619-1625.

Bateman, D. F., and Basham, H. G. (1976).Degradation of plant cell walls and membranes by microbial enzymes. In *Encyclopedia of Plant Physiologyogy* (eds Heitefuss, R. and Williams, P. H.), Springer- Verlag, New York, 316-355.

Baucher, M., Halpin, C., Petit-Conil, M., and Boerjan, W. (2003). Lignin: Genetic engineering and impact on pulping. *Critical Reviews inBiochemistry and Molecular Biology, 38*(4), 305-350.

Benhamou, N., Grenier, J., and Asselin, A. (1991).Immunogold Localization of Pathogenesis-Related Protein P14 in Tomato Root-Cells Infected by *Fusarium-Oxysporum*-F Sp *Radicis-Lycopersici.Physiological andMolecular Plant Pathology, 38*(4), 237-253.

Berger, D. K., Oelofse, D., Arendse, M. S., Du Plessis, E., and Dubery, I. A. (2000). Bean polygalacturonase inhibitor protein-1 (PGIP-1) inhibits polygalacturonases from *Stenocarpella maydis*. *Physiological and Molecular Plant Pathology, 57*(1), 5-14.

Bertazzon,N., Raiola, A., Castiglioni,C., Gardiman,M., Angelini,E.,Borgo,M.,Ferrari,Y. (2011). Transient silencing of the grapevine gene VvPGIP1 by agroinfiltration with a construct for RNA interference.*Plant Cell Rep Report,* DOI 10.1007/s00299-011-1147-2.

Bestel-Corre, G., Dumas-Gaudot, E., Poinsot, V., Dieu, M., Dierick, J. F., van, T. D., et al. (2002).Proteome analysis and identification of symbiosis-related proteins from*Medicago*

dimensional electrophoresis and mass spectrometry. 7.

Bhadauria, V., Wang, L. X., and Peng, Y. L. (2010). Proteomic changes associated with deletion of the Magnaporthe oryzae conidial morphology-regulating gene COM1. *Biol Direct, 5*, 61.

Bhonwong,A., Stout, M.J., Attajarusit, J., Tantasawat,P. (2009). Defensive role of tomato Polyphenol oxidase against cotton bollworm (*Helicoverpa armigera*) and Beet armyworm (*Spodoptera exigua*).*Journal of Chemical Ecology (35)*, 28–38.

Biesiadka, J., Bujacz, G., Sikorski, M. M., and Jaskolski, M. (2002). Crystal structures of two homologous pathogenesis-related proteins from yellow lupine. *Journal of Molecular Biology, 319*(5), 1223-1234.

Billotte, N., Marseillac, N., Risterucci, A. M., Adon, B., Brottier, P., Baurens, F. C., et al. (2005). Microsatellite-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.).*Theoretical and Applied Genetics, 110*(4), 754-765.

Birtic, S., and Kranner, I. (2006).Isolation of high-quality RNA from polyphenol-, polysaccharide- and lipid-rich seeds.*Phytochemical Analysis, 17*(3), 144-148.

Blanchette, R.A. (1984). Selective delignification of eastern hemlock by *Ganoderma tsugae*.*Phytopathology 74*(1), 153-160.

Blein, J. P., Coutos-Thevenot, P., Marion, D., and Ponchet, M. (2002). From elicitins to lipid transfer proteins: a new insight in cell signalling involved in plant defence mechanisms. *Trends in Plant Science*, 7(7), 293-296.

Blilou, I., Ocampo, J. A., and Garcia-Garrido, J. M. (2000). Induction of Ltp (lipid transfer protein) and Pal (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. *Journal of Experimental Botany, 51*(353), 1969-1977.

Blueggel, M., Chamrad, D., and Meyer, H. E. (2004).Bioinformatics in proteomics.*Current Pharmaceutical Biotechnology, 5*(1), 79-88.

Baucher, M. (2003).Lignin biosynthesis.*Annual Review of*

Bohmer, M., Colby, T., Bohmer, C., Brautigam, A., Schmidt, J., and Bolker, M. (2007). Proteomic analysis of dimorphic transition in the phytopathogenic fungus *Ustilago maydis*.*Proteomics, 7*(5), 675-685.

Bookout, A. L., and Mangelsdorf, D. J. (2003). Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways.*Nuclear Receptor Signaling journal, 1*, e012.

Boulila-Zoghlami, L., Gallusci, P., Holzer, F. M., Basset, G. J., Djebali, W., Chaibi, W., et al. (2011). UP-regulation of leucine aminopeptidase-A in cadmium-treated tomato roots. *Planta, 234*(4), 857-863.

Bowles, D. J. (1990).Defense-related proteins in higher plants.*Annual Review of Biochemistry, 59*, 873-907.

Brencic, A., and Winans, S. C. (2005). Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiology and Molecular Biology Reviews, 69*(1), 155-194.

Bringans, S., Eriksen, S., Kendrick, T., Gopalakrishnakone, P., Livk, A., Lock, R., et al. (2008). Proteomic analysis of the venom of *Heterometrus longimanus* (Asian black scorpion).*Proteomics, 8*(5), 1081-1096.

Brown, E., and Michael, F. J. (2005).*Cruel Oil: How Palm Oil Harms Health, Rainforest and Wildlife*, Center for Science in the Public Interest, Washington, DC. www.cspinet.org/palmoilreport/.

Bruschi, M., Seppi, C., Arena, S., Musante, L., Santucci, L., Balduini, C., Scaloni, A., Lanciotti, M., Righetti, P.G. and Candiano, G. (2005).Proteomic analysis of erythrocyte membranes by soft Immobiline gels combined with differential protein extraction. *Journal of Proteome Research, 4*(4)1304-1309.

Buhot,N.,Douliez,J.P.,Jacquemard,A.,Marion,D.,Tran,V.,Maume,B.F.,Milat,M.L.,Poncht, M., Mikes, V.,Kadar,J.C.,Blein,J.P. (2001). A lipid transfer protein binds to a receptor involved in the control of plant defense responses. *FEBS Letter*, (509), 27-30.

Cairns, J. R. K., and Esen, A. (2010).beta-Glucosidases.*Cellular and Molecular Life Sciences, 67*(20), 3389-3405.

Cameron,K.D., Teece,M.A., Smart,L.B. (2006). Increased accumulation of cutical wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco.*Plant Physiologyogy, 140*(1), 176-183.

Cánovas, F.M., Dumas-Gaudot, E., Recorbet, G., Jorrin, J., Mock, H.P., Rossignol, M.(2004).Plant proteome analysis.*Proteomics, 4*(2):285-98.

Cantu, M. D., Mariano, A. G., Palma, M. S., Carrilho, E., and Wulff, N. A.(2008). Proteomic analysis reveals suppression of bark chitinases and proteinase inhibitors in citrus plants affected by the citrus sudden death disease. *Phytopathology, 98*(10), 1084-1092.

Cao,T., Srivastava,S., Rahman,M.H., Kavk,N.N.V., Hotte,N., Deyholos,M.K., Strelkov,S.E. (2008).Proteome-level changes in the roots of Brassica napus as a result of Plansmodiophora brassicae infection. *Plant Science 174*(1), 97–115.

Capitani, G., Hohenester, E., Feng, L., Storici, P., Kirsch, J. F., and Jansonius, J. N. (1999).Structure of 1-aminocyclopropane-1-carboxylate synthase, a key enzyme in the biosynthesis of the plant hormone ethylene.*Journal of Molecular Biology, 294*(3), 745-756.

Carlsson, U., Elmqvist, T., Wennstrom, A., and Ericson, L. (1990).Infection by Pathogens and Population Age of Host Plants.*Journal of Ecology, 78*(4), 1094-1105.

Carpentier, S. C., Witters, E., Laukens, K., Deckers, P., Swennen, R., and Panis, B. (2005). Preparation of protein extracts from recalcitrant plant tissues: An evaluation of different methods for two-dimensional gel electrophoresis analysis. *Proteomics, 5*(10), 2497-2507.

Carter, C., Finley, W., Fry, J., Jackson, D., and Willis, L. (2007). Palm oil markets and future supply. *European Journal of Lipid Science and Technology, 109*(4), 307-314.

Castillejo, M. A., Susin, R., Madrid, E., Fernandez-Aparicio, M., Jorrin, J. V., and Rubiales, D. (2010a).Two-dimensional gel electrophoresis-based proteomic analysis of the *Medicago truncatula*-rust (*Uromyces striatus*) interaction.*Annals of Applied Biology, 157*(2), 243-257.

Castillejo, M. A., Curto, M., Fondevilla, S., Rubiales, D., and Jorrin, J. V. (2010b). Two-Dimensional Electrophoresis Based Proteomic Analysis of the Pea (*Pisum sativum*) in Response to *Mycosphaerella pinodes*. *Journal of Agricultural and Food Chemistry, 58*(24), 12822-12832.

Cater, N. B., Heller, H. J., and Denke, M. A. (1997). Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. *American Journal of Clinical Nutrition, 65*(1), 41-45.

Cervone, F., De Lorenzo, G., Degra, L., Salvi, G., and Bergami, M. (1987). Purification and Characterization of a Polygalacturonase-Inhibiting Protein from *Phaseolus vulgaris* L. *Plant Physiologyogy, 85*(3), 631-637.

Cervone, F., De Lorenzo, G., Presey, R., Darvil, A. G. and Albersheim, P. (1990). Can *Phaseolus* PGIP inhibit pectic enzymes from microbes and plants *Phytochemistry,29*(2), 447–449.

Chadha, P., and Das, R.H. (2006). A pathogenesis related protein, AhPR10 from peanut: an insight of its mode of antifungal activity. *Planta, 225*(1), 213-222.

Chang, M. M., Chiang, C. C., Martin, M. W., and Hadwiger, L. A. (1993). Expression of a Pea Disease Resistance Response Gene in the Potato Cultivar Shepody. *American Potato Journal, 70*(9), 635-647.

Chang, W.W., Huang, L., Shen, M., Webster, C., Burlingame, A.L., and Roberts, J.K. (2000). Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment and identification of proteins by mass spectrometry. *Plant Physiology, 122*(2), 295-318.

Cheah,S.C. (2003). Understanding the causes of oil palm tissue culture abnormalities on the road to commercialization. Proceedings of the PIPOC 2003 International Palm Oil Congress. *Agriculture Conference, pp. 121 – 128. MPOB.*

Chen, C.G., Cornish, E.C., and Clarke, A.E. (1992). Specific expression of an extensin-like gene in the style of Nicotiana alata. *Plant Cell, 4*(9), 1053-1062.

I., Burggraeve, B., De Rycke, R.M., Inoue, K., De 000). Cell-specific and conditional expression of caffeoyl-coenzyme A-3-O-methyltransferase in poplar.*Plant Physiologyogy, 123*(3), 853-867.

Chen, G., Gharib, T.G., Huang, C.C., Taylor, J.M., Misek, D.E., Kardia, S.L., Giordano, T.J., Iannettoni, M.D., Orringer, M.B., Hanash, S.M. and Beer, D.G. (2002) *Mol. Cell. Proteomics, 1,* 304- 313.

Chen, H. (2007). Stability of plant defense proteins in the gut of insect herbivores (vol 143, pg 1954, 2007).*Plant Physiologyogy, 144*(2), 1233-1233.

Chevalier, F., Martin, O., Rofidal, V., Devauchelle, A. D., Barteau, S., Sommerer, N., et al. (2004).Proteomic investigation of natural variation between Arabidopsis ecotypes.*Proteomics, 4*(5), 1372-1381.

Christensen, A. B., Cho, B.H., Naesby, M., Gregersen, P.L., Brandt, J., Madriz-Ordenana, K., et al. (2002). The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins. *Molecular Plant Pathology, 3*(3), 135-144.

Cilia, M., Bereman, M., Fish, T., MacCoss, M. J., & Gray, S. (2012). Homopteran Vector Biomarkers for Efficient Circulative Plant Virus Transmission are conserved in Multiple Aphid Species and the Whitefly *Bemisia tabaci*. *Journal of Integrative Agriculture, 11*(2), 249-262.

Claverie, J.-M., and Notredame, C. (2007).*Bioinformatics for dummies* (2nd ed.). Hoboken, N.J.: Wiley Pub.

Cochard, B., Adon, B., Rekima, S., Billotte, N., de Chenon, R.D., Koutou, A., et al. (2009). Geographic and genetic structure of African oil palm diversity suggests new approaches to breeding. *Tree Genetics and Genomes, 5*(3), 493-504.

Colditz, F., Braun, H.P., Jacquet, C., Niehaus, K., and Krajinski, F. (2005). Proteomic profiling unravels insights into the molecular background underlying increased *Aphanomyces euteiches*-tolerance of *Medicago truncatula*. *Plant Molecular Biology, 59*(3), 387-406.

iehaus, K., Eubel, H., Braun, H. P., and Krajinski, F. (2004). ation of *Medicago truncatula* proteins induced in roots after infection with the pathogenic oomycete *Aphanomyces euteiches*. *Plant Molecular Biology, 55*(1), 109-120.

Collins, R. M., Afzal, M., Ward, D.A., Prescott, M.C., Sait, S. M., Rees, H. H., et al. (2010).Differential Proteomic Analysis of Arabidopsis thaliana Genotypes Exhibiting Resistance or Susceptibility to the Insect Herbivore, Plutella xylostella.*Plos One, 5*(4).

Conway, W.S., Yao, C., Ross, G.S. and Sams, C.E. (1998).*Apple polygalacturonase-inhibiting protein cDNA cloning and transcription.Proceedings of 7th International Congress of Plant Pathology,* Edinburgh, Scotlan.

Cook B.J., Clay, R.P., Bergmann, C.W., Albersheim, P., and Darvill, A.G. (1999). Fungal polygalacturonases exhibit different substrate degradation patterns and differ in their susceptibilities to polygalacturonase-inhibiting proteins. *Molecular Plant-Microbe Interactions, 12*(8), 703-711.

Cooper, R.M., and Wood, R.K.S. (1975).Regulation of Synthesis of Cell-Wall Degrading Enzymes by *Verticillium-Albo-Atrum* and *Fusarium oxysporum* F Sp Lycopersici.*Physiological Plant Pathology, 5*(2), 135-156.

Corley, R.H.V., Tinker, P.B., and Hartley, C.W.S. (2003).*The oil palm* (4th ed.). Oxford: Blackwell Science.

Corthals, G. L., Wasinger, V. C., Hochstrasser, D. F., and Sanchez, J. C. (2000). The dynamic range of protein expression: a challenge for proteomic research. *Electrophoresis, 21*(6), 1104-1115.

Cox, K. D., Layne, D. R., Scorza, R., and Schnabel, G. (2006). Gastrodia anti-fungal protein from the orchid Gastrodia elata confers disease resistance to root pathogens in transgenic tobacco. *Planta, 224*(6), 1373-1383.

Curto, M., Camafeita, E., Lopez, J.A., Maldonado, A.M., Rubiales, D., and Jorrin, J.V. (2006).A proteomic approach to study pea (Pisum sativum) responses to powdery mildew (Erysiphe pisi).*Proteomics, 6 Suppl 1*, S163-174.

Cullis, C.A. (2004). *Plant Genomics and Proteomics*. Hoboken, New Jersey: John Wiley and Sons, Inc.

and Mackey, D. (2006). Innate immunity in plants: a
. *Microbes and Infection, 8*(5), 1372-1381.

Dahal, D., Pich, A., Braun, H.P., and Wydra, K. (2010). Analysis of cell wall proteins regulated in stem of susceptible and resistant tomato species after inoculation with *Ralstonia solanacearum*: a proteomic approach. *Plant Molecular Biology, 73*(6), 643-658.

Danielsen, F., Beukema, H., Burgess, N.D., Parish, F., Bruhl, C.A., Donald, P.F., et al. (2009). Biofuel Plantations on Forested Lands: Double Jeopardy for Biodiversity and Climate. *Conservation Biology,23*(2), 348-358.

David, H., Ozcelik, I. S., Hofmann, G., and Nielsen, J. (2008).Analysis of *Aspergillus nidulans* metabolism at the genome-scale.*Bmc Genomics, 9*. (163).

D'Ovidio, R., Roberti, S., Di Giovanni, M., Capodicasa, C., Melaragni, M., Sella, L., et al. (2006). The characterization of the soybean polygalacturonase-inhibiting proteins (Pgip) gene family reveals that a single member is responsible for the activity detected in soybean tissues. *Planta, 224*(3), 633-645.

De Keukeleire, J., Roldan-Ruiz, I., Van Bockstaele, E., Heyerick, A., and De Keukeleire, D. (2006).Efficient extraction of High-Quality total RNA from various hop tissues (*Humulus lupulus* L.).*Preparative Biochemistry and Biotechnology, 36*(4), 355-362.

De Lorenzo, G., D'Ovidio, R., and Cervone, F. (2001).The role of polygalacturonase-inhibiting proteins (PGIPS) in defense against pathogenic fungi.*Annual Review of Phytopathology, 39*, 313-335.

De Vries, R.P., and Visser, J. (2001). Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews, 65*(4), 497-522.

Deacon, J.W. (1997).*Modern mycology* (3rd ed.). Oxford: Blackwell Science. Rajanaidu,N., and Jalani,B.S. (1994a). Oil palm germplasm collection in Senegal. ISOPB *Newsletterer* 10(1), 1-18.

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., et al. (2005).The genome sequence of the rice blast fungus *Magnaporthe grisea*.*Nature, 434*(7036), 980-986.

003). Purification and characterization of polygalacturonase-*um guajava* Linn. (guava) fruit. *Plant Science, 164*(2), 147-156.

Desiderio, A., Aracri, B., Leckie, F., Mattei, B., Salvi, G., Tigelaar, H., et al. (1997). Polygalacturonase-inhibiting proteins (PGIPs) with different specificities are expressed in Phaseolus vulgaris. *Molecular Plant-Microbe Interactions, 10*(7), 852-860.

Devoto, A., Leckie, F., Lupotto, E., Cervone, F., and De Lorenzo, G. (1998). The promoter of a gene encoding a polygalacturonase-inhibiting protein of *Phaseolus vulgaris* L. is activated by wounding but not by elicitors or pathogen infection. *Planta, 205*(2), 165-174.

Di Matteo, A., Federici, L., Mattei, B., Salvi, G., Johnson, K. A., Savino, C., et al. (2003). The crystal structure of polygalacturonase-inhibiting protein (PGIP), a leucine-rich repeat protein involved in plant defense.*Proceedings of the National Academy of Sciences U S A, 100*(17), 10124-10128.

Dimelu, M.U., and Anyaiwe, V. (2011). Priorities of Smallholder Oil Palm Producers in Ika Local Government Area of Delta State: Implication for Agricultural Extension Service in Nigeria. *World Journal of Agricultural Sciences 7* (2): 117-123.

Ding, C. Q., You, J., Liu, Z. H., Rehmani, M. I. A., Wang, S. H., Li, G. H., et al. (2011). Proteomic Analysis of Low Nitrogen Stress-Responsive Proteins in Roots of Rice.*Plant Molecular Biology Reporter, 29*(3), 618-625.

Dinham, B. (2005). Agrochemical markets soar: pest pressures or corporate design? *Pesticides News 68*: 9-11.

Dodds, P.N., and Rathjen, J.P. (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics, 11*(8), 539-548.

Donald, P. F. (2004). Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology, 18* (1), 17-37.

Douady, D., Grosbois, M., Guerbette, F., and Kader, J.(1986). Phospholipid transfer protein from maize seedlings is partly membrane-bound. *Plant Sci, 45*(3) 151-156.

Isolation of Plant DNA from fresh tissue. Focus 12:13-15.

Dubos, C., and Plomion, C. (2001). Drought differentially affects expression of a PR-10 protein, in needles of maritime pine (*Pinus pinaster* Ait.) seedlings. *Journal of Experimental Botany, 52*(358), 1143-1144.

Duke, J.A. (1983). Handbook of Energy Crops, unpublished. Available online on Purdue university's website (http://www.hort.purdue.edu/newcrop/ duke_energy/).

Dumas-Gaudot, E., Amiour, N., Weidmann, S., Bestel-Correl, G., Valot, B., Lenogue, S., et al. (2004).A technical trick for studying proteomics in parallel to transcriptomics in symbiotic root-fungus interactions.*Proteomics, 4*(2), 451-453.

Durand-Gasselin, T., Asmady, H., Flori, A., Jacquemard, J.c., Hayun, Z., Breton, F., and de FranqueVille,H. (2005). Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninse* – prospects for future breeding. *Mycopathologia, 15*9, 93-100.

Edreva, A. (2005). Pathogenesis-related proteins: research progress in the last 15 years.*Gen. Appl. Plant Physiologyogy, 31*(1-2), 105-124.

Eichmann, R., Biemelt, S., Schafer, P., Scholz, U., Jansen, C., Felk, A., et al. (2006).Macroarray expression analysis of barley susceptibility and nonhost resistance to Blumeria graminis.*Journal of Plant Physiology, 163*(6), 657-670.

Ekramoddoullah, A.K. (1993). Analysis of needle proteins and N-terminal amino acid sequences of two photosystem II proteins of western white pine (*Pinus monticola* D. Don).*Tree Physiology, 12*(1), 101-106.

Enkerli, K., Hahn, M. G., and Mims, C.W. (1997). Ultrastructure of compatible and incompatible interactions of soybean roots infected with the plant pathogenic oomycete Phytophthora sojae. *Canadian Journal ofBotany-Revue Canadienne De Botanique, 75*(9), 1493-1508.

Fang, Y., Macool, D.J., Xue, Z., Heppard, E.P., Hainey, C.F., Tingey, S.V., et al. (2002). Development of a high-throughput yeast two-hybrid screening system to study protein-protein interactions in plants.*Molecular Genetics & Genomics, 267*(2), 142-153.

statistical service. FAO, Rome. Available from
                    d January 2008.

Favaron, F. (2001). Gel detection of *Allium porrum* polygalacturonase inhibiting protein reveals a high number of isoforms. *Physiological andMolecular Plant Pathology, 58*(6), 239-245.

Favaron, F., Castiglioni, C., and Dilenna, P. (1993).Inhibition of Some Rot Fungi Polygalacturonases by *Allium-Cepa* L and *Allium-Porrum* L Extracts.*Journal of Phytopathology-Phytopathologische Zeitschrift, 139*(3), 201-206.

Favaron, F., Castiglioni, C., Dovidio, R., and Alghisi, P. (1997).Polygalacturonase inhibiting proteins from *Allium porrum* L. and their role in plant tissue against fungal endo-polygalacturonases.*Physiological andMolecular Plant Pathology, 50*(6), 403-417.

Favaron, F., Dovidio, R., Porceddu, E., and Alghisi, P. (1994).Purification and Molecular Characterization of a Soybean Polygalacturonase Inhibiting Protein.*Planta, 195*(1), 80-87.

Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F., and Whitehouse, C.M. (1989).Electrospray ionization for mass spectrometry of large biomolecules.*Science, 246*(4926), 64-71.

Fernandez,C., Szyperski,T., Bruyere,T., Ramage,P., Mosinger,E., Wutrich,K.(1997).NMR solution structure of thepathogenesis-related protein p14a.*Journal of Molecular Biology, 266*(3), 576-93.

Fernandez, P., Di Rienzo, J., Fernandez, L., Hopp, H. E., Paniego, N., and Heinz, R. A. (2008). Transcriptomic identification of candidate genes involved in sunflower responses to chilling and salt stresses based on cDNA microarray analysis. *BMC Plant Biology, 8*, 11.

Ferrari,S., Vairo,D., Ausubel,F.M., Cervone,F., De Lorenzo,G. (2003). Arabidopsis polygalacturonase-inhibiting proteins (PGIP) are regulated by different signal transduction pathways during fungal infection. *Plant Cell,* (15), 93–106.

Ferro, M., Salvi, D., Brugiere, S., Miras, S., Kowalski, S., Louwagie, M., et al. (2003). Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana.Molcular Cell Proteomics, 2*(5), 325-345.

...l Inhibitors of Fungal Polygalacturonases in Infected Fruit ...*crobiology, 123*(Apr), 377-381.

Fisher, M.L., Anderson, A.J., and Albershe, P. (1973).Host-Pathogen Interactions .6. A Single Plant Protein Efficiently Inhibits Endopolygalacturonases Secreted by *Colletotrichum-Lindemuthianum* and *Aspergillus-Niger*. *Plant Physiologyogy, 51*(3), 489-491.

Fizames, C., Munos, S., Cazettes, C., Nacry, P., Boucherez, J., Gaymard, F., et al. (2004). The Arabidopsis root transcriptome by serial analysis of gene expression. Gene identification using the genome sequence.*Plant Physiologyogy, 134*(1), 67-80.

Flood, J., Bridge, P.D., Holderness, M.(2000).Preface.*Ganoderma Diseases of Perennial Crops*.CABI Publishing, Wallingford, UK.

Flores, F., El Yahyaoui, F., de Billerbeck, G., Romojaro, F., Latche, A., Bouzayen, M., et al. (2002).Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais *Cantaloupe melons*.*Journal of Experimental Botany, 53*(367), 201-206.

Fort, F., Hayoun, L., Valls, J., Canals, J. M., Arola, L., and Zamora, F. (2008). A new and simple method for rapid extraction and isolation of high-quality RNA from grape (*Vitis vinifera*) berries. *Journal of the Science of Food and Agriculture, 88*(2), 179-184.

Francis, G.L., Upton, F.M., Ballard, F.J., Mcneil, K.A., and Wallace, J.C. (1988). Insulin-Like Growth Factor-I and Factor-Ii in *Bovine Colostrum* - Sequences and Biological-Activities Compared with Those of a Potent Truncated Form. *Biochemical Journal, 251*(1), 95-103.

Freeman, W.M., Walker, S.J., and Vrana, K.E. (1999). Quantitative RT PCR: pitfalls and potential. *Biotechniques, 26*(1), 112-122, 124-115.

French A, Ubeda-Tomas S, Holman TJ, Bennett MJ, Pridmore T (2009) High-throughput quantification of root growth using a novel image-analysis tool. *Plant Physiologyogy* 150: 1784–1795.

Fukao,T., Kennedy,R.A., Yamasue,Y., Rumpho,M.E. (2003).Genetic and biochemical analysis of anaerobically induced enzymes during seed germination of *Echinochloa crus-galli* varieties tolerance and intolerant of anoxia.*Journal of experimental biology*.*54*(386), 1421-1429.

Gallardo, K., Le Signor, C., Vandekerckhove, J., Thompson, R. D., and Burstin, J. (2003). Proteomics of Medicago truncatula seed development establishes the time frame of diverse metabolic processes related to reserve accumulation. *Plant Physiologyogy, 133*(2), 664-682.

Gao, M. J., Lydiate, D. J., Li, X., Lui, H., Gjetvaj, B., Hegedus, D. D., et al. (2009). Repression of Seed Maturation Genes by a Trihelix Transcriptional Repressor in Arabidopsis Seedlings.*Plant Cell, 21*(1), 54-71.

Gao, S., and Shain, L. (1995). Activity of Polygalacturonase Produced by *Cryphonectria parasitica* in Chestnut Bark and Its Inhibition by Extracts from American and Chinese Chestnut. *Physiological and Molecular Plant Pathology, 46*(3), 199-213.

Garcia-Olmedo, F., Molina, A., Segura, A., and Moreno, M. (1995).The defensive role of nonspecific lipid-transfer proteins in plants.*Trends in Microbiology, 3*(2), 72-74.

Gaudet, D.A., Wang, Y., Penniket, C., Lu, Z.X., Bakkeren, G., and Laroche, A. (2010).Morphological and molecular analyses of host and nonhost interactions involving barley and wheat and the covered smut pathogen *Ustilago hordei*.*Molecular Plant-Microbe Interactions, 23*(12), 1619-1634.

Ge, X., Chen, J., Li, N., Lin, Y., Sun, C., and Cao, K. (2003).Resistance function of rice lipid transfer protein LTP110. *The Journal of Steroid Biochemistry and Molecular Biology, 36*(6), 603-607.

Geddes, J., Eudes, F., Laroche, A., and Selinger, L.B. (2008).Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, *Hordeum vulgare*.*Proteomics, 8*(3), 545-554.

George,S., Parida,A. (2010).Characterization of an oxidative stress inducible nonspecific lipid transfer protein coding cDNA and its promoter from drought tolerant plant Prosopsis juliflora.*Plant Molecular Biology Reports, 28*(32), 32–40.

Glazener, J. A. (1992). Accumulation of Phenolic-Compounds in Cells and Formation of Lignin-Like Polymers in Cell-Walls of Young Tomato Fruits after Inoculation with Botrytis-Cinerea.*Physiological Plant Pathology, 20*(1), 11-25.

......P., Sy.D., Marion.D.,ader,J.C., Vovelle,F., Ptak,M. (1996). ......nding of a nonspecific lipid transfer protein extracted from maize seeds. *Protein Science ,5*(4) 565-577.

Gomathi, V. (2001).Studies on the antifungal effects of plant extracts on *Colletotrichum* spp. infecting *Capsicum annum* L. PhD thesis, Pondicherry University, Pondicherry.

Gomathi, V., and Gnanamanickam, S.S. (2004). Polygalacturonase-inhibiting proteins in plant defence. *Current Scienceence, 87*(9), 1211-1217.

Gomathi, V., Rajathilagam, R. and Kannabiran, B. (2001).*In-vitro* and *in-vivo* inhibition of pectinolytic enzymes in *Colletotrichum capsici* by plant extracts. National Symposium on Eco-friendly Approaches for Plant diseaseease Management, Chennai.

Gonzalez-Camacho, F. and Medina, F.J. (2007) *Methods Mol. Biol., 355*, 63-72.

Gonzalez-Fernandez, R., and Jorrin-Novo, J.V. (2012).Contribution of Proteomics to the Study of Plant Pathogenic Fungi.*Journal of Proteome Research, 11*(1), 3-16.

Gopal, J. (2001). The Development of Malaysia's Palm Oil Refining Industry: Obstacles, Policy and Performance, PhD. Thesis submitted to Imperial College, London.

Gorg, A. (1991). 2-Dimensional Electrophoresis.*Nature, 349*(6309), 545-546.

Gorg, A., Weiss, W., and Dunn, M. J. (2004).Current two-dimensional electrophoresis technology for proteomics.*Proteomics, 4*(12), 3665-3685.

Gotoh, Y., Nalumpang, S., Isshiki,A., Utsumi,T., Gomi,K., Yamamoto,H., Akimitsu,K.(2002).A cDNA encoding polygalacturonase inhibiting protein induced in citrus leaves by polygalacturonase of *Alternaria citri. General Plant Pathology*, (68), 57–61.

Grenville-Briggs, L. J., Avrova, A. O., Bruce, C. R., Williams, A., Whisson, S. C., Birch, P. R., et al. (2005). Elevated amino acid biosynthesis in *Phytophthora infestans* during appressorium formation and potato infection.*Fungal Genetics and Biology, 42*(3), 244-256.

Vignols, F., Garcia-Garrido, J.M., Lescot, M., Tharreau, D., ...thogen attack and developmental regulation of the rice Ltp1 gene.*Plant Molecular Biology, 49*(6), 683-699.

Gygi, S.P., Corthals, G.L., Zhang, Y., Rochon, Y., and Aebersold, R. (2000).Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology.*Proceedings of the National Academy of Scienceences U S A, 97*(17), 9390-9395.

Gygi, S.P., Rochon, Y., Franza, B.R. and Aebersold, R. (1999) *Molecular and Cellular Biology., 19,* 1720-30.

Harcharan, S. K. (1976). Oil Palm Industry: An Economic Study, Kuala Lumpur: University of Malaya Press.

Hale, J. E., Gelfanova, V., Ludwig, J. R., & Knierman, M. D. (2003). Application of proteomics for discovery of protein biomarkers.*Brief Funct Genomic Proteomic, 2*(3), 185-193.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. (2004).Trichoderma species - Opportunistic, avirulent plant symbionts.*Nature Reviews Microbiology, 2*(1), 43-56.

Hartley, C.W.S. (1988).*The Oil Palm.*Third Edition. Harlow, England: Longman.

Hashiguchi, A., Ahsan, N., Komatsu, S. (2010).Proteomics application of crops in the context of climate changes.*Food Research International43*(7), 1803–1813.

Hashimoto, M., Kisseleva, L., Sawa, S., Furukawa, T., Komatsu, S., and Koshiba, T. (2004). A novel rice PR10 protein, RSOsPR10, specifically induced in roots by biotic and abiotic stresses, possibly via the jasmonic acid signaling pathway. *Plant and Cell Physiology, 45*(5), 550-559.

Havaux, M., Ksas, B., Szewczyk, A., Rumeau, D., Franck, F., Caffarri, S., et al. (2009). Vitamin B6 deficient plants display increased sensitivity to high light and photo-oxidative stress. *Bmc Plant Biology, 9,*130.

J., and van Kan, J. A. L. (1998). The endopolygalacturonase
...all virulence of *Botrytis cinerea*. *Molecular Plant-Microbe Interactions, 11*(10), 1009-1016.

He,J., Chen,F., Chen,S., Lv,G., Deng,Y., Fang,Z., Guan,Z., He,C. (2011).Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation.*Journal of Plant Physiology 168*(7), 687–693.

Henson, I.E., and Mohd Tayed, D. (2003).Physiological analysis of an oil palm density trial on a peat soil. *Journal of Oil Palm Research,15*(2), 1-27.

Henson, I.E.(1999).Comparative ecophysiology of oil palm andtropical rain forest. In: Oil Palm and the Environment – A Malaysian Perspective. Eds. G. Singh, L. K. Huan, T. Leng, D. L. Kow, Malaysian Oil Palm Growers Council, Kuala Lumpur (Malaysia), pp. 9–39.

Herrero, S., Gonzalez, E., Gillikin, J.W., Velez, H., and Daub, M.E. (2011). Identification and characterization of a pyridoxal reductase involved in the vitamin B6 salvage pathway in Arabidopsis. *Plant Molecular Biology, 76*(1-2), 157-169.

Heukeshoven, J., and Dernick, R. (1988).Improved silver staining procedure for fast staining in PhastSystem Development Unit. I. Staining of sodium dodecyl sulfate gels. *Electrophoresis, 9*(1), 28-32.

Hisano, H., Nandakumar, R., and Wang, Z. Y. (2009).Genetic modification of lignin biosynthesis for improved biofuel production.*In Vitro Cellular and Developmental Biology-Plant, 45*(3), 306-313.

Hochholdinger, F., Woll, K., Guo, L., and Schnable, P. S. (2005). The accumulation of abundant soluble proteins changes early in the development of the primary roots of maize (*Zea mays* L.). *Proteomics, 5*(18), 4885-4893.

Hodenius, M., De Cuyper, M., Desender, L., Muller-Schulte, D., Steigel, A., and Lueken, H. (2002). Biotinylated Stealth magnetoliposomes.*Chemistry andPhysics of Lipids, 120*(1-2), 75-85.

Hoffmann-Sommergruber, K. (2002). Pathogenesis-related (PR)-proteins identified as allergens. *Biochemical Society Transactions, 30*, 930-935.

M. C., and Reski, R. (2002).Plant functional *, 89*(6), 235-249.

Howe,G.A., Jander,G. (2008).Plant immunity to herbivores.*Ann Rev Plant Biology(*59)*,*41–66.

Hu, D., Dai, R., Wang, Y., Zhang, Y., Liu, Z., Fang, R., et al. (2011). Molecular Cloning, Sequence Analysis, and Expression of the Polygalacturonase-inhibiting Protein (PGIP) Gene in Mulberry. Plant Molecular Biology Reporter, DOI: 10.1007/s11105-011-0324-3.

Hu,J Y., Zeng,Y., Sang,Y.Y. (2002). Two-dimensional polyacrylamide gel electrophoresis analysis of proteins from albino- green chimeric leaves of *Iris japonicaActa Botanica Yunnanica. 24*(3): 387–391.

Huang, Q., and Allen, C. (2000). Polygalacturonases are required for rapid colonization and full virulence of *Ralstonia solanacearum* on tomato plants. *Physiological and Molecular Plant Pathology, 57*(2), 77-83.

Huang, Y.M., Xiao, B.Z., and Xiong, L.Z. (2007).Characterization of a stress responsive proteinase inhibitor gene with positive effect in improving drought resistance in rice.*Planta, 226*(1), 73-85.

Hunter, T.C., Andon, N.L., Koller, A., Yates, J.R., and Haynes, P.A. (2002). The functional proteomics toolbox: methods and applications. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Scienceess, 782*(1-2), 165-181.

Hyun,T.K., Uddin,M.N., Rim,Y., Kim,J.Y. (2011).Cell-to-cell trafficking of RNA and RNA silencing through plasmodesmata. *Protoplasma 248*, 101-116.

Iandolino, A.B., da Silva, F. G., Lim, H., Choi, H., Williams, L. E., and Cook, D. R. (2004). High-quality RNA, cDNA, and derived EST libraries from grapevine (*Vitis vinifera* L.).*Plant Molecular Biology Reporter, 22*(3), 269-278.

Idris, A.S., Ismail, S., Ariffin, D., and Ahmad, H. (2003). *Control of Ganoderma-infected palm-development of pressure injection and field applications.*MPOB Information Series No. 131.

ne, R., and Watt, A. (2000). The identity of *Ganoderma* SR disease of oil palm in MalaysiaMorphological Characteristics.*Malaysian Palm OilBoard* 102: 77a.

Idris, S., KhusairI, D., Ariffin, D. and Basri, W. (2006). Technique for inoculation of oil palm germinated seeds with *Ganoderma. MPOB Information Series*, 314, pp. 1–4.

Imin, N., De Jong, F., Mathesius, U., van Noorden, G., Saeed, N. A., Wang, X. D., et al. (2004). Proteome reference maps of *Medicago truncatula* embryogenic cell cultures generated from single protoplasts. *Proteomics, 4*(7), 1883-1896.

Isshiki, A., Akimitsu, K., Yamamoto, M, Yamamoto H. (2001). Endopolygalacturonase is essential for citrus black rot caused by *Alternaria citri* but not brown spot caused by *Alternaria alternata.Molecular Plant-Microbe Interactions,14*(6):749-57.

Nur Ain Izzati, M.Z., Abdullah, F. (2008). Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum. Plant Protection Science 3,*101–107.

Jacobsen,S.E., Sakai, H., Finnegan,E.J., Cao, X., Meyerowitz,E.M.(2000). Ectopic hypermethylation of flower-specific genes in Arabidopsis.*Current Biology 10*, 179–186.

Jakobek, J. L., Smith, J. A., and Lindgren, P. B. (1993).Suppression of Bean Defense Responses by *Pseudomonas syringae.Plant Cell, 5*(1), 57-63.

Jaligot, E., Adler, S., Debladis, E., Beule, T., Richaud, F., Ilbert, P., et al. (2011). Epigenetic imbalance and the floral developmental abnormality of the in vitro-regenerated oil palm *Elaeis guineensis. Annals of Botany, 108*(8), 1453-1462.

Jang,S., Lee,B., Kim,C., Yim,J., Han,J., Lee,S., Kim,R., An,G. (2003). The OsFOR1 gene encodes a polygalacturonaseinhibiting protein (PGIP) that regulates Xoral organ number in rice. *Plant Molecular Biology, (53)*3,*357–369.

Janni, M., Sella, L., Favaron, F., Blechl, A. E., De Lorenzo, G., and D'Ovidio, R. (2008). The expression of a bean PGIP in transgenic wheat confers increased resistance to the fungal pathogen *Bipolaris sorokiniana. Molecular Plant-Microbe Interactions, 21*(2), 171-177.

...ting the protein language using proteomics.*Nature Reviews* ...391-403.

Jeun, Y.C., and Buchenauer, H. (2001).Infection structures and localization of the pathogenesis-related protein AP24 in leaves of tomato plants exhibiting systemic acquired resistance against Phytophthora infestans after pre-treatment with 3-aminobutyric acid or tobacco necrosis virus.*Journal of Phytopathology, 149*(3-4), 141-153.

Jeun, Y.-Ch. (2000). Immunolocalization of PR-protein P14 in leaves of tomato plant exhibiting systemic acquired resistance against *Phytophthora infestans* induced by pretreatment with 3-aminobutyric acid and preinoculation with Tobacco necrosis virus.*J.Plant disease. Prot* 107, 352-367.

Jiang, R. H. Y., Tripathy, S., Govers, F., and Tyler, B. M. (2008). RXLR effector reservoir in two Phytophthora species is dominated by a single rapidly evolving superfamily with more than 700 members. *Proceedings of the National Academy of Scienceences of the United States of America, 105*(12), 4874-4879.

Jiang, S., Park, P., and Ishii, H. (2008). Ultrastructural study on acibenzolar S-methyl-induced scab resistance in epidermal pectin layers of Japanese pear leaves. *Phytopathology, 98*(5), 585-591.

Jiang, Y., and Deyholos, M.K. (2006). Comprehensive transcriptional profiling of NaCl-stressed Arabidopsis roots reveals novel classes of responsive genes. *BMC Plant Biology, 6*, 25.

Johnston, D.J., Ramanathan, V. and Williamson, B. (1993).A protein from immature raspberry fruits which inhibits endopolygalacturonases from *Botrytis cinereai* and other microorganisms.*Journal of experimental biology, 44*(6) 971–976.

Jones, B. A., Hatfield, R.D., and Muck, R.E. (1995).Characterization of Proteolysis in Alfalfa and Red-Clover.*Crop Sciencne, 35*(2), 537-541.

Jorrin-Novo, J. V., Maldonado, A. M., Echevarria-Zomeno, S., Valledor, L., Castillejo, M. A., Curto, M., et al. (2009). Plant proteomics update (2007-2008): Second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge. *Journal of Proteomics, 72*(3), 285-314.

...s, V. L., Garrett, W. M., Whitaker, B. D., Janisiewicz, W. J., and Biochemical Characterization of Polygalacturonase Produced by *Penicillium expansum* During Postharvest Decay of 'Anjou' Pear.*Phytopathology, 100*(1), 42-48.

Kader, J. C. (1996). Lipid-Transfer Proteins in Plants.*Annual Review of Plant Physiology and Plant Molecular Biology, 47*, 627-654.

Kader, J. C. (1997). Lipid-transfer proteins: A puzzling family of plant proteins. *Trends in Plant Scienceence, 2*(2), 66-70.

Khan, A., Khan, A.U (2012). Biomarker Discovery and Drug Development: A Proteomics Approach. *Journal of Proteomics & Bioinformatics* 5: v-vi. doi:10.4172/jpb.10000e12

Kamoun, S. (2006).A catalogue of the effector secretome of plant pathogenic oomycetes.*Annual Review of Phytopathology, 44*, 41-60.

Kanneganti, T. D., Bai, X., Tsai, C. W., Win, J., Meulia, T., Goodin, M., et al. (2007). A functional genetic assay for nuclear trafficking in plants.*Plant Journal, 50*(1), 149-158.

Karas, M., and Hillenkamp, F. (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Analytical Chemistry, 60*(20), 2299-2301.

Karim,K.,Hatem,C.M.,Mokhtar,T., and Mbarek,B.N.(2011).Identification of salt stress-induced transcripts in barley leaves by differential display reverse transcriptase polymerase chain reaction (DDRT-PCR).*African Journal of Biotechnology,10*(83), 19356-19364.

Kater, M. M., Koningstein, G. M., Nijkamp, H. J. J., and Stuitje, A. R. (1991).cDNA Cloning and Expression of *Brassica-Napus* Enoyl-Acyl Carrier Protein Reductase in Escherichia-Coli. *Plant Molecular Biology, 17*(4), 895-909.

Kav, N. V. N., Srivastava, S., Yajima, W., and Sharma, N. (2007).Application of proteomics to investigate plant-microbe interactions.*Current Proteomics*, 4, 28–43.

Kawamura, Y., and Uemura, M. (2003). Mass spectrometric approach for identifying putative plasma membrane proteins of Arabidopsis leaves associated with cold acclimation. *Plant Journal, 36*(2), 141-154.

Kemp, G., Bergmann, C. W., Clay, R., Van der Westhuizen, A. J., and Pretorius, Z. A. (2003). Isolation of a polygalacturonase-inhibiting protein (PGIP) from wheat.*Molecular Plant-Microbe Interactions, 16*(11), 955-961.

Kersten, B., Burkle, L., Kuhn, E. J., Giavalisco, P., Konthur, Z., Lueking, A., et al. (2002). Large-scale plant proteomics.*Plant Molecular Biology, 48*(1-2), 133-141.

Khemvong, S., & Suvachittanont, W. (2005). Molecular cloning and expression of a cDNA encoding 1-deoxy-D-xylulose-5-phosphate synthase from oil palm *Elaeis guineensis* Jacq. *Plant Science, 169*(3), 571-578.

Khoo, K.M. (2001). Developing trends in the Malaysian palm oil industry.Proc.of the 2001 PIPOC International Palm Oil Congress – Economics and Marketing Conference.MPOB, Bangi.

Kiefer, E., Heller, W., and Ernst, D. (2000).A simple and efficient protocol for isolation of functional RNA from plant tissues rich in secondary metabolites.*Plant Molecular Biology Reporter, 18*(1), 33-39.

Kim, J. Y., Park, S. C., Hwang, I., Cheong, H., Nah, J. W., Hahm, K. S., et al. (2009). Protease inhibitors from plants with antimicrobial activity.*International Journal of Molecular Sciences, 10*(6), 2860-2872.

King, B. C., Waxman, K. D., Nenni, N. V., Walker, L. P., Bergstrom, G. C., and Gibson, D. M. (2011). Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnology for Biofuels, 4*.4.

Kloetzel, P. M., and Ossendorp, F. (2004). Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Current Opinion in Immunology, 16*(1), 76-81.

Koh, L.P., and Wilcove, D.S. (2007).Cashing in palm oil for conservation.*Nature, 448*(7157), 993-994.

Koh, L.P., and Wilcove, D.S. (2008). Is oil palm agriculture really destroying tropical biodiversity? *Conservation Letterers, 1*(2), 60-64.

...rtin, D., Encelot, N., and Martin, F. (2003). The poplar root ...0 expressed sequence tags. *Febs Letters, 542*(1-3), 37-41.

Koistinen,K.M., Hassinen,V.H., Gynther,P., Lehesranta,S.J., Keinanen,S.I., Kokko,H.I., et al. (2002). Birch PR-10c is induced by factors causing oxidative stress but appears not to confer tolerance to these agents. *New Phytology 155*(3), 381–391.

Koiwa,H., Bressan,R.A., Hasegawa,P.M.(1997). Regulation of protease inhibitors and plant defence.*Trends in Plant Science2*(10), 379–384.

Koltai, H., and Volpin, H. (2003). Agricultural Genomics: An Approach to Plant Protection. *European Journal of Plant Pathology, 109*(2), 101-108.

Komjanc, M., Festi, S., Rizzotti, L., Cattivelli, L., Cervone, F., and De Lorenzo, G. (1999). A leucine-rich repeat receptor-like protein kinase (LRPKm1) gene is induced in Malus x domestica by *Venturia inaequalis* infection and salicylic acid treatment. *Plant Molecular Biology, 40*(6), 945-957.

Kortstee, A.J., Appeldoorn, N.J. G., Oortwijn, M.E. P., and Visser, R.G.F. (2007).Differences in regulation of carbohydrate metabolism during early fruit development between domesticated tomato and two wild relatives.*Planta, 226*(4), 929-939.

Kovtun, Y., Chiu, W.L., Tena, G., and Sheen, J. (2000).Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants.*Proceedings of the National Academy of Scienceences of the United States of America, 97*(6), 2940-2945.

Kwasniewski, M., and Szarejko, I. (2006).Molecular cloning and characterization of beta expansin gene related to root hair formation in barley. *Plant Physiology, 141*(4), 1149-58.

Lance, C., and Rustin, P. (1984).The Central Role of Malate in Plant-Metabolism.*Physiologie Vegetale, 22*(5), 625-641.

Larson, T.R., Edgell, T., Byrne, J., Dehesh, K., and Graham, I.A. (2003).Acyl CoA profiles of transgenic plants that accumulate medium-chain fatty acids indicate inefficient storage lipid synthesis in developing oilseeds. *Plant Journalournal, 32*(4), 519-527.

...., Cardinali,A., (2006).Role of phenolics in the resistance .... fungal pathogens and insects. Phytochemistry: *Advances in Research*. Kerala, India, Research Signpost: 23–67.

Laurance, W.F. (2003). Slow burn: the insidious effects of surface fires on tropical forests. *Trends in Ecology and Evolution, 18*(5): 209–212.

Le Provost, G., Herrera, R., Paiva, J. A., Chaumeil, P., Salin, F., and Plomion, C. (2007).A micromethod for high throughput RNA extraction in forest trees.*Biological Research, 40*(3), 291-297.

Leung, D.W.M. (1992). Involvement of Plant Chitinase in Sexual Reproduction of Higher-Plants.*Phytochemistry, 31*(6), 1899-1900.

Li, C.W., Bonnema, G., Che, D., Dong, L., Lindhout, P., Visser, R., et al. (2007). Biochemical and molecular mechanisms involved in monogenic resistance responses to tomato powdery mildew. *Molecular Plant-Microbe Interactions, 20*(9), 1161-1172.

Lin,W., Li,Z. (2002). The partial structure of wheat polygalacturonase- inhibiting protein.*Chinese Journal of Biochemistry and Molecular Biology, 2* (18), 197–201.

Liu, J. J., Ekramoddoullah, A.K.M., and Yu, X.S. (2003).Differential expression of multiple PR10 proteins in western white pine following wounding, fungal infection and cold-hardening.*Physiologia Plantarum, 119*(4), 544-553.

Liu, X., Huang, B., Lin, J., Fei, J., Chen, Z., Pang, Y., et al. (2006). A novel pathogenesis-related protein (SsPR10) from *Solanum surattense* with ribonucleolytic and antimicrobial activity is stress- and pathogen-inducible. *Journal of Plant Physiology, 163*(5), 546-556.

Liu, Y., Lamkemeyer, T., Jakob, A., Mi, G., Zhang, F., Nordheim, A., et al. (2006). Comparative proteome analyses of maize (*Zea mays* L.) primary roots prior to lateral root initiation reveal differential protein expression in the lateral root initiation mutant rum1. *Proteomics, 6*(15), 4300-4308.

Liu,Q., Kasuga,M., Sakuma,Y., Abe,H., Miura,S., Yamaguchi-Shinozaki,K., Shinozaki,K. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 binding domain separate two signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. *The Plant Cell, 10*(8), 1391–1406.

d Nicholson, R.L. (1999). cDNA cloning of a sorghum PR-10) and differential expression of defense-related genes following inoculation with Cochliobolus heterostrophus or *Colletotrichum sublineolum*. *Molecular Plant-Microbe Interactions, 12*(6), 479-489.

Lotan, T., Ori, N., and Fluhr, R. (1989). Pathogenesis-Related Proteins Are Developmentally Regulated in Tobacco Flowers. *Plant Cell, 1*(9), 881-887.

Lucas, J.A. (2004). Survival, surfaces and susceptibility - the sensory biology of pathogens. *Plant Pathology, 53*(6), 679-691.

Lyngved, R., Renaut, J., Hausman, J. F., Iversen, T. H., and Hvoslef-Eide, A. K. (2008). Embryo-specific Proteins in Cyclamen persicum Analyzed with 2-D DIGE. *Journal of Plant Growth Regulation, 27*(4), 353-369.

Mackintosh, C.A., Lewis, J., Radmer, L.E., Shin, S., Heinen, S.J., Smith, L.A., et al. (2007). Overexpression of defense response genes in transgenic wheat enhances resistance to *Fusarium* head blight. *Plant Cell Reporter, 26*(4), 479-488.

Mahalingam, R., Wang, G.J., and Knap, H.T. (1999). Polygalacturonase and polygalacturonase inhibitor protein: Gene isolation and transcription in Glycine max - Heterodera glycines interactions. *Molecular Plant-Microbe Interactions, 12*(6), 490-498.

Mahmud,A. (2009). The use of micro filter recovered palm oil mill effluent (pome) sludge as fish feed ingredient. Master's thesis, Universiti Sains Malaysia, Penang.

Maizura, I., Rajanaidu, N., Zakri, A.H., and Cheah, S.C. (2006). Assessment of genetic diversity in oil palm (*Elaeis guineensis* Jacq.) using Restriction Fragment Length Polymorphism (RFLP). *Genetic Resources and Crop Evolution, 53*(1), 187-195.

Maizura,I. (1999). Genetic variability of oil palm (*Elaeis guineensis* Jacq.) germplasm collection using RFLP markers. *PhD thesis*, Universiti Kebangsaan Malaysia.

Markom MA, Shakaff AYM, Adom AH, Ahmad MN, Hidayat W, Abdullah AH, Fikri NA, (2009). Intelligent electronic nose system for basal stem rot disease detection. *Computer Electronics Agriculture, 66* (2):140-146

..., Dixon, R.A., Lamb, C.J., and Cameron, R.K. (2002). A ... involved in systemic resistance signalling in Arabidopsis. *Nature, 419*(6905), 399-403.

Maleck, K., and Dietrich, R.A. (1999). Defense on multiple fronts: How do plants cope with diverse enemies? *Trends in Plant Science, 4*(6), 215-219.

Manfredini, C., Sicilia, F., Ferrari, S., Pontiggia, D., Salvi, G., Caprari, C., et al. (2005). Polygalacturonase-inhibiting protein 2 of *Phaseolus vulgaris* inhibits BcPG1, a polygalacturonase of *Botrytis cinerea* important for pathogenicity, and protects transgenic plants from infection. *Physiological and Molecular Plant Pathology, 67*(2), 108-115.

Maor, R., Jones, A., Nuhse, T. S., Studholme, D. J., Peck, S. C., and Shirasu, K. (2007).Multidimensional protein identification technology (MudPIT) analysis of ubiquitinated proteins in plants.*Molecular and Cellular Proteomics, 6*(4), 601-610.

Marra, R., Ambrosino, P., Carbone, V., Vinale, F., Woo, S. L., Ruocco, M., et al. (2006). Study of the three-way interaction between Trichoderma atroviride, plant and fungal pathogens by using a proteomic approach.*Current Genetics, 50*(5), 307-321.

Massengo-Tiasse, R. P., and Cronan, J. E. (2009).Diversity in enoyl-acyl carrier protein reductases.*Cellular and Molecular Life Sciences, 66*(9), 1507-1517.

Mayes,S., Hafeez,F., Price,Z., MacDonald,D., Billotte,N., Roberts,J. (2008). Molecular research in oil palm, the key oil crop for the future. In: Moore PH, Ming R. eds. Genomics of Tropical Crop Plants. New York, NY: Springer, 371–404.

McCarty, R.E., Evron, Y., and Johnson, E.A. (2000). The chloroplast ATP synthase: A Rotary Enzyme? *Annu Rev Plant Physiology Plant Mol Biol, 51*, 83-109.

McDowell, J.M., and Dangl, J.L. (2000). Signal transduction in the plant immune response. *Trends Biochem Science, 25*(2), 79-82.

Mechin, V., Thevenot, C., Le Guilloux, M., Prioul, J.L., and Damerval, C. (2007). Developmental analysis of maize endosperm proteome suggests a pivotal role for pyruvate orthophosphate dikinase. *Plant Physiologyogy, 143*(3), 1203-1219.

me,A.L. (1994). The advantages and versatility of a high-
sociation-based strategy for the sequence and structural
determination of proteins.*Methods: A comparison to Methods in Enzymology* 6,284–303.

Mehta, A., Brasileiro, A.C.M., Souza, D.S.L., Romano, E., Campos, M.A., Grossi-De-Sa, M.F., et al. (2008). Plant-pathogen interactions: what is proteomics telling us? *Febs Journal, 275*(15), 3731-3746.

Meisel, L., Fonseca, B., Gonzalez, S., Baeza-Yates, R., Cambiazo, V., Campos, R., et al. (2005).A rapid and efficient method for purifying high quality total RNA from peaches (*Prunus persica*) for functional genomics analyses.*Biological Research, 38*(1), 83-88.

Melo, G.A., Shimizu, M.M., and Mazzafera, P. (2006). Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry, 67*(3), 277-285.

Meskiene, I., Ligterink, W., Bogre, L., Jonak, C., Kiegerl, S., Balog, J., et al. (1998). The SAM kinase pathway: An integrated circuit for stress signaling in plants. *Journal of Plant Research, 111*(1102), 339-344.

Miernyk, J.A., and Dennis, D.T. (1982).Isozymes of the Glycolytic-Enzymes in Endosperm from Developing Castor-Oil Seeds.*Plant Physiologyogy, 69*(4), 825-828.

Miller, R.N.G., Holderness, M., Bridge, P.D., Chung, G.F., and Zakaria, M.H. (1999).Genetic diversity of *Ganoderma* in oil palm plantings.*Plant Pathology, 48*(5), 595-603.

Miles, L.A., Dahlberg, C.M., PleSciencea, J., Felez, J., Kato, K., and Plow, E.F. (1991).Role of Cell-Surface Lysines in Plasminogen Binding to Cells - Identification of Alpha-Enolase as a Candidate Plasminogen Receptor.*Biochemistry, 30*(6), 1682-1691.

Mitprasat, M., Roytrakul, S., Jiemsup, S., Boonseng, O., and Yokthongwattana, K. (2011).Leaf proteomic analysis in cassava (*Manihot esculenta*, Crantz) during plant development, from planting of stem cutting to storage root formation.*Planta, 233*(6), 1209-1221.

enson, I.H. (2004) "Oil Palm – Achievements and Potential" *ational Crop Scienceence Congress*, 26 Sep – 1 Oct 2004, Brisbane, Australia.

Moldenhauer, J., Moerschbacher, B. M., and Van der Westhuizen, A. J. (2006). Histological investigation of stripe rust (*Puccinia striiformis* f.sp tritici) development in resistant and susceptible wheat cultivars. *Plant Pathology, 55*(4), 469-474.

Mooney, B.P., Miernyk, J.A., Greenlief, C.M. and Thelen, J.J. (2006) *Physiologia Plantarum*, *128*, 237-250.

Mooney, R.A., Davis, S.E., Peters, J.M., Rowland, J.L., Ansari, A. Z., and Landick, R. (2009). Regulator trafficking on bacterial transcription units in vivo. *Mol Cell, 33*(1), 97-108.

Morcillo, F., Gagneur, C., Adam, H., Richaud, F., Singh, R., Cheah, S. C., et al. (2006). Somaclonal variation in micropropagated oil palm. Characterization of two novel genes with enhanced expression in epigenetically abnormal cell lines and in response to auxin. *Tree Physiology, 26*(5), 585-594.

Mou, Z.L., He, Y.K., Dai, Y., Liu, X.F., and Li, J.Y. (2000). Deficiency in fatty acid synthase leads to premature cell death and dramatic alterations in plant morphology. *Plant Cell, 12*(3), 405-417.

Mouchel, C.F., Osmont, K.S., and Hardtke, C.S. (2006). BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature, 443*(7110), 458-461.

MPOB and APOC, (2010), Palm oil development and performance in Malaysia, presentation to USITC, Washington DC, accessible at: http://www.americanpalmoil.com/pdf/USITCpre-PublicHearing-V2.pdf, last accessed 28 February, 2010.

Müller, O., Schreier, P., and Uhrig, J. (2008). Identification and characterization of secreted and pathogenesis-related proteins in *Ustilago maydis*. *Molecular Genetics and Genomics*, 279(1), 27-39.

Murad, A. M., Laumann, R. A., Lima, T. D., Sarmento, R. B. C., Noronha, E. F., Rocha, T. L., et al. (2006). Screening of entomopathogenic *Metarhizium anisopliae* isolates and proteomic analysis of secretion synthesized in response to cowpea weevil (*Callosobruchus*

omparative Biochemistry and Physiology C-Toxicology 65-370.

Murad, A.M., Laumann, R.A., Mehta, A., Noronha, E. F., and Franco, O.L. (2007). Screening and secretomic analysis of enthornopatogenic *Beauveria bassiana* isolates in response to cowpea weevil (*Callosobruchus maculatus*) exoskeleton. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology, 145*(3), 333-338.

Natarajan, S., Xu, C.P., Caperna, T.J., and Garrett, W.A. (2005).Comparison of protein solubilization methods suitable for proteomic analysis of soybean seed proteins.*Analytical Biochemistry, 342*(2), 214-220.

Niture, S.K. (2008). Comparative biochemical and structural characterizations of fungal polygalacturonases.*Biologia, 63*(1), 1-19.

Nodichao, L., Chopart, J. L., Roupsard, O., Vauclin, M., Ake, S., and Jourdan, C. (2011).Genotypic variability of oil palm root system distribution in the field.Consequences for water uptake.*Plant and Soil, 341*(1-2), 505-520.

Norton, G. (1991). Proteinase inhibitors. In J. P. F. D' Mello, C. M. Duffus, and J. H. Duffus (Eds.), Toxic substances in crop plants (pp. 68–106). Cambridge: The Royal Society of Chemistry.

Oeser,B., Heidrichm,P.M., Muller,U., Tudzynski,P., Tenberge,K.B. (2002). Polygalacturonase is a pathogenicity factor in the Claviceps purpurea/rye interaction. *Fungal Genetics and Biology,36*(3), 176–186.

O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins.*The Journal of Biological Chemistry, 250*(10), 4007-4021.

Ornstein, D.K., and Petricoin, E.F. (2004).Proteomics to diagnose human tumors and provide prognostic information.*Oncology (Williston Park), 18*(4), 521-529; discussion 529-532.

Ostergaard, O., Finnie, C., Laugesen, S., Roepstorff, P., and Svensson, B. (2004). Proteome analysis of barley seeds: Identification of major proteins from two-dimensional gels (pl 4-7). *Proteomics, 4*(8), 2437-2447.

..., M. (2000).Proteomics to study genes and ...37-846.

Park, C.J., Shin, R., Park, J.M., Lee, G.J., You, J.S., and Paek, K.H. (2002). Induction of pepper cDNA encoding a lipid transfer protein during the resistance response to tobacco mosaic virus. *Plant Molecular Biology, 48*(3), 243-254.

Park, C.J., Kim, K.J., Shin, R., Park, J.M., Shin, Y.C. and Paek, K.H. (2004).Pathogenesis-related protein 10 isolated from hot pepper functions as a ribonuclease in an antiviral pathway. *Plant Journal, 37*(2) 186–198.

Paterson, R.R.M. (2007). *Ganoderma* disease of oil palm - A white rot perspective necessary for integrated control.*Crop Protection, 26*(9), 1369-1376.

Paterson, R.R.M., Holderness, M., Kelley, J., Miller, R., O'Grady, E. (2000). In vitro diodegradation of oil-palm stem using macroscopic fungi from South East Asia: a preliminary investigation. In: Flood, J., Bridge, P.D., Holderness, M. (Eds.), *Ganoderma* Diseases of PerennialCrops. CABI Publishing, Wallingford, UK, pp. 129–138.

Patil, S.S., and Dimond, A.E. (1967).Inhibition of Verticillium Polygalacturonase by Oxidation Products of Polyphenols.*Phytopathology, 57*(5), 492-496.

Paul, K.S., Bacchi, C.J., and Englund, P.T. (2004). Multiple triclosan targets in Trypanosoma brucei. *Eukaryot Cell, 3*(4), 855-861.

Pedras, M.S., Zaharia, I.L., Gai, Y., Zhou, Y., and Ward, D.E. (2001). In planta sequential hydroxylation and glycosylation of a fungal phytotoxin: Avoiding cell death and overcoming the fungal invader. *Proceedings of the National Academy of Scienceences U S A, 98*(2), 747-752.

Peltier, J.B., Friso, G., Kalume, D.E., Roepstorff, P., Nilsson, F., Adamska, I., et al. (2000). Proteomics of the chloroplast: systematic identification and targeting analysis of lumenal and peripheral thylakoid proteins. *Plant Cell, 12*(3), 319-341.

Phizicky, E., Bastiaens, P. I., Zhu, H., Snyder, M., and Fields, S. (2003). Protein analysis on a proteomic scale.*Nature, 422*(6928), 208-215.

Mueller,L.N., Domon,B., Aebersold,R.(2009).Full dynamic ...cerevisiae by targetedproteomics.*Cell,138*(4)795-806.

Pilotti, C.A. (2005). Stem rots of oil palm caused by Ganoderma boninense: pathogen biology and epidemiology. *Mycopathologia, 159*(1), 129-137.

Pilotti, C.A., Sanderson, F.R., and Aitken, E.A.B. (2003).Genetic structure of a population of *Ganoderma boninense* on oil palm.*Plant Pathology, 52*(4), 455-463.

Pincon, G., Chabannes, M., Lapierre, C., Pollet, B., Ruel, K., Joseleau, J. P., et al. (2001).Simultaneous down-regulation of caffeic/5-hydroxy ferulic acid-O-methyltransferase I and cinnamoyl-coenzyme a reductase in the progeny from a cross between tobacco lines homozygous for each transgene.Consequences for plant development and lignin synthesis.*Plant Physiologyogy, 126*(1), 145-155.

Piquemal, J., Lapierre, C., Myton, K., O'Connell, A., Schuch, W., Grima-Pettenati, J., et al. (1998). Down-regulation of cinnamoyl-CoA reductase induces significant changes of lignin profiles in transgenic tobacco plants. *Plant Journalournal, 13*(1), 71-83.

Powell, A. L., van Kan, J., ten Have, A., Visser, J., Greve, L. C., Bennett, A. B., et al. (2000). Transgenic expression of pear PGIP in tomato limits fungal colonization. *Molecular Plant-Microbe Interactions, 13*(9), 942-950.

Przymusinski, R., Rucinska, R., and Gwozdz, E.A. (2004).Increased accumulation of pathogenesis-related proteins in response of lupine roots to various abiotic stresses.*Environmental and Experimental Botany, 52*(1), 53-61.

Pyee,J., Yu,H., Kolattukudy,P.E. (1994). Indentification of lipid transfer protein as the maijor protein in the surface wax of broccoli (*Brassuca olearcea*) leaves. *Arch Biochem Biophys 311*(2),460-468.

Qin, X.Y., Liu, Y., Mao, S.J., Li, T.B., Wu, H.K., Chu, C.C., and Wang,Y.P.(2011).Genetic transformation of lipid transfer protein encoding gene in *Phalaenopsis amabilis* to enhance cold resistance.*Euphytica, 177* (1), 33–43.

Raja,N., Albert,S., Babu,A., Ignacimuthu,S., Dorn,S. (2000). Role of botanical protectants and larval parasitoid *Dinarmus vagabundus* (Timberlake) (Hymenoptera: Pteromalidae)

latus Fab. (Coleoptera: Bruchidae) infesting cowpea seeds. (1-2):55–60.

Rajanaidu,N., and Jalani,B.S. (1994a). Germplasm collection in Gambia. ISOPB *Newsletterer 10*(2), 1-16.

Rajanaidu,N., and Jalani,B.S. (1994b). Oil palm genetic resources – collection, evaluation, utilization and conservation. A paper presented at PORIM Colloquium on Oil Palm Genetic Resources. PORIM, Malaysia 13 September 1994.

Rampitsch, C., Bykova, N.V., McCallum, B., Beimcik, E., andEns, W. (2006). Analysis of the wheat and *Puccinia triticina* (leaf rust) proteomes during a susceptible host-pathogen interaction.*Proteomics, 6*(6), 1897-1907.

Rasiah,R. (2006). Explaining Malaysia s Export Expansion in Palm Oil and Related Products , Vandana Chandra (ed), *The How and the Why of Technology Development in Developing Economies*, Washington DC: World Bank, pp 163-192.

Rathna Kumar,A.L., Balasubramanian,P. (2000). Induct ion of phenols in groundnut rust resistance. *Int Arach Newslet,* (20), 55–57.

Ravigadevi, S., Mohamad Arif, A.M., and Abrizah, O. (2005) Molecular/Biotechnological approach towards understanding Ganoderma. Paper presented at the *MPOB* Workshop on Prioritizing Ganoderma Research in Oil Palm. 28 March. Extended Abstract 20 pages.Hotel Equatorial Bangi.

Rees, R. W.,Flood, J., Hasan, Y., Potter, U., and Cooper, R.M. (2009).Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense.Plant Pathology, 58*(5), 982-989.

Rees,R., (2006). *Ganoderma* stem rot of oil palm *(Elaeis guineensi*s); mode of infection, epidemiology and biological control.PhD thesis, University of Bath, UK.

Ricardo, C. (2006). Oil Palm. From Cosmetics to Biodiesel - Colonization Lives On", retrieved 18 March 2010 from: http://www.wrm.org.uy/plantations/material.

Ricardo,C. (2006).Oil Palm.From Cosmetics to Biodiesel - Colonization Lives On.pp.6-8.

B., and Hadwiger, L.A. (1985). The Disease Resistance
d with Increased Levels of Specific Messenger-Rnas. *Plant Molecular Biology, 4*(2-3), 81-86.

Rinaldi, C., Kohler, A., Frey, P., Duchaussoy, F., Ningre, N., Couloux, A., et al. (2007). Transcript profiling of poplar leaves upon infection with compatible and incompatible strains of the foliar rust *Melampsora larici* populina. *Plant Physiologyogy, 144*(1), 347-366.

Rode, C., Gallien, S., Heintz, D., Van Dorsselaer, A., Braun, H.P., and Winkelmann, T. (2011). Enolases: storage compounds in seeds? Evidence from a proteomic comparison of zygotic and somatic embryos of *Cyclamen persicum* Mill.*Plant Molecular Biology, 75*(3), 305-319.

Rojo, E., Leon, J., and Sanchez-Serrano, J.J. (1999). Cross-talk between wound signalling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. *Plant Journal, 20*(2), 135-142.

Rolph, H., Wijesekara, R., Lardner, R., Abdullah, F., Kirk, P.M., Holderness, M., Bridge, P.D., Flood, J. (2000). Molecular variation in Ganoderma from oil palm, coconut and betelnut. In: Flood, J., Bridge, P.D., Holderness, M. (Eds.), Ganoderma Diseases of Perennial Crops. CABI Publishing, Wallingford, UK, pp. 205–221.

Rouquie, D., Peltier, J. B., MarquisMansion, M., Tournaire, C., Doumas, P., and Rossignol, M. (1997).Construction of a directory of tobacco plasma membrane proteins by combined two-dimensional gel electrophoresis and protein sequencing.*Electrophoresis, 18*(3-4), 654-660.

Ruiz-Rivero, O.J., and Prat, S., (1998). A –308 deletion of the tomato LAP promoters is able to direct flowerspecific and MeJAinduced expression in transgenic plants. *Plant Molecular Biology 36*(5), 639–648.

Ryu, H.S., Han, M., Lee, S. K., Cho, J.I., Ryoo, N., Heu, S., et al. (2006). A comprehensive expression analysis of the WRKY gene superfamily in rice plants during defense response. *Plant Cell Report, 25*(8), 836-847.

Ryu, H. S., Song, M. Y., Kim, C. Y., Han, M. H., Lee, S. K., Ryoo, N., et al. (2009). Proteomic analysis of rice mutants susceptible to *Magnaporthe oryzae.Plant Biotechnology Reports, 3*(2), 167-174.

Salekdeh,G.H., Siopongco,G., Wade,L.J., Ghareyazie,B., Bennett,J. (2002) Proteomic analysis of rice leaves during drought stress and recovery. *Proteomics, 2*(9)1131–1145.

Salvi, G., Giarrizzo, F., Delorenzo, G., and Cervone, F. (1990). A Polygalacturonase-Inhibiting Protein in the Flowers of *Phaseolus vulgaris* L. *Journal of Plant Physiologyogy, 136*(5), 513-518.

Samanani, N., Liscombe, D.K.,and Facchini, P.J. (2004).Molecular cloning and characterization of norcoclaurine synthase, an enzyme catalyzing the first committed step in benzylisoquinoline alkaloid biosynthesis.*Plant Journal, 40*(2), 302-313.

Sambrook ,J., Fritsch,E.F., Maniatis,T. (1989). *Molecular cloning: A laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sambrook,J., Russell,D.W., and Sambrook,J. (2001).*Molecular Cloning.A Laboratory Manual*, 3rd ed. Cold Spring Harbor, Cold Spring Harbor, New York, USA.

Sangha, J. S., GU, K., Kaur, J., and Yin, Z. (2010).An improved method for RNA isolation and cDNA library construction from immature seeds of *Jatropha curcas* L. *BMC Research Notes, 3*, 126.

Santoni, V., Kieffer, S., Desclaux, D., Masson, F., and Rabilloud, T. (2000). Membrane proteomics: Use of additive main effects with multiplicative interaction model to classify plasma membrane proteins according to their solubility and electrophoretic properties. *Electrophoresis, 21*(16), 3329-3344.

Santoni, V., Rouquie, D., Doumas, P., Mansion, M., Boutry, M., Degand, H., et al. (1998).Use of a proteome strategy for tagging proteins present at the plasma membrane.*Plant Journalournal, 16*(5), 633-641.

Santoso, H., Gunawan, T., Jatmiko, R. H., Darmosarkoro, W., and Minasny, B. (2011). Mapping and identifying basal stem rot disease in oil palms in North Sumatra with QuickBird imagery. *Precision Agriculture, 12*(2), 233-248.

J.K.C. (2004).A critical evaluation of sample extraction …omic analysis of recalcitrant plant tissues.*Proteomics, 4*(9), 2522-2532.

Sarowar,S.,Kim,Y.J.,Kim,K.D.,Hwang,B.K.,Ok,H.S.,Shin,J.S. (2009). Overexpression of lipid transfer protein (LTP) genes enhances resistance to plant pathogens and LTP functions in long-distance systemic signaling in tobacco.*Plant Cell Rep Report, 28*(3) 419–427.

Scheibe, R. (2004). Malate valves to balance cellular energy supply. *Physiologia Plantarum, 120*(1), 21-26.

Scheres, B., Benfey, P., and Dolan,L. (2002) In *The Arabidopsis book* (Somerville, C.R. and Meyerowitz, E.M. Eds) Rockville, MD:American Society of Plant Biologyogists.

Schlink, K. (2010). Down-regulation of defense genes and resource allocation into infected roots as factors for compatibility between *Fagus sylvatica* and *Phytophthora citricola*. *Functional and Integrative Genomics, 10*(2), 253-264.

Segura,A., Moreno,M., Garcia-Olmedo,F.(1993).Purification and antipathogeneic activity pf lipid transfer protein (LTPs) from the leaves of Arabidopsis and spinach. *FEBS Letter, 332*,(3) 243-246.

Sekine, K.T., Ishihara, T., Hase, S., Kusano, T., Shah, J., and Takahashi, H. (2006). Single amino acid alterations in *Arabidopsis thaliana* RCY1 compromise resistance to Cucumber mosaic virus, but differentially suppress hypersensitive response-like cell death. *Plant Molecular Biology, 62*(4-5), 669-682.

Sella, L., Castiglioni,C., Roberti,S., D'Ovidio,R., Favaron,F. (2004). An endo-polygalacturonase (PG) of *Fusarium moniliforme* escaping inhibition by plant polygalacturonase-inhibiting proteins (PGIPs) provides new insights into the PG-PGIP interaction. *FEMS Microbiol.Letter. 240*(1), 117-124.

Sengupta, D., Kannan,M., Reddy,A.R.(2011).A root proteomics-based insight reveals dynamic regulation of root proteins under progressive drought stress and recovery in *Vigna radiata* (L.) Wilczek.*Planta, 233*(6) 1111-27.

Rao, D.M. (2009).Morphological and chemical components *icoverpa armigera* in wild relatives of pigeonpea.*Arthropod-Plant Interactions, 3*(3), 151-161.

Sharma, R., Tan, F., Jung, K.H., Sharma, M.K., Peng, Z., and Ronald, P.C. (2011). Transcriptional dynamics during cell wall removal and regeneration reveals key genes involved in cell wall development in rice. *Plant Molecular Biology, 77*(4-5), 391-406.

Sheffield, J., Taylor, N., Fauquet, C., and Chen, S. (2006). The cassava (*Manihot esculenta* Crantz) root proteome: protein identification and differential expression. *Proteomics, 6*(5), 1588-1598.

Shevchenko, A., Jensen, O.N., Podtelejnikov, A.V., Sagliocco, F., Wilm, M., Vorm, O., et al. (1996).Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels.*Proceedings of the National Academy of Scienceences U S A, 93*(25), 14440-14445.

Shibayama, M., Kazuhiro, O., Johnson, R., Scott, B., and Itoh, Y. (2002).Suppression of tandem-multimer formation during genetic transformation of the mycotoxin-producing fungus *Penicillium paxilli* by disrupting an orthologue of *Aspergillus nidulans* uvsC.*Current Genetics, 42*(1), 59-65.

Shimizu, M., Yuda, N., Nakamura, T., Tanaka, H., and Wariishi, H. (2005). Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycete *Phanerochaete chrysosporium* against exogenous addition of vanillin. *Proteomics, 5*(15), 3919-3931.

Shin,D.H., Lee,J.Y., Hwang,K.Y., Kim,K.K., Huh,S.W. (1995).High-resolution crystal structure of the nonspecific lipid-transfer protein from maize seedlings.*Structure, 3*(2)189-199.

Singh, R., Ibrahim, M., Esa, N., and Iliyana, M. (2010).Composting of waste from palm oil mill: a sustainable waste management practice.*Reviews in Environmental Scienceence and Biotechnology*, 9(4), 331-344.

Skylas,D.J., Mackintosh,J.A., Cordwell,S,J., Basseal,D.J., Walsh,B.J., Harry ,J., et al. (2000). Proteome approach to the characterization of protein composition in the developing and mature wheat-grain endosperm.*Journal of Cereal Scienceence,32*(2), 169–18.

..., S., Back, K., Yoon, J.B., Park, H.G., et al. (2012). ...ogenesis-related protein 10 (PR10) genes between fungal resistant and susceptible peppers.*European Journal of Plant Pathology, 132*(1), 37-48.

Somssich, I.E., Schmelzer, E., Bollmann, J., and Hahlbrock, K. (1986).Rapid activation by fungal elicitor of genes encoding "pathogenesis-related" proteins in cultured parsley cells.*Proceedings of the National Academy of Scienceences U S A, 83*(8), 2427-2430.

Somssich, I.E., Schmelzer, E., Kawalleck, P., and Hahlbrock, K. (1988).Gene structure and in situ transcript localization of pathogenesis-related protein 1 in parsley.*Moecularl Genetics, 213*(1), 93-98.

Song, Y., Zhang, H., Wang, G., & Shen, Z. (2012). DMSO, an Organic Cleanup Solvent for TCA/Acetone-Precipitated Proteins, Improves 2-DE Protein Analysis of Rice Roots. *Plant Molecular Biology Reporter*, 1-6.

Sossountzov, L., Ruiz-Avila, L., Vignols, F., Jolliot, A., Arondel, V., Tchang, F., et al. (1991). Spatial and temporal expression of a maize lipid transfer protein gene. *Plant Cell, 3*(9), 923-933.

Srivastava, S., Rahman, M. H., Shah, S., and Kav, N. N. V. (2006a). Constitutive expression of the pea ABA-responsive 17 (ABR17) cDNA confers multiple stress tolerance in *Arabidopsis thaliana*. *Plant Biotechnology Journal, 4*(5), 529-549.

Srivastava, S., Emery, R.J.N., Kurepin, L.V., Reid, D.M., Fristensky, B., and Kav, N.N.V. (2006b). Pea PR 10.1 is a ribonuclease and its transgenic expression elevates cytokinin levels. *Plant Growth Regulation, 49*(1), 17-25.

Steenblik,R.(September 2007).*Biofuels — At What Cost?Government support for ethanol and biodiesel in selected OECD countries.*31 January 2012.From, http://www.globalsubsidies.org/files/assets/oecdbiofuels.pdf.

Steyaert, R.L. (1975). *Ganoderma boninense.*CMI descriptions of pathogenic fungi and bacteria No. 444.Commonwealth Mycological Institute.

Stotz, H.U., Contos, J.J., Powell, A.L., Bennett, A.B., and Labavitch, J.M. (1994).Structure and expression of an inhibitor of fungal polygalacturonases from tomato.*Plant Molecular Biology, 25*(4), 607-617.

Strange, R.N., and Scott, P.R. (2005).Plant diseaseease: A threat to global food security.*Annual Review of Phytopathology, 43*, 83-116.

Suarez, M.B., Sanz, L., Chamorro, M.I., Rey, M., Gonzalez, F.J., Llobell, A., et al. (2005).Proteomic analysis of secreted proteins from *Trichoderma harzianum*.Identification of a fungal cell wall-induced aspartic protease.*Fungal Genetics and Biology, 42*(11), 924-934.

Subhankar, R. B., Sautter, C., Chattoo, B. B., and Sautter, C. (2006). Expression of the lipid transfer protein Ace-AMP1 in transgenic wheat enhances antifungal activity and defense responses. *Transgenic Research, 15*(4), 435-446.

Sun, J.Y.,Gaudet,A.,Lu,Z,X.,Frick, M., Puchalski, B., andLaroche, A. (2008).Characterization and Antifungal Properties of Wheat Nonspecific Lipid Transfer Proteins.*Molecular Plant-Microbe interaction, 21*(3), 346-360.

Susanto, A., Sudharto, P.S., and Purba, R.Y. (2005). Enhancing biological control of basal stem rot disease (andlt; I and gt;*Ganoderma boninense* and lt;/I and gt;) in oil palm plantations. *Mycopathologia, 159*(1), 153-157.

Syed, R.A., Law, I.H. and Corley, R.H.V. (1982). Insect pollination of oil palm: Introduction , establishment and pollinating efficiency of *Elaeidobius kamerunicus* in Malaysia. *The Planter,* Kuala Lumpur. 58:547.

Tahiri-Alaoui, A., Dumas, E., Gianinazzi, S. (1990). Detection of PR-b proteins in tobacco roots infected with Chalara elegans. *Plant Molecular Biology, 14*(5), 869-871.

Takagi, M., Shimomura, S., and Fukui, T. (1981). Function of the phosphate group of pyridoxal 5'-phosphate in the glycogen phosphorylase reaction. *The Journal of Biological Chemistry, 256*(2), 728-730.

Tan, K.T., Lee, K.T., Mohamed, A.R., and Bhatia, S. (2009). Palm oil: Addressing issues and towards sustainable development. *Renewable and Sustainable Energy Reviews, 13*(2), 420-427.

W., Glazebrook, J., Chang, H. S., Han, B., et al. Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae.Plant Cell, 15*(2), 317-330.

Tate, D.J.M. (1996). The RGA History of the Plantation Industry in the Malaysian Peninsula, Oxford University Press, New York.688 pp.

Tattersall, E. A. R., Ergul, A., AlKayal, F., DeLuc, L., Cushman, J. C., and Cramer, G. R. (2005).Comparison of methods for isolating high-quality RNA from leaves of grapevine.*American Journal of Enology and Viticulture, 56*(4), 400-406.

Thoma, S., Hecht, U., Kippers, A., Botella, J., De Vries, S., and Somerville, C. (1994). Tissue-specific expression of a gene encoding a cell wall- localized lipid transfer protein from Arabidopsis. *Plant Physiology, 105*(1), 35-45.

Toh,A.S. (2008). A Shattered Dream Daunted by Pests and Incurable Palm Diseases. Retrieved July 15, 2010, from Chapter3 pg2.htm.

Tregear, J.W., Morcillo, F., Richaud, F., Berger, A., Singh, R., Cheah, S. C., et al. (2002). Characterization of a defensin gene expressed in oil palm inflorescences: induction during tissue culture and possible association with epigenetic somaclonal variation events. *Journal ofExperimental Botany, 53*(373), 1387-1396.

Trostle,R.(2008). *Global Agricultural Supply and Demand: Factors Contributing to the Recent Increase in Food Commodity Prices.*21 Deceber 2011, from www.ers.usda.gov.

Tsang, E. W., Yang, J., Chang, Q., Nowak, G., Kolenovsky, A., McGregor, D. I., et al. (2003).Chlorophyll reduction in the seed of *Brassica napus* with a glutamate 1-semialdehyde aminotransferase antisense gene.*Plant Molecular Biology, 51*(2), 191-201.

Tsunezuka, H., Fujiwara, M., Kawasaki, T., and Shimamoto, K. (2005). Proteome analysis of programmed cell death and defense signaling using the rice lesion mimic mutant cdr2. *Molecular Plant-Microbe Interactions, 18*(1), 52-59.

Turner, E.C., Snaddon, J.L., Fayle, T.M., and Foster, W.A. (2008). Oil Palm Research in Context: Identifying the Need for Biodiversity Assessment. *Plos One, 3*(2).

and Kramer, F.R. (2000).Wavelength-shifting molecular *(11), 1191-1196.

Ukaji, N., Kuwabara, C., Takezawa, D., Arakawa, K., and Fujikawa, S. (2004). Accumulation of pathogenesis-related (PR) 10/Bet v 1 protein homologues in mulberry (*Morus bombycis* Koidz.) tree during winter.*Plant Cell and Environment, 27*(9), 1112-1121.

Ussuf, K.K., Laxmi, N.H., and Mitra, R. (2001). Proteinase inhibitors: Plant derived genes of insecticidal protein for developing insect-resistant transgenic plants. *Current Scienceence, 80*(7), 847-853.

USDA , Foreign Agriculture Service (2009). Indonesia: Palm Oil Production Prospects Continue to Grow. Retrieved from http://www.pecad.fas.usda.gov/highlights/2007/12 /Indonesia_palmoil/

Utriainen, M., Kokko, H., Auriola, S., Sarrazin, O., and Karenlampi, S. (1998). PR-10 protein is induced by copper stress in roots and leaves of a Cu/Zn tolerant clone of birch, *Betula pendula*. *Plant Cell and Environment, 21*(8), 821-828.

Van Loon, L. C., Rep, M., and Pieterse, C. M. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology, 44*, 135-162.

Van Loon, L.C.(1999). *Occurrence and properties of plant pathogenesis-related proteins.* In: Pathogenesis-related proteins in plants. Eds. S.K. Datta, S. Muthukrishnan, CRC Press LLC, Boca Raton, 1-19.

Van Loon,L.C., and Van Strien,E.A.(1999).The family of pathogenesis-related proteins.*Physiol Molecular Plant Pathology, 55*(2)85–97.

Van Loon,L.C., Pierpoint, W.S., Boller, T., Conejero, V. (1994).Recommendation for naming plant pathogenesis-related proteins.*Plant Molecular Biology Reports, 12*(3)245–64.

Vanholme, R., Morreel, K., Ralph, J., and Boerjan, W. (2008).Lignin engineering.*Current Opinion in Plant Biologyogy, 11*(3), 278-285.

Walling, L.L. (2006). Recycling or regulation?The role of amino-terminal modifying enzymes.*Current Opinion in Plant Biologyogy, 9*(3), 227-233.

Wang, J.Y., Ruan, S.L., Wu, W.H., Xu, X. F., Wang, Y., and Han, Z.H. (2010a). Proteomics Approach to Identify Differentially Expressed Proteins Induced by Iron Deficiency in Roots of Malus. *Pakistan Journal of Botany, 42*(5), 3055-3064.

Wang, T., Zhang, X., and Li, C. (2007). Growth, Abscisic acid content, and carbon isotope composition in wheat cultivars grown under different soil moisture.*Biologia Plantarum, 51*(1), 181-184.

Wang, X., Tian, W., and Li, Y. (2008). Development of an efficient protocol of RNA isolation from recalcitrant tree tissues.*Molecular Biotechnology, 38*(1), 57-64.

Wang, Y.P., Nowak, G., Culley, D., Hadwiger, L.A., and Fristensky, B. (1999). Constitutive expression of pea defense gene DRR206 confers resistance to blackleg (*Leptosphaeria maculans*) disease in transgenic canola (*Brassica napus*). *Molecular Plant-Microbe Interactions, 12*(5), 410-418.

Wang, Z.F., Wang, Z.H., Shi, L., Wang, L.J., and Xu, F.S. (2010b). Proteomic alterations of *Brassica napus* root in response to boron deficiency. *Plant Molecular Biology, 74*(3), 265-278.

War, A.R., Paulraj, M.G., War, M.Y., Ignacimuthu, S. (2011). Jasmonic acidmediated induced resistance in groundnut (*Arachis hypogaea* L.) against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae).*Journal of Plant Growth Regulator. 30*(4), 512-523.

Washburn, M.P., Wolters, D., and Yates, J.R. (2001).Large-scale analysis of the yeast proteome by multidimensional protein identification technology.*Nature Biotechnology, 19*(3), 242-247.

Webb, C.A., and Fellers, J.P. (2006).Cereal rust fungi genomics and the pursuit of virulence and avirulence factors.*FEMS Microbiology Letterers, 264*(1), 1-7.

Weiss, W., and Gorg, A. (2007).Two-dimensional electrophoresis for plant proteomics.*Methods of Molecular Biology, 355*, 121-143.

...I.P. (2002). Biochemical dissection of the mitochondrial ...idopsis thaliana by three-dimensional gel electrophoresis.*Electrophoresis, 23*(4), 640-646.

Werner,P., Voigt,M., Keinanen,K., Wisden,W., Seeburg,P.H. (1991). Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. *Nature ,351*(6329)742–744.

Wilkins, M.R., Pasquali, C., Appel, R.D., Ou, K., Golaz, O., Sanchez, J.C., et al. (1996). From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology (NY), 14*(1), 61-65.

Wilkins, M.R., Appel, R.D., Van Eyk, J.E., Chung, M.C., Gorg, A., Hecker, M., et al. (2006).Guidelines for the next 10 years of proteomics.*Proteomics, 6*(1), 4-8.

Williams, N. K., Lucet, I. S., Klinken, S. P., Ingley, E., and Rossjohn, J. (2009). Crystal structures of the Lyn protein tyrosine kinase domain in its Apo- and inhibitor-bound state. *Journal of Biological Chemistry, 284*(1), 284-291.

Wirtz, K.W. (1991). Phospholipid transfer proteins. *Annual Review of Biochemistry, 60*, 73-99.

Wolters, D.A., Washburn, M.P., and Yates, J.R. (2001).An automated multidimensional protein identification technology for shotgun proteomics.*Analytical Chemistry, 73*(23), 5683-5690.

Wong, J.H., Ng, T.B., Cheung, R.C.F., Ye, X.J., Wang, H.X., Lam, S.K., et al. (2010). Proteins with antifungal properties and other medicinal applications from plants and mushrooms.*Applied Microbiology and Biotechnology, 87*(4), 1221-1235.

Wongpia, A., and Lomthaisong, K. (2010).Changes in the 2DE protein profiles of chilli pepper (*Capsicum annuum*) leaves in response to *Fusarium oxysporum* infection.*Science, 36*(4), 259-270.

Xie, Y.R., Chen, Z.Y., Brown, R.L., and Bhatnagar, D. (2010).Expression and functional characterization of two pathogenesis-related protein 10 genes from *Zea mays.Journal of Plant Physiology, 167*(2), 121-130.

Van, L., Zheng, Y., Zhou, P., et al. (2011). Identification and gene expression in the mesocarp and kernel of oil palm nuts using suppression subtractive hybridization. *Tree Genetics and Genomes, 7*(5), 999-1010.

Xue, G. P., McIntyre, C. L., Jenkins, C. L., Glassop, D., van Herwaarden, A. F., and Shorter, R. (2008). Molecular dissection of variation in carbohydrate metabolism related to water-soluble carbohydrate accumulation in stems of wheat. *Plant Physiologyogy, 146*(2), 441-454.

Yamada, M. (1992). Lipid Transfer Proteins in Plants and Microorganisms. *Plant and Cell Physiology, 33*(1), 1-6.

Yamaguchi, M., Valliyodan, B., Zhang, J., Lenoble, M.E., Yu, O., Rogers, E.E., Nguyen, H.T., Sharp, R.E. (2010). Regulation of growth response to water stress in the soybean primary root. I. Proteomic analysis reveals region-specific regulation of phenylpropanoid metabolism and control of free iron in the elongation zone. *Plant, Cell & Environment, 33*(23)223–243.

Yan, S., Tang, Z., Su, W., Sun, W. (2005). Proteomic analysis of salt stressresponsive proteins in rice root. *Proteomics, 5*(1) 235–244.

Yao, C.L., Conway, W.S., Ren, R.H., Smith, D., Ross, G.S., and Sams, C.E. (1999). Gene encoding polygalacturonase inhibitor in apple fruit is developmentally regulated and activated by wounding and fungal infection. *Plant Molecular Biology, 39*(6), 1231-1241.

Ying, Z., and Tao, Y. (2002). RNA Isolation from Highly Viscous Samples Rich in Polyphenols and Polysaccharides. *Plant Molecular Biology Reporter, 20*, 417a–417e.

Yoo, B. C., Kragler, F., Varkonyi-Gasic, E., Haywood, V., Archer-Evans, S., Lee, Y. M., et al. (2004). A systemic small RNA signaling system in plants. *Plant Cell, 16*(8), 1979-2000.

Yun, Z., Li, W. Y., Pan, Z. Y., Xu, J., Cheng, Y. J., and Deng, X. X. (2010). Comparative proteomics analysis of differentially accumulated proteins in juice sacs of ponkan (*Citrus reticulata*) fruit during postharvest cold storage. *Postharvest Biology and Technology, 56*(3), 189-201.

talk for sustainability. *Global Oils and Fats Business*

Yusof, B. (2001).*The Role of Palm Oil in the Global Supply and Demand Chai*, Paper prepared for the 72nd World Congress of the International Association of Seed Clusters,Industry Challenges of the 21st Century", Sydney.

Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G. B., Zago, E., Delledonne, M., et al. (2008).Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling.*J Exp Bot, 59*(15), 4145-4159.

Zavala,J.A., Casteel,C.L., DeLucia,E.H., Berenbaum,M.R .(2008).Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *Proceedings of the National Academy of Scienceences of the United States of America*, 105, 5129-5133.

Zavallo,D., Bilbao,M.L., Hopp,H.E., Heinz,R. (2010). Isolation and functional characterization of two novel seed-specific promoters from sunflower (*Helianthus annuus* L.).*Plant Cell Rep Report 29*(6), 239–248.

Zhang, J., and Erickson, L. R. (2012). Harvest-inducibility of the promoter of alfalfa S-adenosyl-L-methionine: trans-caffeoyl-CoA3-O-methyltransferase gene. *Molecular Biology Reports, 39*(3), 2489-2495.

Zhu,H., Ma,X.J., Ma,X.H., Wang,T.C. (2001). Two-dimensional electrophoresis analysis of leaf proteins in theat. *Journal of Harbin Normal University (Natural Scienceences Edition), 17*(1), 98-101.

Zivy,M., and de Vienne,D.(2000).Proteomics: a link between genomics, genetics and physiology.*Plant Molecular Biology 44*(5) 575–580.

Appendix A: List of chemicals and brands

Chemicals	Sources
Acetic acid	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Agarose	SIGMA, Spain
Ammonium Persulphate	Amersham BioScienceence, Sweden
Boric acid	Vivantis, Malaysia
Bromophenol Blue	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Butanol	Merck, USA
Chloroform	Fisher Scienceentific, UK
Chloroform	Fisher Scienceentific, Malaysia
Coomasie Brilliant Blue R	Sigma,Aldrich (M) Sdn Bhd, Malaysia
CTAB	BDH GPR™, UK
DEPC	Bio Basic INC., Canada
Drystrip Cover Fluid	Amersham BioScienceence, Sweden
EDTA	BDH Laboratory , UK
Enzyme:*RNAse Ecor1*	Fermentas,USA
Ethidiumbromide	SIGMA, France
Ethyl Alcohol	Fisher Scienceentific, UK
Formaldehyde (solution)	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Glutaraldehyde (solution)	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Iodoacetamide (IDD)	Amersham BioScienceence, Sweden
IPG buffer 3-10 and 4-7	Amersham BioScienceence, Sweden
Iso-Amyl alcohol	Fisher Scienceentific, UK

	MOX, Malaysia
Methanol	Fisher Scienceentific, Malaysia
NaCl	SYSTEM^R, Malaysia
NaoAC	[BDH] Prolabo (VWR^{TM}), UK
NaOH	Fisher Scienceentific, Malaysia
Perfect^{TM} 100bp DNA Ladder, 50µl	EURx, Poland
Perfect^{TM} 1kb DNA Ladder, 100µl	EURx, Poland
Phenol	Fisher Scienceentific, Malaysia
Power SYBR green	Applied Biosystems, UK
Proteinase K	Sigma,Aldrich (M) Sdn Bhd, Malaysia
PVPP	MP BIOMEDICALS, Germany
QIAquick PCR purification Kit	Qiagen Inc., Netherland
SDS (sodium dodecyl sulphate)	Promega, USA
Silver nitrate	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Sodium Acetate	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Sodium carbonate	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Sodium thiosulphate	Merck, USA
Sucrose	BDH laboratory, UK
TEMED	Amersham BioScienceence, Sweden
Thiourea	Amersham BioScienceence, Sweden
Tris-Base	Promega, USA
Trise Base	Promega, USA
Tris-HCl	Promega, USA
Urea	Amersham BioScienceence, Sweden
β-Mercaptoethanol	SIGMA, France

	Sigma,Aldrich (M) Sdn Bhd, Malaysia
Cover fluid oil	Amersham BioScienceence, Sweden
Acrylmide ready solution 30%	Bio-Rad, USA
Acrylmide ready solution 40%	Bio-Rad, USA
DTT (Dithiothretol)	Amersham BioScienceence, Sweden

Keys

BDG = British Drug House Ltd, Poole, UK

CTAB =Cetyl timethylammonium bromide

TEMED = N,N,N',N' – Tetramethyl-ethelendiamine

Appendix B: List of instrument, consumables, software and their respective source, stock solutions/reagents

Appendix B1: List of instrument, consumables, software and their respective source

Instruments	Sources
5800 Proteomics Analyzer	AB Scienceex, USA
Autoclave	Tommy ,Japan
Bio Photometer	Amersham BioScienceence
Centrifuge 5415 D	eppendorf, Germany
Centrifuge 5417 R	eppendorf, Germany
Dry Bath	Aosheng, China
ETTAN *Dalt six*	GE Healthcare, Sweden
Gel Documentation system	Alpha Innotech,USA
Ice Maker	nuove tecnologie del freddo, Italy
ImageScanner™ III	GE Healthcare, Sweden
LEGEND MICRO 17 centrifuge	Thermo Scienceentific, Germany

	National, Japan
mini-PROTEAN Tetra handcast System	Bio-Rad, USA
Multiphore II	GE Healthcare, Sweden
Nanovuy (nanospectrometer)	GE Healthcare, Sweden
PCR machine :PTC 100 ™ Programmable thermal cycler	MJ research Inc., USA
PCR machine DNA engine	MJ research Inc., USA
Personal Thermal Cycler	Bio-Rad, USA
pH Meter	Sartorius, Germany
Real-Time Quantitative PCR 7500	Applied Biosystems, USA
Stirring hotplate	Favourit, Australia
Tecan GENios Microplate Reader	Tecan, Switzerland
Top Pan Balance	Setra, USA
Ultrafreezer	Denley, UK
Vortex mixer	Stuart, UK
Water bath	Memmert, Germany
Water bath Shaker	Innova^{TM} 3100, USA
Water Distiller	Sartorius, Germany
Software	**Source**
Image master	Melanie © Swiss institute of bioinformatics
Chromas software	http://www.flu.org.cn/en/download-49.html
ClustalW	http://www.clustal.org/clustal2/#Download
Primer Express	Applied Biosystems, USA
ImageScanner™ III	GE Healthcare, Sweden

olutions/reagents

Genomic investigation /Proteomic investigation
0.5 M EDTA pH 8.0
0.5 M Tris-HCl,pH 6.8
1% Agarose Gel
1 M MgCl2.6H$_2$O
1 M Tris-HCl pH 7.5
1 X TE buffer
10% APS
10% SDS
10 X Running buffer (Laemmli buffer)
10 X TBE buffer
2% Agarose Gel
30:8% w/v acrylamid:Bis acrylamide
3X sample loading buffer
4 X resolving solution (1.5 M Tris-HCl,pH 8.8)
5 X Protease K buffer
6 M NaCl
6X bromophenol blue dye (Gel loading buffer)
70% ethanol
Agarose sealing solution
Bromophenol blue stock solution
Buffer EB (purification kit)
Buffer PB (purification kit)
Buffer PE(purification kit)
Commasie Blue Staining
Destaining solution (Commasie Staining)

...ning solution (Silver solution)
Developing solution
Equilibration Buffer
Ethidium Bromide (EtBr) 10mg/ml
Fixing solution (Commasie Blue Staining)
Fixing solution (Silver staining)
lyses solution
Phenol-Chlorophorm
Preserving solution
Rehydration solution with IPG buffer
RNAse A solution (10 mg/ml)
Running buffer
Sample Buffer
Staining solution
Stopping solution

Appendix C: Sterilizations of materials

All glassware and solutions were steam sterilized at 121ºC (15 psi) for 15 min in an autoclave (TOMY, JAPAN). Distilled water, polypropylene centrifuge tubes, micropipette tips, micro-centrifuge tunes and PCR tubes were autoclaved for 20 minutes, except RNA working stuff autoclaved for 45 min.

All stock solutions were sterilized by filtration through sterile 0.2 μm (pore size) disposable nylon membrane filters.

```
LOCUS       AM180653                1029bp    DNA       linear    PLN 25-
OCT-2006
DEFINITION  Oryza sativa pgip2 gene for polygalacturonase inhibiting
protein 2.
ACCESSION   AM180653
VERSION     AM180653.1  GI:116743148
KEYWORDS    pgip2 gene; polygalacturonase inhibiting protein 2.
SOURCE      Oryza sativa (rice)
ORGANISM    Oryza sativa
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Ehrhartoideae; Oryzeae; Oryza.
REFERENCE   1
  AUTHORS   Janni,M., Di Giovanni,M., Roberti,S., Capodicasa,C. and
D'Ovidio,R.
  TITLE     Characterization of expressed Pgip genes in rice and wheat
reveals
similar extent of sequence variation to dicot PGIPs and identifies
an active PGIP lacking an entire LRR repeat
JOURNAL   Theor. Appl. Genet. 113 (7), 1233-1245 (2006)
  PUBMED   16906405
REFERENCE   2  (bases 1 to 1029)
  AUTHORS   D'Ovidio,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-DEC-2005) D'Ovidio R., Agrobiologia e
Agrochimica,
            University of TuSciencea, Via San Camillo de Lellis, s.n.c.,
Viterbo,
            I-1100, ITALY
FEATURES             Location/Qualifiers
source          1..1029
                /organism="Oryza sativa"
                /mol_type="genomic DNA"
                /cultivar="Roma"
                /db_xref="taxon:4530"
                /dev_stage="seedling"
                /country="Italy"
gene            1..1029
                /gene="pgip2"
CDS             1..1029
                /gene="pgip2"
                /function="inhibitor of fungal polygalacturonase"
                /codon_start=1
                /product="polygalacturonase inhibiting protein 2"
                /protein_id="CAJ55692.1"
                /db_xref="GI:116743149"

/translation="MDVKLLLLLLLSVLLLSAASMAKDDDEQQQKVQCHEDDEAALLA

IDDALGNPYNLASWTRNTSCCDWYDVDCDQDSGRVVSLSVFQDTNLTGAIPDAIANLT
```

219

TDLTHLTISWTAVSGPVPSFLANLTSLTMLDLSF

NSLTGLIPPSLAALTNLSAINLSRNRLSGPIPPGLFSNLQLQDDDDEVYLRLSHNNLS

GSVPADLLAAANLALVDLSRNALTGDASAVFRRARYVDLSRNGFVFNMSGVEFAEETY

YVDVSHNAIRGGIPAQAANLTNLQTFNVSYDKMCGAVPAMPRFDAYCYQHNKCLCGAP
                         LATACRRR"
sig peptide   1..66
                     /gene="pgip2"
mat peptide   67..1026
                     /gene="pgip2"
                     /product="polygalacturonase inhibiting protein 2"
ORIGIN
        1 atggatgtga agctcctgct gctgctgctg ctgtccgtcc tcctcctgtc
tgctgcctcc
       61 atggccaagg acgacgatga gcagcagcag aaggtgcaat gccatgagga
cgacgaggcg
      121 gcgcttctgg ccatcgacga cgccctcggg aacccctaca atttggcgtc
gtggacgcgc
      181 aacacctcct gctgcgactg gtacgacgtg gactgcgacc aggactctgg
ccgcgtcgtc
      241 tccctctccg tcttccagga caccaacctc accggcgcca tccccgacgc
catcgccaac
      301 ctcacccacc tccgcaccct cctcctccac cacctcccca gcctctccgg
ccccatcccc
      361 gactccctcg ccgccctcac cgacctcacc cacctcacca tctcctggac
cgccgtctcc
      421 ggccccgtcc cctccttcct cgccaacctc acctccctca ccatgctcga
tctctccttc
      481 aactccctca ccggcctcat cccgccctcc ctcgccgccc tcaccaatct
ctccgccatc
      541 aacctcagcc gcaaccgcct ctccggcccc atcccgccgg gcctcttctc
caacctgcag
      601 ctgcaggacg acgacgacga ggtgtacctc cggctgtcgc acaacaacct
gtccgggagc
      661 gtccggcgg acctgctggc ggcggcgaac ctggcgctgg tggacctgtc
gcggaacgcg
      721 ctgaccggcg acgcgtcggc ggtgttccgg cgggcaaggt acgtggacct
gtcgcgaaac
      781 ggcttcgtct tcaacatgtc cggcgtggag ttcgcggagg agacgtacta
cgtggacgtg
      841 agccacaacg ccatccgcgg ggggatcccg gcgcaggcgg ccaacctcac
caacctgcag
      901 acgttcaacg tcagctacga caagatgtgc ggcgcggtgc ccgccatgcc
caggttcgac
      961 gcctactgct accaacacaa caaatgcctc tgcggcgctc ccctcgccac
cgcctgccgt
     1021 cgtcgataa

842bp   DNA   linear   PLN 28-

DEFINITION   Stephanandra chinensis isolate St_ch_A polygalacturonase inhibitor
protein (PGIP) gene, partial cds.
ACCESSION   AF196951
VERSION   AF196951.1 GI:13873279
KEYWORDS   .
SOURCE   Stephanandra chinensis
ORGANISM Stephanandra chinensis
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicotyledons;
rosids; fabids; Rosales; Rosaceae; Spiraeoideae; Neillieae;
Stephanandra.
REFERENCE   1 (bases 1 to 842)
  AUTHORS   Potter,D., Oh,S.-H., Gao,F. and Baggett,S.
  TITLE   Phylogenetic relationships among putative genes encoding
polygalacturonase inhibitor proteins (PGIPs) in Rosaceae
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 842)
  AUTHORS   Potter,D., Oh,S.-H., Gao,F. and Baggett,S.
  TITLE   Direct Submission
  JOURNAL   Submitted (20-OCT-1999) Pomology, University of California, Davis,
            1045 Wickson Hall, One Shields Avenue, Davis, CA 95616, USA
FEATURES   Location/Qualifiers
source   1..842
                /organism="Stephanandra chinensis"
                /mol_type="genomic DNA"
                /isolate="St_ch_A"
                /db_xref="taxon:141009"
gene<1..>842
                /gene="PGIP"
join(<1..394,486..>842)
                /gene="PGIP"
                /product="polygalacturonase inhibitor protein"
CDSjoin(<1..394,486..>842)
                /gene="PGIP"
                /codon_start=1
                /product="polygalacturonase inhibitor protein"
                /protein_id="AAK43460.1"
                /db_xref="GI:13873280"

/translation="SWNPDNDCCDWYSVTCDSTTNRINSLTIFAGEVSGQIPTQVGDL

PYLETLEFHKQPNLTGPIQPSIVKLKSLKFLRLSWTNLSGSVPDFLSQLKNLTFLDLS

FSNFTGSIPSSLSKLPNLNALHLDRNKLTGHIPESFGEFQGSVPDLYLSHNQLSGSIP

TSFAKMDFSSIDLSRNKLEGDASMIFGSNKTTQIVDLSRNLLEFNLSKVVFPKSLTSL
                DLNHNQITGSIPVELTQLDLQFLNVSYNRLCG"
ORIGIN
        1 tcatggaacc cagacaatga ctgctgtgac tggtactctg tcacctgtga
ttccaccacc
       61 aaccgcatca actccctcac catcttcgcc ggagaagtct ccggccaaat
cccgacccaa

```
                               cgtatct tgaaaccctt gaatttcaca agcaacccaa
      101 ccaatccagc ccccattgt gaagctcaaa agcctcaagt tcctgcgcct
cagctggacc
      241 aacctctcag gctctgtccc tgacttcctc agccaactca agaacctcac
ctttcttgat
      301 ctctctttca gcaacttcac aggctccatc cccagctcac tttcaaagct
cccgaacctg
      361 aacgctcttc atctcgatcg taacaagctc acaggtttca tactcctttg
atatttctct
      421 gtctggctaa caagtactgg cattagatac aaaatttgca tatgaaatta
acacctcgct
      481 tacaggtcat atcccggagt cattcggaga gttccagggc agcgttccag
atctctatct
      541 ctcccacaac cagctctcag gcagcatacc aacctcattt gccaaaatgg
atttcagcag
      601 tatagactta tcccgtaaca agcttgaagg cgatgcatcc atgatattcg
ggtcgaacaa
      661 gacaacccag attgtggatc tgtcgaggaa cttgctggaa tttaatctgt
caaaggtggt
      721 gtttccgaag agcttgactt ccctggatct caaccataac caaatcacag
ggagtattcc
      781 tgtggagctg acccaattgg atttgcagtt cctgaacgtg agctacaaca
ggctgtgtgg
      841 ta
//
```

_____

```
LOCUS       AM180658                    1008bp    DNA     linear   PLN 08-
JAN-2007
DEFINITION  Triticum monococcum pgip1 gene for polygalacturonase
inhibiting
protein 1.
ACCESSION   AM180658
VERSION     AM180658.1  GI:121489498
KEYWORDS    pgip1 gene; polygalacturonase inhibiting protein 1.
SOURCE      Triticum monococcum
ORGANISM    Triticum monococcum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Pooideae; Triticeae; Triticum.
REFERENCE   1
  AUTHORS   Janni,M., Di Giovanni,M., Roberti,S., Favaron,F. and
D'Ovidio,R.
  TITLE     Molecular characterization and chromosomal localization of
polygalacturonase inhibiting protein genes (PGIPs) in wheat and
rice
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1008)
  AUTHORS   D'Ovidio,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-DEC-2005) D'Ovidio R., Agrobiologia e
Agrochimica,
```

f TuSciencea, Via San Camillo de Lellis, s.n.c.,

```
FEATURES             Location/Qualifiers
source          1..1008
                     /organism="Triticum monococcum"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:4568"
                     /tissue_type="leaves"
gene            1..1008
                     /gene="pgip1"
CDS             1..1008
                     /gene="pgip1"
                     /function="inhibitor of fungal polygalacturonase"
                     /codon_start=1
                     /product="polygalacturonase inhibiting protein 1"
                     /protein_id="CAJ55697.1"
                     /db_xref="GI:121489499"

/translation="MSTPPSAHAFLVLLLVVLAGAAIASAENNKDCHPSDKAALLAVK

SAFSNQSHFASWTPSTPCCDWHDITCNDAGRVIILLFFEDVNLTGTIPDAISGLTELL

VLNLYYLPAISGPIPKGIAKLSKLTSLSISLTSVSGPIPSFLGALTKLRELTLSSNFL

SGTIPASLGGLRYLDSVDLRNNSLTGTIPPLFSRSTLLPVLLLSSNHLSGSIPGEFAA

VGFLQIDLSHNALTGDASVFFGRTKELQSIDLSHNALSFDLSSVEFPEEMQTMDASHN

AIRGGIPAQVANVTNLRQFNVSYNKLCGQVPAALARLDAYSFRGNKCLCGAPLPDPCK
                     K"
sig peptide     1..78
                     /gene="pgip1"
mat peptide     79..1005
                     /gene="pgip1"
                     /product="polygalacturonase inhibiting protein 1"
ORIGIN
        1 atgagcactc caccctcggc acatgccttc ctcgtcctcc tccttgtcgt
cctcgccgga
       61 gcggccatcg cctccgccga gaataacaaa gactgccacc ctagcgataa
ggccgcgctt
      121 ctcgccgtca agtccgcctt cagcaatcaa agccacttcg cctcgtggac
tccttccacc
      181 ccgtgctgcg actggcacga catcacttgc aacgacgccg gccgcgtcat
catcctcctc
      241 ttcttcgagg atgtcaacct cacgggcacc atcccggacg ccatctccgg
cctcaccgag
      301 cttctcgtcc tcaacctcta ctacctcccg gccatctccg gccctatccc
caagggaatc
      361 gccaaactat ccaagctcac ctcgctctct atctctctca cctccgtttc
gggccctatt
      421 ccgtccttcc tgggcgcact gaccaagctc agagaactca ccctctcctc
caacttcctc
      481 tccggcacga tcccggcctc gctcgggggc ctccgttact tagactccgt
cgacctcaga
      541 aacaacagcc tcacaggcac catcccgcca ctcttctcca gatccactct
tctcccagtc
```

```
                            gcaacca cctctccggt tccatcccgg gcgagttcgc
      661 cccctgcaaa ccgacctctc ccacaacgct ctcaccggcg acgcgtccgt
gttcttcggc
      721 aggacaaagg agctgcaaag cattgacctg tcccacaacg ccctcagctt
cgacctctcc
      781 agcgtggagt ccccggagga gatgcaaacc atggatgcga gccacaacgc
catccgcggc
      841 ggcatcccag cgcaggtggc gaatgtaacg aacctgcggc agttcaacgt
gagctacaac
      901 aagctgtgcg gccaggttcc ggccgccttg gcgaggttgg atgcctacag
cttccggggc
      961 aacaagtgcc tctgcggtgc gcccctccca gatccatgca agaaataa
//
```

```
LOCUS       DQ995511                1044bp   DNA       linear   PLN 14-
NOV-2006
DEFINITION  Hordeum vulgare clone 509d02_c-84 genomic sequence.
ACCESSION   DQ995511 REGION: 4565..5608
VERSION     DQ995511.1  GI:117670146
KEYWORDS    .
SOURCE      Hordeum vulgare
ORGANISM    Hordeum vulgare
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Pooideae; Triticeae; Hordeum.
REFERENCE   1  (bases 1 to 1044)
  AUTHORS   Wicker,T., Schlagenhauf,E., Graner,A., Close,T.J., Keller,B.
and
            Stein,N.
  TITLE     454 sequencing put to the test using the complex genome of
barley
  JOURNAL   BMC Genomics 7, 275 (2006)
   PUBMED   17067373
  REMARK    Publication Status: Online-Only
REFERENCE   2  (bases 1 to 1044)
  AUTHORS   Wicker,T., Schlagenhauf,E., Graner,A., Close,T.J., Keller,B.
and
            Stein,N.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2006) Institute of Plant Biologyogy,
University
            Zurich, Zollikerstrasse 107, Zurich 8008, Switzerland
FEATURES             Location/Qualifiers
source          1..1044
                /organism="Hordeum vulgare"
                /mol_type="genomic DNA"
                /db_xref="taxon:4513"
                /clone="509d02_c-84"
<1..>1044
```

                        oduct="polygalacturonase inhibiting protein-like"
                        1044
                        /note="509D2.5; similar to Oryza sativa
polygalacturonase
inhibiting protein Os05g01370"
                        /codon_start=1
                        /product="polygalacturonase inhibiting protein-like"
                        /protein_id="ABK56716.1"
                        /db_xref="GI:117670147"

/translation="MMRMHPHALLLLLFLFSVLAGGANAEPSRELTYKDCHPDDKAVL

LSFPESWSSSWTPDTPCCDWFAVACDRSTGRIVGFSVFQDPDFTGTIPSAIAGLTHLQ

DLFLHHLPGLSGPIPPEIGKLSNLSRLEISWTGVSGPVPSSLGALKKLAQLNLSFNSL

SGAIPVSLGTIPGITLSRNRLTGAIPNMFLGESAGRDVYLDVSHNNLTGAIPAEFAAV

NFTHLDLSRNALTGDASGLFGRGKELQYIDLSRNAFDFDLSGVVVLPEKLFHLDLSHN

AIHGSIPAQVADVANLLAFNVSYNRLCGAVPTGGNMARFDLYNFQHNKCLCGAPPPLP
                        ARTRWIILWVLTE"
ORIGIN
        1 atgatgagga tgcacccaca cgccctgctg ctgctcctct tcctcttctc
tgtcctcgcc
       61 ggcggagcca acgccgagcc ctctagagag ctcacttaca aggattgcca
ccctgacgac
      121 aaggcggtgc tgctctcctt cccggagtcc tggtcctcgt catggacacc
cgacacaccc
      181 tgctgcgact ggttcgccgt cgcctgcgac cgctccaccg ggcgcatcgt
gggcttctcc
      241 gtcttccagg accccgactt caccggcaca atccccagcg ccatcgccgg
cctcacccac
      301 ctgcaggacc tcttcctgca ccacctcccg gggctgtctg gccccatacc
gccggagata
      361 ggcaagctct ccaacctctc caggctcgaa atctcatgga cggggggtgtc
cggcccccgtg
      421 ccgtcctccc tgggcgcgct caagaagctc gcccagctca acctctcctt
caactcgctc
      481 agcggcgcca tcccggtgtc gctggggaca atccccggca tcaccctcag
ccgcaaccgc
      541 ctcaccgggg ccatccccaa catgttcctc ggcgagtccg cgggccggga
cgtctacctc
      601 gacgtgtcgc acaacaacct caccggggcc atcccggccg agttcgccgc
cgtgaacttc
      661 acgcacctcg acctctcgcg caacgccctg accggcgacg cgtcgggcct
cttcggccgt
      721 gggaaggagc tgcagtacat cgacctgtcc cgcaacgcct tcgacttcga
cctgtcaggc
      781 gtggtggtgc tcccggagaa gctcttccac ctggacctga gccacaatgc
catccatggc
      841 agcatcccgg cgcaggtcgc ggacgtggcc aacctgctgg cgttcaacgt
cagctacaac
      901 aggctctgcg gcgccgtgcc caccggcggg aacatggcga ggttcgatct
ctacaacttc
      961 cagcacaaca agtgcctctg cggtgctccc cctcccctcc ctgcaagaac
tagatggatt
     1021 atcttgtggg ttttgacaga ataa

225

```
                             CTCCTGCTGCTGCTGCTGCTGTCCGTCCTCCTCCTGTCTGCTGCCTCC
                             GACAATGACTGCTGTGACTGGTACTCTGTCACCTGTGATTCCACCACC
Triticum        ATGAGCACTCCACCCTCGGCACATGCCTTCCTCGTCCTCCTCCTTGTCGTCCTCGCCGGA
Hordeum         ATGATGAGGATGCACCCACACGCCCTGCTGCTGCTCCTCTTCCTCTTCTCTGTCCTCGCC
                            *                                  *   **              *

Oryza           ATGGCCAAGGACGACGATGAGCAGCAGCAGAAGGTGCAATGCCATGAGGACGACGAGGCG
Stephanandra    AACCGCATCAACTCCCTCACCATCTTCGCCGGAGAAGTCTCCGGCCAAATCCCGACCCAA
Triticum        GCGGCCATCGCCTCCGCCGAGAATAACAAAGACTGCCACCCTAGCGATAAGGCCGCGCTT
Hordeum         GGCGGAGCCAACGCCGAGCCCTCTAGAGAGCTCACTTACAAGGATTGCCACCCTGACGAC
                           *    *

Oryza           GCGCTTCTGGCCATCGACGACGCCCTCGGGAACCCCTACAATTTGGCGTCGTGGACGCGC
Stephanandra    GTCGGGGACTTGCCGTATCTTGAAACCCTTGAAATTTCACAAGCAACCCAATCTCACTGGC
Triticum        CTCGCCGTCAAGTCCGCCTTCAGCAATCAAAGCCACTTCGCCTCGTGGACTCCTTCCACC
Hordeum         AACACCTCCTGCTGCGACTGGTACGACGTGGACTGCGACCAGGACTCTGGCCGCGTCGTC
                                                          *

Oryza           AACACCTCCTGCTGCGACTGGTACGACGTGGACTGCGACCAGGACTCTGGCCGCGTCGTC
Stephanandra    AACACCTCCTGCTGCGACTGGTACGACGTGGACTGCGACCAGGACTCTGGCCGCGTCGTC
Triticum        AACACCTCCTGCTGCGACTGGTACGACGTGGACTGCGACCAGGACTCTGGCCGCGTCGTC
Hordeum         TCCCTCTCCGTCTTCCAGGACACCAACCTCACCGGCGCCATCCCCGACGCCATCGCCAAC
                 *   ****    ** *  *        *  **  *      *   ***   *        *    *   *      **  *    *

Oryza           TCCCTCTCCGTCTTCCAGGACACCAACCTCACCGGCGCCATCCCCGACGCCATCGCCAAC
Stephanandra    TCCCTCTCCGTCTTCCAGGACACCAACCTCACCGGCGCCATCCCCGACGCCATCGCCAAC
Triticum        TCCCTCTCCGTCTTCCAGGACACCAACCTCACCGGCGCCATCCCCGACGCCATCGCCAAC
Hordeum         GTGGTGGTGCTCCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATCCATGGC
                      *          **       ** *     *    ** *       *  ** *  * ******       *

Oryza           GTGGTGGTGCTCCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATCCATGGC
Stephanandra    GTGGTGGTGCTCCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATCCATGGC
Triticum        GTGGTGGTGCTCCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATCCATGGC
Hordeum         CTGCAGGACCTCTTCCTGCACCACCTCCCGGGGCTGTCTGGCCCCATACCGCCGGAGATA
                   *      **        ***               * *  *  * ***             ***  **       *

Oryza           AGCATCCCGGCGCAGGTCGCGGACGTGGCCAACCTGCTGGCGTTCAACGTCAGCTACAAC
Stephanandra    AGCATCCCGGCGCAGGTCGCGGACGTGGCCAACCTGCTGGCGTTCAACGTCAGCTACAAC
Triticum        GCCAAACTATCCAAGCTCACCTCGCTCTCTATCTCTCTCACCTCCGTTTCGGGCCCTATT
Hordeum         GGCAAGCTCTCCAACCTCTCCAGGCTCGAAATCTCATGGACGGGGGTGTCCGGCCCCGTG
                     **    *      *    *    **  *             *       *   *                   **

Oryza           GGCCCCGTCCCCTCCTTCCTCGCCAACCTCACCTCCCTCACCATGCTCGATCTCTCCTTC
Stephanandra    GTCTGGCTAACAAGTACTGGCATTAGATACAAAATTTGCATATGAAATTAACACCTCGCT
Triticum        CCGTCCTTCCTGGGCGCACTGACCAAGCTCAGAGAACTCACCCTCTCCTCCAACTTCCTC
Hordeum         CCGTCCTCCCTGGGCGCGCTCAAGAAGCTCGCCCAGCTCAACCTCTCCTTCAACTCGCTC
                                   *         *                        **                *

Oryza           AACTCCCTCACCGGCCTCATCCCGCCCTCCCTCGCCGCCCTCACCAATCTCTCCGCCATC
Stephanandra    TACAGGTCATATCCCGGAGTCATTCGGAGAGTTCCAGGGCAGCGTTCCAGATCTCTATCT
Triticum        GTGGTGGTGCTCCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATCCATGGC
Hordeum         AGCGGCGCCATCCCGGTGTCGCTGGGGACAATCCCCGGCATCACCCTCAGCCGCAACCGC
                                                            *

Oryza           AACCTCAGCCGCAACCGCCTCTCCGGCCCCATCCCGCCGGGCCTCTTCTCCAACCTGCAG
Stephanandra    CTCCCACAACCAGCTCTCAGGCAGCATACCAACCCTGCGACTGGTACGACGTGGACTGCG
Triticum        AGCATCCCGGCGCAGGTCGCGGACGTGGCCAACCTGCTGGCGTTCAACGTCAGCTACAAC
Hordeum         CTGCGACTGGTACGACGTGGACTGCGACCAGGACTCTGGCCGCGTCGTCTCCCTCTCCGT
                             *               *     *

Oryza           CTGCAGGACGACGACGACGAGGTGTACCTCCGGCTGTCGCACAACAACCTGTCCGGGAGC
Stephanandra    ACCAGGACTCTGGCCGCGTCGTCTCCCTCTCCGTCTTCCAGGACACCAACCTCACCGGCG
Triticum        CTCTTGCTCTCCAGCAACCACCTCTCCGGTTCCATCCCGGGCGAGTTCGCTGCAGTCGGC
Hordeum         CTTCCAGGACACCAACCTCACCGGCGCCATCCCCGACGCCATCGCCAATTCGCCGCCGTG
                                         *                                    *

|
Oryza           GTCCCGGCGGACCTGCTGGCGGCGGCGAACCTGGCGCTGGTGGACCTGTCGCGGAACGCG
Stephanandra    CCATCCCCGACGCCATCGCCAAGACAACCCAGATTGTGGATCTGTCGAGGAACTTGCTGG
Triticum        TTCCTGCAAATCGACCTCTCCCACAACGCTCTCACCGGCGACGCGTCCGTGTTCTTCGGC
Hordeum         AACTTCACGCACCTCGACCTCTCGCGCAACGCCCTGACCCGGCGACGCGTCGGGCCTCTTC
                                         *                                       *

Oryza           CTGACCGGCGACGCGTCGGCGGTGTTCCGGCGGGCAAGGTACGTGGACCTGTCGCGAAAC
Stephanandra    AATTTAATCTGTCAAAGGTGGTGTTTCCGAAGAGCTTGACTTCCCTGGATCTCAACCATA
Triticum        AGGACAAAGGAGCTGCAAAGCATTGACCTGTCCCACAACGCCCTCAGCTTCGACCTCTCC
Hordeum         GGCCGTGGGAAGGAGCTGCAGTACATCGACCTGTCCCGCAACGCCTTCGACTTCGACCTG
                                         *

Oryza           GGCTTCGTCTTCAACATGTCCGGCGTGGAGTTCGCGGAGGAGACGTACTACGTGGACGTG
Stephanandra    ACCAAATCACAGGGAGTATTCCTGTGGAGCTGACCCAATTGGATTTGCAGTTCCTGAACG
Triticum        AGCGTGGAGTTCCCGGAGGAGATGCAAACCATGGATGCGAGCCACAACGCCATCCGCGGC
Hordeum         TCAGGCGTGGTGGTGCTCCCGGAGAAGCTCTTCCACCTGGACCTGAGCCACAATGCCATC
                                                              *

Oryza           AGCCACAACGCCATCCGCGGGGGGGATCCCGGCGCAGGCGGCCAACCTCACCAACCTGCAG
Stephanandra    TGAGCTACAACAGGCTGTGTGTGGTA------------------------------------
Triticum        GGCATCCCAGCGCAGGTGGCGAATGTAACGAACCTGCGGCAGTTCAACGTGAGCTACAAC
Hordeum         CATGGCAGCATCCCGGCGCAGGTCGCGGACGTGGCCAACCTGCTGGCGTTCAACGTCAGC
                                                   *

Oryza           ACGTTCAACGTCAGCTACGACAAGATGTGCGGCGCGGTGCCCGCCATGCCCAGGTTCGAC
Stephanandra    ------------------------------------------------------------
Triticum        AAGCTGTGCGGCCAGGTTCCGGCCGCCTTGGCGAGGTTGGATGCCTACAGCTTCCGGGGC
Hordeum         TACAACAGGCTCTGCGGCGCCGTGCCCACCGGCGGGAACATGGCGAGGTTCGATCTCTAC

Oryza           GCCTACTGCTACCAACACAACAAATGCCTCTGCGGCGCTCCCCTCGCCACCGCCTGCCGT
Stephanandra    ------------------------------------------------------------
Triticum        AACAAGTGCCTCTGCGGTGCGCCCCTCCCAGATCCATGCAAGAAATAA-------------
Hordeum         AACTTCCAGCACAACAAGTGCCTCTGCGGTGCTCCCCCTCCCCTCCCTGCAAGAACTAGA

Oryza           CGTCGATAA----------------------
Stephanandra    -------------------------------
Triticum        -------------------------------
Hordeum         TGGATTATCTTGTGGGTTTTGACAGAATAA
```

226

LTP genes from gene bank and clustal alignment

```
LOCUS       OSU77295               799bp           linear   PLN 13-NOV-
1996
DEFINITION  Oryza sativa lipid transfer protein (LTP) , complete cds.
ACCESSION   U77295
VERSION     U77295.1  GI:1667589
KEYWORDS    .
SOURCE      Oryza sativa Indica Group
ORGANISM    Oryza sativa Indica Group
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Ehrhartoideae; Oryzeae; Oryza.
REFERENCE   1  (bases 1 to 799)
  AUTHORS   Lee,M.C., Park,J.Y., Lee,J.S. and Eun,M.Y.
  TITLE     Molecular cloning and characterization of LTP gene in rice
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 799)
  AUTHORS   Lee,M.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-OCT-1996) Cytogenetics Division, National
Institute
of Agricultural Scienceence and Technology, Rural Development
            Administration, Suwon 441-707, Korea
FEATURES             Location/Qualifiers
source          1..799
                /organism="Oryza sativa Indica Group"
                /mol_type=""
                /cultivar="Milyang 23"
                /db_xref="taxon:39946"
                /clone="KCDL860"
                /tissue_type="immature seed"
gene            1..799
                /gene="LTP"
CDS             101..451
                /gene="LTP"
                /codon_start=1
                /product="lipid transfer protein"
                /protein_id="AAB18815.1"
                /db_xref="GI:1667590"

/translation="MARAQLVLVALVAALLLAAPHAAVAITCGQVNSAVGPCLTYARG

GAGPSAACCSGVRSLFAAASTTADRRTACNCLKNAARGIKGLNAGNAASIPSKCGVSV
                PYTISASIDCSRVS"
ORIGIN
        1 caaaagctcg tgccggaacc cggcacgaat catcatcaac caattcgcac
cgatcgatcg
       61 atcgatccag caactagatc aacagtacta gctaggaagc atggcccgtg
cacagttggt
      121 gttggtcgcc ctggtggcag ctctgctcct cgccgccccg cacgccgccg
tggccatcac
      181 ctgcggccag gtcaactccg ccgtcgggcc ctgcctgacc tacgcccgcg
gcggcgccgg
```

227

```
                             cgctgca gcggcgtgag gagccttttt gctgcagcaa
     301 agacggcgc accgcctgca actgcctcaa gaacgcggcc cgcggcatca
aggggctcaa
     361 cgccggcaac gccgccagca tcccctccaa gtgcggcgtc agcgtcccct
acaccatcag
     421 cgcttccatc gattgctcca gggtgagctg agccatcgat cagagacgga
tcatatatat
     481 acagcagcgc gccggttgcc acctagtaga ttttgcctgg gtcgatgtgt
ggagccgaat
     541 tatgtatcca gtactagtag tatcatatct gtattctgga ataaaagatg
agctagctaa
     601 ggtttcgatc aatcaccatg catccatggt tgatcggccc ggtcacgcta
gctagctttc
     661 ttcttcttgt gtgttcgttt cgtacgtttt gctcctttttc gaggggtacg
tgtaccagag
     721 agagctagag attctacatg catgtactgc aactccttgt actacgtgct
tgttttggat
     781 tattacacat aaaaaaaaa
//
```

LOCUS   NM_001112535   551bp   linear PLN 16-NOV-2008

DEFINITION Zea mays LTP-like protein (LOC100101507), .
ACCESSION   NM_001112535
VERSION   NM_001112535.1 GI:162463863
KEYWORDS   .
SOURCE   Zea mays
ORGANISM   Zea mays
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
PACCAD
clade; Panicoideae; Andropogoneae; Zea.
REFERENCE   1 (bases 1 to 551)
  AUTHORS   Alexandrov,N.N., Brover,V.V., Freidin,S., Troukhan,M.E.,
            Tatarinova,T.V., Zhang,H., Swaller,T.J., Lu,Y.P., Bouck,J.,
            Flavell,R.B. and Feldmann,K.A.
  TITLE   Insights into corn genes derived from large-scale cDNA
sequencing
  JOURNAL   Plant Mol. Biol. (2008) In press
   PUBMED   18937034
   REMARK   Publication Status: Available-Online prior to print
COMMENT   PROVISIONAL REFSEQ: This record has not yet been subject to
final
NCBI review. The reference sequence was derived from AJ006702.1.
FEATURES   Location/Qualifiers
source   1..551
                     /organism="Zea mays"
                     /mol_type=""
                     /db_xref="taxon:4577"
gene   1..551
                     /gene="LOC100101507"
                     /note="LTP-like protein"
                     /db_xref="GeneID:100101507"
CDS   55..366

```
                        e="LOC100101507"
                        te="anther specific protein"
                        /codon_start=1
                        /product="LTP-like protein"
                        /protein_id="NP_001106005.1"
                        /db_xref="GI:162463864"
                        /db_xref="GeneID:100101507"

/translation="MALEAATAPRALLAACLVLLVLGGGTGPSSVLRGAGAQAGGQCL

PQLNRLLACRAYLVPGAPDPSADCCSALSAVSHECACSTMGIINSLPGRCHLAQANCS
                  A"
sig_peptide     55..126
                        /gene="LOC100101507"
polyA_signal    534..539
                        /gene="LOC100101507"
ORIGIN
        1 tgccagcaga gcagaaagca gccgcagccc cagcccccac aagacgaggc
aacaatggcg
       61 ctagaagcag ccaccgcccc ccgcgcactc ctcgccgcgt gcctcgtcct
gctggtcctc
      121 ggcggcggca ccggcccgtc gtcggtgctg cgcggcgccg gggcgɶggc
cggcgggcag
      181 tgcctgccgc agctgaaccg cctcctggcg tgccgcgcgt acctggtgcc
cggcgcgccg
      241 gacccagcg cggactgctg cagcgcgctg agcgccgtgt cgcacgagtg
cgcctgcagc
      301 accatgggca tcatcaacag cctgcccggc cggtgccacc tcgcccaagc
caactgctcc
      361 gcttgaagca gggacctggc acgcgtgctg caatggatgg caggagggga
gaggaataag
      421 aagtgtttcc atttcacagt gagagcagtc gagctccaac gttgtcgtcg
tcgtcgtctt
      481 cttcttttga tattcagact ctgtcttgcg gtctatatca tcagcataat
aataataaaa
      541 taagtaaaac c
```

```
LOCUS       EF432573                683bp           linear   PLN 23-SEP-
2008
DEFINITION  Triticum aestivum lipid transfer protein , complete cds.
ACCESSION   EF432573
VERSION     EF432573.1  GI:127519389
KEYWORDS    .
SOURCE      Triticum aestivum (bread wheat)
ORGANISM  Triticum aestivum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceæ;
BEP
clade; Pooideae; Triticeae; Triticum.
REFERENCE   1  (bases 1 to 683)
  AUTHORS   Kirubakaran,S.I., Begum,S.M., Ulaganathan,K. and Sakthivel,N.
```

...tion of a new antifungal lipid transfer protein

wheat
JOURNAL   Plant Physiology.Biochem. 46 (10), 918-927 (2008)
   PUBMED   18595724
REFERENCE   2 (bases 1 to 683)
  AUTHORS   Sakthivel,N., Li,W.L., Gill,B.S. and Muthukrishnan,S.
  TITLE   Isolation and characterization of wheat protein gene with high
homology to lipid transfer protein
  JOURNAL   Unpublished
REFERENCE   3 (bases 1 to 683)
  AUTHORS   Sakthivel,N., Li,W.L., Gill,B.S. and Muthukrishnan,S.
  TITLE   Direct Submission
  JOURNAL   Submitted (10-FEB-2007) Department of Biotechnology, Pondicherry
            University, Kalapet, Pondicherry 605014, India
FEATURES   Location/Qualifiers
source   1..683
                    /organism="Triticum aestivum"
                    /mol_type=""
                    /cultivar="Sumai 3"
                    /db_xref="taxon:4565"
CDS   53..400
                    /note="LTP"
                    /codon_start=1
                    /product="lipid transfer protein"
                    /protein_id="ABO28527.1"
                    /db_xref="GI:127519390"

/translation="MARSALAQVVLVAVVAAMLLAVTEAAVSCGQVSSALSPCISYAR

GNGASPSAACCSGVRSLVSSARSTADKQAACKCIKSAAAGLNAGKAAGIPTKCGVSVP
                    YAISSSVDCSKIR"
ORIGIN
        1 ctcatcatct ctgcctgagc tcactaccac tactattgct agcttgatcg
agatggcccg
       61 ttctgctctt gctcaggtcg tgctcgtcgc cgtggtggct gctatgctcc
tcgcagtcac
      121 ggaggcggct gtatcgtgcg gtcaggtgag ctctgccttg agcccctgca
tctcctatgc
      181 acgcggcaac ggcgccagcc catctgcggc ctgctgcagc ggcgttagga
gtctagtcag
      241 ctcagcccgg agcaccgctg acaagcaagc ggcgtgcaag tgcatcaaga
gcgctgctgc
      301 tgggctcaac gctggcaagg ccgccggcat ccccacaaag tgcggcgtta
gcgtccctta
      361 cgccatcagc tcttcggtcg actgctctaa gattcgctga tcgagcactt
gctgccatcg
      421 ctgttgccat cgtcccctac gccatcgttg ctggatctac gcttagtacg
ttgaggtcac
      481 acacacgcac acccacatat atatatgaat aaatgctctc atattatctc
actgcgtgag
      541 agagagagga gtacgtacgt ccaagcagct ctgcatggcc ggccacactg
ttgtatcgat
      601 gtttggttgt tcttccactc cccgagtttg ctgtacttcg taccatgtgt
acttttgata
      661 tatggattgt atactcagct gat

```
LOCUS       X96979                         755bp         linear   PLN 18-APR-
2005
DEFINITION  H.vulgare for lipid transfer protein 7a2b.
ACCESSION   X96979
VERSION     X96979.1  GI:1261916
KEYWORDS    lipid transfer protein.
SOURCE      Hordeum vulgare subsp. vulgare (domesticated barley)
ORGANISM    Hordeum vulgare subsp. vulgare
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Pooideae; Triticeae; Hordeum.
REFERENCE   1
  AUTHORS   Hollenbach,B., Schreiber,L., Hartung,W. and Dietz,K.J.
  TITLE     Expression of lipid transfer protein (ltp) is stimulated by
cadmium, but not nickel and zinc: Implications for the involvement
of LTP in wax assembly
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 755)
  AUTHORS   Hollenbach,B.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-MAR-1996) B. Hollenbach, Julius-von-Sachs
Institute,
            Botanik I, Mittlerer Dallenbergweg 64, 97082 Wuerzburg, FRG
FEATURES             Location/Qualifiers
source          1..755
                /organism="Hordeum vulgare subsp. vulgare"
                /mol_type=""
                /cultivar="Gerbel"
                /sub_species="vulgare"
                /db_xref="taxon:112509"
                /clone_lib="lambda-XR Uni-Zap"
                /dev_stage="primary leaves"
CDS             96..464
                /note="non-specific"
                /codon_start=1
                /product="lipid transfer protein 7a2b"
                /protein_id="CAA65680.1"
                /db_xref="GI:1261917"
                /db_xref="GOA:Q42848"
                /db_xref="HSSP:Q42952"
                /db_xref="InterPro:IPR000528"
                /db_xref="InterPro:IPR003612"
                /db_xref="UniProtKB/TrEMBL:Q42848"

/translation="MARLNSKAVAAAVVLAAVVLMMAGREASAALSCGQVDSKLAPCV

AYVTGRASAISKECCSGVQGLNGLARSSPDRQTACRCLKSLATSIKAINMGKVSGVPG
                KCGVSVPFPISMSTDCNKVH"
ORIGIN
        1 tgcatgtaca gtgcagggca accgtaccct gcctagatac acacacatca
ccacacccac
```

```
              cgctagc cagatagctt aaccgatggc tcgtctcaac
       121 gggggggggc ggctgtcctg gcggcggtgg tgctgatgat ggccggcagg
gaggcctcgg
       181 cggcgctgtc gtgcgggcag gtggactcca agctcgcgcc gtgcgtggcg
tacgtcacgg
       241 ggagggcttc tgcgatcagc aaggagtgct gctccggcgt gcaggggctg
aacggcctgg
       301 cccgcagcag cccggaccgg cagacggcgt gcaggtgcct caagagcctc
gctaccagca
       361 tcaaggccat caacatgggc aaggtctccg gagtgcccgg caagtgcggc
gtcagcgtgc
       421 ccttccccat cagcatgtcc accgactgca acaaggtcca ctagcaggga
tcagatactc
       481 cttcctacgt gcatgcatgg acgcgcttgt gtggagctta atatctacgt
tgatggagtg
       541 ctcatacgta cgatattgag caataaaaaa taaaataaaa gagtatgcct
atgtctgtac
       601 cttgcacaca cactgtgtgt gtgcgcatat atatttacgc ggcatgcatg
cacgcgtccg
       661 tatgagtgac tgaacagtca tctccttgct gtactttagc cgatgtgtat
gtgtgatgtg
       721 tctgacctac ctgttcaggc aattaatgaa tagta
```

232

```
                    ...TGCCGGAACCCGGCACGAATCATCATCAACCAATTCGCACCGATCGATCG
                    ...GCAGAAAGCAGCCGCAGCCCCAGCCCCCACAAGACGAGGCAACAATGGCG
                    ...CTGCCTGAGCTCACTACCACTACTATTGCTTAGCTTGATCGAGATGGCCCG
                    ...GTGCAGGGCAACCGTACCCTGCCTAGATACACACACATCACCACACCCAC

Oryza      ATCGATCCAGCAACTAGATCAACAGTACTAGCTAGGAAGCATGGCCCGTGCACAGTTGGT
Zea        CTAGAAGCAGCCACCGCCCCCCGCGCACTCCTCGCCGCGTGCCTCGTCCTGCTGGTCCTC
Triticum   TTCTGCTCTTGCTCAGGTCGTGCTCGTCGCCGTGGTGGCTGCTATGCTCCTCGCAGTCAC
Hordeum    TCATCTGATCACCTGCTAGCCAGATAGCTTAACCGATGGCTCGTCTCAACAGCAAGGCTG
                                    *

Oryza      GTTGGTCGCCCTGGTGGCAGCTCTGCTCCTCGCCGCCCCGCACGCCGCCGTGGCCATCAC
Zea        CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
Triticum   GGAGGCGGCTGTATCGTGCGGTCAGGTGAGCTCTGCCTTGAGCCCCTGCATCTCCTATGC
Hordeum    TGGCGGCCGCCGTTGTCCTGGCGGCGGTGGTGCTGATGATGGCCGGCAGGGAGGCCTCGG
                  *                *                    *           *

Oryza      CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
Zea        GTTGGTCGCCCTGGTGGCAGCTCTGCTCCTCGCCGCCCCGCACGCCGCCGTGGCCATCAC
Triticum   CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
Hordeum    GTTGGTCGCCCTGGTGGCAGCTCTGCTCCTCGCCGCCCCGCACGCCGCCGTGGCCATCAC
              *    *  *       *       *  **    *      *  *     *

Oryza      GCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCAGCAAGCACGACCGC
Zea        CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
Triticum   GTTGGTCGCCCTGGTGGCAGCTCTGCTCCTCGCCGCCCCGCACGCCGCCGTGGCCATCAC
Hordeum    CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
                                        *            **            *

Oryza      AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Zea        GCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCTGCAGCAAGCACGACCGC
Triticum   CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG
Hordeum    GCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCTGCAGCAAGCACGACCGC
                      *   **  *      *   *              *            *

Oryza      CGCCGGCAACGCCGCCAGCATCCCCTCCAAGTGCGGCGTCAGCGTCCCCTACACCATCAG
Zea        AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Triticum   GCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCTGCAGCAAGCACGACCGC
Hordeum    AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
                    **    *  ***   *    *      *  *    *              *

Oryza      CGCTTCCATCGATTGCTCCAGGGTGAGCTGAGCCATCGATCAGAGACGGATCATATATAT
Zea        GACCCCAGCGCGGACTGCTGCAGCGCGCTGAGCGCCGTGTCGCACGAGTGCGCCTGCAGC
Triticum   AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Hordeum    CTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCCGG

Oryza      AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Zea        AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Triticum   TATGGATTGTATACTCAGCTGATCAACAGCCTGCCCGGCCGGTGCCACCTCGCCCAAGCC
Hordeum    GCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCTGCAGCAAGCACGACCGC
                  *   **                     *              *

Oryza      CTTCCTACGTGCATGCATGGACGCGCTTGTGTGGAGCTTAATATCTACGTTGATGGAGTG
Zea        CTTCCTACGTGCATGCATGGACGCGCTTGTGTGGAGCTTAATATCTACGTTGATGGAGTG
Triticum   GCTTGAAGCAGGGACCTGGCACGCGTGCTGCAATGGATGGCAGGAGGGGAGAGGAATAAG
Hordeum    AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
                      *          **


Oryza      CTCATACGTACGATATTGAGCAATAAAAAAATAAAATAAAAGAGTATGCCTATGTCTGTAC
Zea        CTCATACGTACGATATTGAGCAATAAAAAAATAAAATAAAAGAGTATGCCTATGTCTGTAC
Triticum   AAGTGTTTCCATTTCACAGTGAGAGCAGTCGAGCTCCAACGTTGTCGTCGTCGTCGTCTT
Hordeum    CTTCCTACGTGCATGCATGGACGCGCTTGTGTGGAGCTTAATATCTACGTTGATGGAGTG
                      *                                              *

Oryza      CTTGCACACACACTGTGTGTGTGCGCATATATATTTACGCGGCATGCATGCACGCGTCCG
Zea        CTTGCACACACACTGTGTGTGTGCGCATATATATTTACGCGGCATGCATGCACGCGTCCG
Triticum   CTTCTTTTGATATTCAGACTCTGTCTTGCGGTCTATATCATCAGCATAATAATAATAAAA
Hordeum    CTCATACGTACGATATTGAGCAATAAAAAAATAAAATAAAAGAGTATGCCTATGTCTGTAC
              **          *      *                    **

Oryza      AGAGCTAGAGATTCTACATGCATGTACTGCAACTCCTTGTACTACGTGCTTGTTTTGGAT
Zea        GCTTGAAGCAGGGACCTGGCACGCGTGCTGCAATGGATGGCAGGAGGGGAGAGGAATAAG
Triticum   AGACCGGCGCACCGCCTGCAACTGCCTCAAGAACGCGGCCCGCGGCATCAAGGGGCTCAA
Hordeum    CTTGCACACACACTGTGTGTGTGCGCATATATATTTACGCGGCATGCATGCACGCGTCCG

Oryza      TATTACACATAAAAAAAAAA----------------------------------------
Zea        AAGTGTTTCCATTTCACAGTGAGAGCAGTCGAGCTCCAACGTTGTCGTCGTCGTCGTCTT
Triticum   CTTCCTACGTGCATGCATGGACGCGCTTGTGTGGAGCTTAATATCTACGTTGATGGAGTG
Hordeum    TATGAGTGACTGAACAGTCATCTCCTTGCTGTACTTTAGCCGATGTGTATGTGTGATGTG

Oryza      ------------------------------------------------------------
Zea        CTTCTTTTGATATTCAGACTCTGTCTTGCGGTCTATATCATCAGCATAATAATAATAAAA
Triticum   CTCATACGTACGATATTGAGCAATAAAAAAATAAAATAAAAGAGTATGCCTATGTCTGTAC
Hordeum    TCTGACCTACCTGTTCAGGCAATTAATGAATAGTA-------------------------

Oryza      ------------------------------------------------------------
Zea        TAAGTAAAACC-------------------------------------------------
Triticum   CTTGCACACACACTGTGTGTGTGCGCATATATATTTACGCGGCATGCATGCACGCGTCCG
Hordeum    ------------------------------------------------------------

Oryza      ------------------------------------------------------------
Zea        ------------------------------------------------------------
Triticum   ACACACGCACACCCACATATATATATGAATAAATGCTCTCATATTATCTCACTGCGTGAG
Hordeum    ------------------------------------------------------------

Oryza      ------------------------------------------------------------
Zea        ------------------------------------------------------------
Triticum   AGAGAGAGGAGTACGTACGTCCAAGCAGCTCTGCATGGCCGGCCACACTGTTGTATCGAT
Hordeum    ------------------------------------------------------------

Oryza      ------------------------------------------------------------
Zea        ------------------------------------------------------------
Triticum   GTTTGGTTGTTCTTCCACTCCCCGAGTTTGCTGTACTTCGTACCATGTGTACTTTTGATA
Hordeum    ------------------------------------------------------------

Oryza      ------------------------
Zea        ------------------------
Triticum   TATGGATTGTATACTCAGCTGAT
Hordeum    ------------------------
```

233

PR10 genes from gene bank and clustal alignment

```
LOCUS          AB127580                744bp         linear   PLN 15-FEB-
2008
DEFINITION  Oryza sativa Japonica Group RSOsPR10  for root specific
pathogenesis-related protein 10, complete cds.
ACCESSION   AB127580
VERSION     AB127580.1  GI:38678113
KEYWORDS    .
SOURCE      Oryza sativa Japonica Group
ORGANISM  Oryza sativa Japonica Group
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae;
BEP
clade; Ehrhartoideae; Oryzeae; Oryza.
REFERENCE    1
  AUTHORS   Hashimoto,M., Kisseleva,L., Sawa,S., Furukawa,T., Komatsu,S.
and
            Koshiba,T.
  TITLE     A novel rice PR10 protein, RSOsPR10, specifically induced in
roots
by biotic and abiotic stresses, possibly via the jasmonic acid
signaling pathway
  JOURNAL   Plant Cell Physiol. 45 (5), 550-559 (2004)
   PUBMED   15169937
REFERENCE    2  (bases 1 to 744)
  AUTHORS   Hashimoto,M., Sawa,S., Komatsu,S. and Koshiba,T.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-DEC-2003) Contact:Tomokazu Koshiba Tokyo
Metropolitan
            University, Department of Biological Scienceences; 1-1,
Minami-Osawa,
            Hachioji-shi, Tokyo 192-0397, Japan
FEATURES            Location/Qualifiers
source          1..744
                /organism="Oryza sativa Japonica Group"
                /mol_type=""
                /cultivar="Nipponbare"
                /db_xref="taxon:39947"
gene            1..744
                /gene="RSOsPR10"
CDS             80..562
                /gene="RSOsPR10"
                /codon_start=1
                /product="root specific pathogenesis-related protein
10"
                /protein_id="BAD03969.1"
                /db_xref="GI:38678114"

/translation="MAPVSISDERAVSVSAERVWKVFSDAPAMPKVCAGFIDAIEVEG

DGGAGTVTTMKLNPAVDDGGSFKTRVVARDNAAHIIKSEVLDVPAGSKVGKLKSHVTE

TKIEAAGAGSCLAKINVEYELEDGGSLSPEKEKLILDGYFGMLKMIEDYLVAHPTEYA"
polyA_signal    703..708
                /gene="RSOsPR10"
```

```
             caactag gtatctagct aagcagtggt gtgatcagta
ggaagctgca
        61 ggtgggggat atatcaccaa tggctccggt cagcatctcc gacgagcgcg
ccgtctcggt
       121 gtcggcggag cgggtgtgga aggtcttctc cgacgcgccc gccatgccca
aggtttgtgc
       181 cggcttcatc gacgccattg aggtcgaggg ggatggcggg gcgggcactg
tcaccaccat
       241 gaagctcaac cctgctgtgg atgatggggg gtcattcaaa acacgtgtgg
tggcacgtga
       301 caacgcagct cacattatca agtcagaggt tctggatgtg ccggccggaa
gtaaagtggg
       361 caagctcaag tcgcacgtga cagagacgaa gatcgaggcc gccggtgccg
gctcttgctt
       421 ggccaagata aacgtggagt atgagctcga ggacggcggc tcactgtcgc
cggagaagga
       481 gaagctcatc ctcgacggct acttcggcat gctcaagatg atcgaggact
acctcgtcgc
       541 tcaccctacc gagtatgctt aaaaattgtc ataaaccaaa ataatataca
tccatctccg
       601 tattgctgct tcctgataat taaataatgt gagccacgac aaatccaatc
ttttgtggtg
       661 tttgattttg tgagagtgat ttgtgtttga ggttatgtaa gaaataaatc
ataattgtga
       721 tcgtgttcta aaaaaaaaaa aaaa
```

LOCUS   EU908212   483bp   linear   PLN 18-AUG-2008
DEFINITION Triticum aestivum cultivar Xingzi9104 pathogenesis related protein
            10 (PR10) , complete cds.
ACCESSION   EU908212
VERSION   EU908212.1 GI:196051130
KEYWORDS   .
SOURCE   Triticum aestivum (bread wheat)
  ORGANISM Triticum aestivum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BEP
clade; Pooideae; Triticeae; Triticum.
REFERENCE   1 (bases 1 to 483)
  AUTHORS   Zhang,G., Zhang,Y., Li,Y., Wang,X., Dong,Y., Guo,J., Wei,G.,
            Huang,L. and Kang,Z.
  TITLE   Cloning and Characterization of a Pathogenesis Related Protein Gene
            TaPR10 from Wheat Induced by Stripe Rust Pathogen
  JOURNAL   Zhongguo Nong Ye Ke Xue (2008) In press
REFERENCE   2 (bases 1 to 483)
  AUTHORS   Zhang,G.
  TITLE   Direct Submission
  JOURNAL   Submitted (17-JUL-2008) Plant Protection, Shaanxi Key Laboratory of

...ology for Agriculture, Northwest A&F University, ...ngling, Xi'an, Shaanxi 712100, China

```
FEATURES             Location/Qualifiers
source          1..483
                     /organism="Triticum aestivum"
                     /mol_type=""
                     /cultivar="Xingzi9104"
                     /db_xref="taxon:4565"
                     /PCR_primers="fwd_seq: atggcctccaccgacagc, rev_seq:
tcagttgtaggcgtccggg"
gene            1..483
                     /gene="PR10"
                     /note="TaPR10"
CDS             1..483
                     /gene="PR10"
                     /codon_start=1
                     /product="pathogenesis related protein 10"
                     /protein_id="ACG68733.1"
                     /db_xref="GI:196051131"

/translation="MASTDSWTHEIESPVAAARLFRAGVMDWHTLAPKLAPHIVASAH

PVEGEGGIGSVRQFNFTSAMPFSLMKERLEFIDADKCECKSTLIEGGGIGTAIETTTS

HIKVEPAANGGSVVKVESTYKLLPGVEVNDEITKAKESVTAIFKAAEAYLVANPDAYN"
ORIGIN
        1 atggcctcca ccgacagctg gacccacgag atcgaatcgc cggtggccgc
agcgcgcctc
       61 ttccgcgccg gcgtcatgga ctggcacaca ctggccccta agctcgcacc
gcacatcgtc
      121 gccagcgccc accctgttga gggcgaaggc ggcatcggca gcgtcaggca
gttcaacttc
      181 acctcagcca tgcccttcag cctcatgaaa gagaggctcg agttcatcga
cgccgacaag
      241 tgcgagtgca agtccaccct catcgagggc ggcggcatcg gcaccgccat
cgagacgacc
      301 acctcgcaca tcaaggtgga gccggcggcc aacggtggga gcgtcgttaa
ggtggagtcg
      361 acctacaagc tgctgccggg cgtggaggta aacgatgaga tcaccaaggc
caaggagtct
      421 gtcacggcca tcttcaaggc cgccgaggcc tacctcgtcg ccaacccgga
cgcctacaac
      481 tga
```

```
LOCUS       EU931221                855bp        linear    PLN 01-SEP-
2008
DEFINITION  Rheum australe pathogenesis-related protein 10 , complete
cds.
ACCESSION   EU931221
VERSION     EU931221.1  GI:197312886
KEYWORDS    .
SOURCE      Rheum australe (Rheum emodi)
ORGANISM  Rheum australe
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
```

```
                              a; Magnoliophyta; eudicotyledons; core
Caryophyllales, Polygonaceae; Rheum.
REFERENCE   1  (bases 1 to 855)
  AUTHORS   Ghawana,S., Kumar,S. and Ahuja,P.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (28-JUL-2008) Biotechnology Division, Institute of
            Himalayan Bioresource Technology, Palampur, HP 176061, India
FEATURES             Location/Qualifiers
source          1..855
                     /organism="Rheum australe"
                     /mol_type=""
                     /db_xref="taxon:284363"
CDS             44..526
                     /note="PR10"
                     /codon_start=1
                     /product="pathogenesis-related protein 10"
                     /protein_id="ACH63224.1"
                     /db_xref="GI:197312887"

/translation="MGVQSYSQEISSPVAPGRLFKALCLDSHNFLPKLVPETFKSVEF

VHGDCVAVGAVKQINFSEGTTFKSVKHRVDELDVDKFYYKYTVTEGDVLGDKVEYVVN

EVKFVASGSGSGCSFSNHYHSKEGAVLDEEKIKYGHEKLKGLFKKVEEYLVANPEVYA"
ORIGIN
        1 agactaagta aagcctctcc atcaaccaat ttttcaacaa aaaatgggtg
          tacaatcata
       61 cagccaagaa atttctagcc cagttgcccc aggcaggctg ttcaaggccc
          tgtgcctaga
      121 ctcccacaac ttcctcccca agctcgtccc cgaaaccttc aaatccgtcg
          agtttgtaca
      181 tggcgattgc gttgctgttg gagcagtcaa acagatcaac ttctccgaag
          gaacgacgtt
      241 caagtctgtg aagcacagag tagacgagct tgacgtagac aagttctatt
          acaagtacac
      301 agttacggag ggggacgtcc tgggggacaa ggtggagtac gtggtcaatg
          aggtcaagtt
      361 tgtggcctct gggagcggct ctgggtgcag cttcagcaac cactaccact
          ccaaggaagg
      421 cgccgtgctg gatgaggaga agatcaagta tggacatgag aagttgaagg
          gcttgttcaa
      481 gaaggtggag gagtacctcg ttgccaaccc tgaagtctat gcttaattca
          ccacatccta
      541 cttgtcgtgg ggaggagttc aagtgtgttt ctacgtgtgc atcatcttca
          ttgagtaagt
      601 attgtccttt ttatactctg gttgagtgca ctaaagtgtg gcaataagac
          aatggagttg
      661 taatcagctt gttgtgactt gtgagatatg taatacttgg tttctatgac
          aaataagatc
      721 aataagtaga ctactgttgg gcctaaccca aatcatagct catcatgagg
          aggtcgtctt
      781 tgaaaactct ccaagagtgt attcggagct tattgtctat gcaattatat
          tcattagcaa
      841 atataaaaaa aaaaa
```

```
                                462bp        linear    PLN 16-NOV-
DEFINITION  Lycopersicon chilense pathogenesis related protein PR10-like
,
complete sequence.
ACCESSION   AY899198
VERSION     AY899198.1  GI:66969931
KEYWORDS    .
SOURCE      Solanum chilense (Lycopersicon chilense)
ORGANISM  Solanum chilense
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta;
Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core
eudicotyledons;
asterids; lamiids; Solanales; Solanaceae; Solanoideae; Solaneae;
Solanum; Lycopersicon.
REFERENCE   1  (bases 1 to 462)
  AUTHORS   Tapia,G.M., Yanez,M.L. and Ruiz,S.A.
  TITLE     Identification of genes differentially expressed in plants of
            Lycopersicon chilense by salt stress
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 462)
  AUTHORS   Yanez,M.L., Ruiz,S.A. and Tapia,G.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-JAN-2005) Instituto de Biologia Vegetal y
            Biotecnologia, Universidad de Talca, 2 Norte 685, Talca,
Chile
COMMENT     NCBI staff are still waiting for submitters to provide
appropriate
coding region information.
FEATURES             Location/Qualifiers
source          1..462
                /organism="Solanum chilense"
                /mol_type=""
                /db_xref="taxon:4083"
misc feature    1..462
                /note="similar to pathogenesis related protein PR10"
ORIGIN
        1 atgagtccac aaccacaatt gccccaacaa ggctattcaa aggtttggtt
ccttgatttt
       61 gatagccttg tacctaaatt gttgtcacat gatgttaaga gcattgagat
tgttgagggt
      121 gatggtggtg ctggaagcat cgagcaaatg aactttgttg aaggtggacc
aattaagtgc
      181 ttgaagcgca aaattcatgt gattgatgac aagaatttgg taacaaaata
ttcacttatt
      241 gaaggtgatg ttcttggtga cgaattggaa tcaattggct atgatgtcaa
atttgaagct
      301 gctggagatg gaggttgtgt ttgcaagaca caactgagt atcacacaaa
gggtgatcat
      361 gttgtgagtg aagaagaaca caatgtaggc aaagggaaag ccattgacct
attcaaggcc
      421 atcgaagcgt acctcctcgc taatccttct gtctacgctt aa
```

```
                    CGAGCAACTAGGTATCTAGCTAAGCAGTGGTGTGATCAGTAGGAAGTTGCA
                    ACCGACAGCTGGACCCACGAGATCGAATCGCCGGTGGCCGCAGCGCGCCTC
                    AAAGCCTCTCCATCAACCAATTTTTTCAACAAAAAATGGGTGTACAATCATA
                    CAACCACAATTGCCCCAACAAGGCTATTCAAAGGTTTGGTTCCTTGATTTTT

Oryza         GGTGGGGGATATATCACCAATGGCTCCGGTCAGCATCTCCGACGAGCGCGCCGTCTCGGT
Triticum      TTCCGCGCCGGCGTCATGGACTGGCACACACTGGCCCCTAAGCTCGCACCGCACATCGTC
Rheum         CAGCCAAGAAATTTCTAGCCCAGTTGCCCCAGGCAGGCTGTTCAAGGCCCTGTGCCTAGA
Lycopersicon  GATAGCCTTGTACCTAAATTGTTGTCACATGATGTTAAGAGCATTGAGATTGTTGAGGGT
                                                                        *

Oryza         GTCGGCGGAGCGGGTGTGGAAGGTCTTCTCCGACGCGCCCGCCATGCCCAAGGTTTGTGC
Triticum      GCCAGCGCCCACCCTGTTGAGGGCGAAGGCGGCATCGGCAGCGTCAGGCAGTTCAACTTC
Rheum         CTCCCACAACTTCCTCCCCAAGCTCGTCCCCGAAACCTTCAAATCCGTCGAGTTTGTACA
Lycopersicon  GATGGTGGTGCTGGAAGCATCGAGCAAATGAACTTTGTTGAAGGTGGACCAATTAAGTGC
                                    *                                  *

Oryza         TGGCGATTGCGTAAATGTTGGAGCAGTCAAACAGATCAACGCGGGCACTGTCACCACCAT
Triticum      TGGCGATTGCGTTGCTGTTGGAGCAGTCAAACAGATCAACAGTTCATCGACGCCGACAAG
Rheum         TGGCGATTGCGTTGCTGTTGGAGCAGTCAAACAGATCAACTTCTCCGAAGGAACGACGTT
Lycopersicon  TGGCGATTGCGTTGCTGTTGGAGCAGTCCAACAGATCAACTAACAAAATATTCACTTATT
              *************     *************  ***********

Oryza         GAAGCTCAACCCTGCTGTGGATGATGGGGGGTCATTCAAAACACGTGTGGTGGCACGTGA
Triticum      GAAGCTCAACAGTCAGAGGTGAGGCTCGGTCATTCAAAACACGTGTGGTGGCACGTGACA
Rheum         GAAGCTCAACCCTGCTGTGGATGATGGGGGGTCATTCAAAACACGTGTGGTGGCACGTGA
Lycopersicon  GAAGCTCAACCGAGCAAATGATGATGGGGGGTCATTCAAAACACGTGTGGTGGCACGTGA
              **********                *    **          ** **  ***  *    *   *

Oryza         CAACGCAGCTCACATTATCAAGAATCCAGGTGAGGCTCGCCGGCCGGAAGTAAAGTGGGC
Triticum      ACGCAGCTCACATTATCAAGTCAGAGGTTTCTGGATGTGCCGGCCGGAAGTAAAGTGGGCA
Rheum         CAACGCAGCTCACATTATCAAGTCAGAGGTGAGGCTCGCCGGCCGGAAGTAAAGTGGGCA
Lycopersicon  CAACGCAGCTCACATTATCAAGTCAGAGGTGAGGCTCGAGTTCATCGAGTAAAGTGGGCA
               *        * **   *                        *      **    ** **

Oryza         AAGCTCAAGTCGCACGTGACAGAGACGAACAATGTAGGCAAAGGGAAAGCCATTGACCTA
Triticum      AGCTCAAGTCGCACGTGACAGAGACGAACAATGTAGGCAAAGGGAAAGCCATTGACCTAT
Rheum         AGCTCAAGTCGCACGTGACAGAGACGAACAATGTAGGCAAAGGGAAAGCCATTGACCTAT
Lycopersicon  AGCTCAAGTCGCACGTGACAGAGACGAACAATGTAGGCAAAGGGAAAGCCATTGACCTAT
               *       *                  *   *  ** ** **    *    *    *

Oryza         TTCAAGGCCATCGAAGCGTACCTCCTCGCTAATCCTTCTGTCTACGCTTAAGAAGCTCAT
Triticum      TCAAGGCCATCGAAGCGTACCTCCTCGCTAATCCTTCTGTCTACGCTTAATGA-------
Rheum         TCAAGGCCATCGAAGCGTACCTCCTCGCTAATCCTTCTGTCTACGCTTAAGAAGGTGGAG
Lycopersicon  TCAAGGCCATCGAAGCGTACCTCCTCGCTAATCCTTCTGTCTACGCTTAA----------
               *  *  *  *                   *     *  *  *             *  *

Oryza         CCTCGACGGCTACTTCGGCATGCTCAAGATGATCGAGGACTACCTCGTCGCTCACCCTAC
Triticum      ------------------------------------------------------------
Rheum         GAGTACCTCGTTGCCAACCCTGAAGTCTATGCTTAATTCACCACATCCTACTTGTCGTGG
Lycopersicon  ------------------------------------------------------------

Oryza         CGAGTATGCTTAAAAATTGTCATAAACCAAAATAATATACATCCATCTCCGTATTGCTGC
Triticum      ------------------------------------------------------------
Rheum         GGAGGAGTTCAAGTGTGTTTTCTACGTGTGCATCATCTTCATTGAGTAAGTATTGTCCTTT
Lycopersicon  ------------------------------------------------------------

Oryza         TTCCTGATAATTAAATAATGTGAGCCACGACAAATCCAATCTTTTGTGGTGTTTGATTTT
Triticum      ------------------------------------------------------------
Rheum         TTATACTCTGGTTGAGTGCACTAAAGTGTGGCAATAAGACAATGGAGTTGTAATCAGCTT
Lycopersicon  ------------------------------------------------------------

Oryza         GTGAGAGTGATTTGTGTTTGAGGTTATGTAAGAAATAAATCATAATTGTGATCGTGTTCT
Triticum      ------------------------------------------------------------
Rheum         GTTGTGACTTGTGAGATATGTAATACTTGGTTTCTATGACAAATAAGATCAATAAGTAGA
Lycopersicon  ------------------------------------------------------------

Oryza         AAAAAAAAAAAAAAA---------------------------------------------
Triticum      ------------------------------------------------------------
Rheum         CTACTGTTGGGCCTAACCCAAATCATAGCTCATCATGAGGAGGTCGTCTTTGAAAACTCT
Lycopersicon  ------------------------------------------------------------

Oryza         ------------------------------------------------------------
Triticum      ------------------------------------------------------------
Rheum         CCAAGAGTGTATTCGGAGCTTATTGTCTATGCAATTATATTCATTAGCAAATATAAAAAA
Lycopersicon  ------------------------------------------------------------

Oryza         -----
Triticum      -----
Rheum         AAAAA
Lycopersicon  -----
```

12.5 SDS-PAGE Gel (SDS PAGE Bio-Rad system) and n)

i. 1.5 M Tris-HCL pH 8.8

36.3 g of Trise base was dissolved in 100 ml of sdH2O and pH was adjusted to 8.8 with 10M NaOH before made up to 200 ml with sdH2O.

ii.0.5 M Tris –HCl,pH 6.8 6.057 of Tris base was dissolved in 40 ml of sdH2O and pH was adjusted tp 6.8 with 10M NaOH before made up to 100 ml with sdH2O.

iii.10% APS (w/v)

1.0 g of ammonium persulphate (APS) was dissolved in 10 ml of sdH2O. The solution was prepared fresh each time prior to use.

iv.10% SDS (w/v)

10.0 g of SDS were dissolved in 100 ml of sdH2O. The solution was kept in room temperature.

v.3X sample loading buffer

1 M Tris –Cl pH 6.8	2.4 ml
10% SDS	3.0 ml
Glycerol	3.0 ml
DTT	1.6 ml
Bromophenol Blue	0.006 g

All reagntes were mixed togather and top up to 10.0 ml with sdH2O. The solution was kept in 4 ºC and was diluted to 1X prior to use.

vi.10 X Electrophoresis buffer

	30.0g
Glycine	14.4 g
SDS	1.0 g

All the reagents were mixed together and top up to 1 liter with dH2O.

vii. Coomasie Blue staining

Coomasie Blue 250	0.5 g
Methanol	800 ml
Acetic acid	140 ml

Coomasie Bluewas stirred in methanol until dissolved. Acetic acid was added into the solution and the solution was top up to 2 Liters with sdH2O.

viii. Distaining Solution I

| Methanol | 500.0 ml |
| Acetic Acid | 100.0 ml |

The reagents were mixed together and top up to 1 liter with sdH2O.

ix. Distaining Solution II

| Methanol | 50.0 ml |
| Acetic Acid | 70.0 ml |

The reagents were mixed together and top up to 1 litter with sdH2O.

electrophoresis

I. Sample Buffer (9M urea .0 mM DTT, 2% (v/v) IPG buffer (4-7 or 3-10) and 0.5% (v/v) Triton-X 100)

Urea	2.7 g
DTT	50.00 g
Triton X-100	0.026 ml

All chemicals were mixed and made up to 5 ml with sdH2O. The solution was aliquoted into 200 µl and stored at -20 ºC was kept for up to two month.

ii.Lysis Buffer (7 M urea,2 M Thiourea,4% CHAPS)

Urea	4.2 g
Thiourea	1.5 g
CHAPS	0.4g

All chemicals were mixed together and the solution to up to 10 ml. The solution was aliquot in 1.5 ml and stored -20 ºC. Protease Inhibitor was added prior to use.

iii. Rehydration Buffer (7 M urea,2 M Thiourea,4% CHAPS ,0.002% bromophenol blue)

Urea	4.2 g
Thiourea	1.5 g
CHAPS	0.4g

20µ of 1% solution

All chemicals were mixed together and the solution to up to 10 ml. The solution was aliquoted in 1.5 ml and stored -20 ºC. 5% IPG buffer were added prior to use.

Appendix I: Reagents for 2nd Dimension electrophoresis

I. Equilibration buffer **(**6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% glycerol, 2% SDS, 0.002% bromophenol blue, and 200 m).

Urea (FW 60.06)	72.1 g
Tris-HCl, pH 8.8	10.0 ml
Glycerol (87% w/w)	69 ml (84.2g)
SDS (FW 288.38)	4.0 g
1% Bromophenol blue stock solution	400 µl

All chemicals were mixed together and the solution to up to 200 ml. This is a stock solution. Just prior to use, add DTT or iodoacetamide the solution was aliquoted 20- or 50-ml aliquots at -20 °C.

ii. The solution was aliquoted (1.5 M Tris base, pH 8.8, 1 l)

Tris base (FW 121.1)	181.7 g
Double-distilled water	750 ml
HCl	adjust to pH 8.8

ether and the solution to up to 1 l. The solution was filtered through 0.45-µm filters. Store at 4 °C.
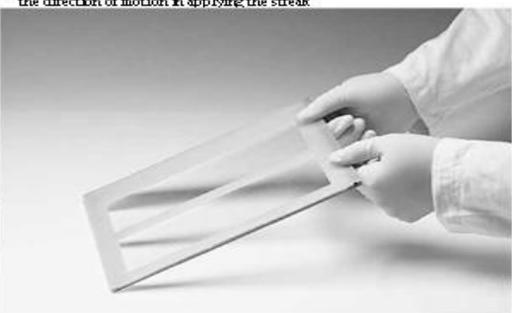
Appendix J: Gel assembly

1 Filling the Ettan DALT*six* electrophoresis unit with anode buffer
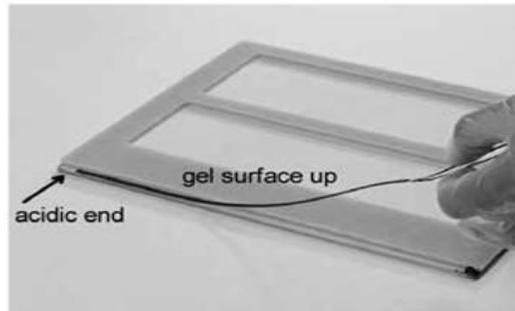
2 Pipetting a streak of gel buffer onto the glass plate. The arrow indicates the direction of motion in applying the streak
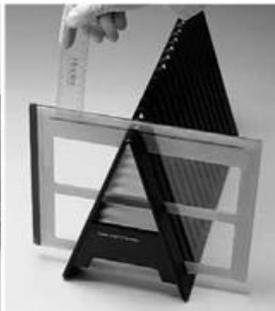
3. Pressing out air pockets between gel and glass plate.

4. Closing the DALT Precast Gel Cassette.

gel surface up

acidic end

5. Positioning an equilibrated Immobiline DryStrip gel on the DALT Precast Gel Cassette.

6. Pushing the Immobiline DryStrip gel down to contact the

7 Sealing the Immobiline DryStrip gel in place on a DALT precast gel using agarose sealing solution.

8. Inserting the cassettes into the cassette carrier

9. Seating the upper buffer chamber.

Appendix K1: Commasei Staining solutions

i. Commasei Staining solution

Commasie brilliant blue R   0.5 g

Methanol   40.00 ml

Acetic acid   7.00 ml

All the solution was mixed and top up to 100 ml with sdH2O. The solution was filtered through Whatman No1 filter to remove any particular matter.

ii. Fixative solution

Ethanol   25 ml

Acetic acid (absolute)   5 ml

iii. Preserving solution (10% (v/v) glycerol)

Glycerol (100 ml) was mixed and top up to 1 liter with sdH2O.

Appendix K2: Reagents for silver staining

i. Fixing solution (40% (v/v) ethanol, 10 % (v/v) acetic acid)

Ethanol   400 ml

Acetic acid   100 ml

All the solution was mixed and top up to 1 litter with sdH2O.

% (v/v) ethanol,0.5 M sodium acetate ,8mM sodium thiosulphate 0.13% (v/v) Formaldehyde)

Ethanol	300 ml
Soduim Acetate	68 g
Soduim thyosulphate	20 g

All the soultions was mixed and top up to 1 liter with sdH2O. Formaldehyde was added just prior to use.

iii. Silver solution (5.9 mM silver nitrate ,0.02% (v/v) Formaldehyde)

Silver nitrate (1g) was dissolved in 1 liter of sdH2O. Formaldehyde was added just prior to use.

iv. Developing solution (0.24 M sodium carbonate ,0.2% (v/v) formaldehyde)

Soduim carbonate (15 g) was dissolved in 1 liter of sdH2O,100 µl of formaldehyde was added to the solution prior to use.

v. Stopping solution (40mM EDTA)

EDTA (14.6 g) was dissolved and top up to 1 liter with sdH2O.

vi. Preserving solution (10% (v/v) glycerol)

Glycerol (100 ml) was mixed and top up to 1 liter with sdH2O.

Appendix L: Purity and concentration of RNA and cDNA from oil palm roots

Appendix L1: Purity and concentration of RNA from oil palm roots

Time of infection	Purity A260/A280	Concentration µg/µl	Control	Purity A260/A280	Concentration µg/µl
0 week/1	1.96	1.26	0 week	1.87	1.827
0 week/2	1.847	0.68	0 week/2	1.89	0.45
0 week/3	1.809	0.98	0 week/3	2.012	1.2
0 week/4	1.97	0.23	0 week/4	2.0	1.23
2 weeks/1	1.87	0.847	2 weeks/1	1.82	0.744
2 weeks/2	1.96	0.91	2 weeks/2	1.92	1.37
2 weeks/3	1.87	1.4	2 weeks/3	1.902	0.54
2 weeks/4	1.807	0.65	2 weeks/4	1.813	0.733
4 weeks/1	1.84	1.48	4 weeks/1	1.80	0.853
4 weeks/2	2.01	0.19	4 weeks/2	1.72	0.247
4 weeks/3	1.97	0.85	4 weeks/3	1.96	0.74
4 weeks/4	1.78	0.612	4 weeks/4	2.03	1.3
6 weeks/1	1.80	0.667	6 weeks/1	1.81	0.392

		0.79	6 weeks/2	1.87	0.48
6 weeks/3	1.802	1.54	6 weeks/3	1.89	0.954
6 weeks/4	1.74	1.025	6 weeks/4	1.7805	0.53
8 weeks/1	1.85	0.43	8 weeks	1.88	0.139
8 weeks/2	1.74	1.06	8 weeks/2	1.78	0.42
8 weeks/3	1.65	0.98	8 weeks/3	1.9	0.85
8 weeks/4	1.87	1.4	8 weeks/4	2.01	1.0

Appendix L2: Purity and concentration of constructed cDNA from oil palm roots

Time of infection	Purity A260/A280	Concentration ng/µl	Control	Purity A260/A280	Concentration ng/µl
0 week/1	1.74	1689	0 week	1.92	1899
0 week/2	1.72	1298	0 week/2	1.73	1053
0 week/3	1.8	1650	0 week/3	1.77	1334
0 week/4	1.79	1331	0 week/4	1.76	1341
2 weeks/1	1.70	1562	2 weeks/1	1.79	1782
2 weeks/2	1.96	1091	2 weeks/2	1.92	1094

|					
		1400	2 weeks/3	1.902	1762
2 weeks/4	1.807	1365	2 weeks/4	1.813	1387
4 weeks/1	1.73	1730	4 weeks/1	1.71	1699
4 weeks/2	2.01	1019	4 weeks/2	1.72	1740
4 weeks/3	1.97	1085	4 weeks/3	1.96	1790
4 weeks/4	1.78	1612	4 weeks/4	2.03	1677
6 weeks/1	1.72	1627	6 weeks/1	1.77	1563
6 weeks/2	1.95	1450	6 weeks/2	1.87	1234
6 weeks/3	1.802	1334	6 weeks/3	1.89	1766
6 weeks/4	1.74	1087	6 weeks/4	1.7805	1340
8 weeks/1	1.74	1540	8 weeks	1.75	1834
8 weeks/2	1.74	1445	8 weeks/2	1.78	1098
8 weeks/3	1.65	670	8 weeks/3	1.9	1699
8 weeks/4	1.87	1865	8 weeks/4	1.76	1345

ncentration of RNA from oil palm roots extracted after tation) time

Time of incubation	Purity A260/A280	Concentrationµg/µl
Over night 1	1.09	.37
Over night 2	1.17	0. 47
Over night 3	1.081	0.08
2 days1	1.23	0.23
2 days 2	1.37	0. 784
2 days 3	1.11	0.904
4 days 1	1.87	1.4
4 days 2	1.807	1.68
4 days 3	1.89	1.55
6 days 1	1.85	1.68
6 days 2	1.78	1.47
6 days 3	1.80	1.66

lts

PGIP

sis
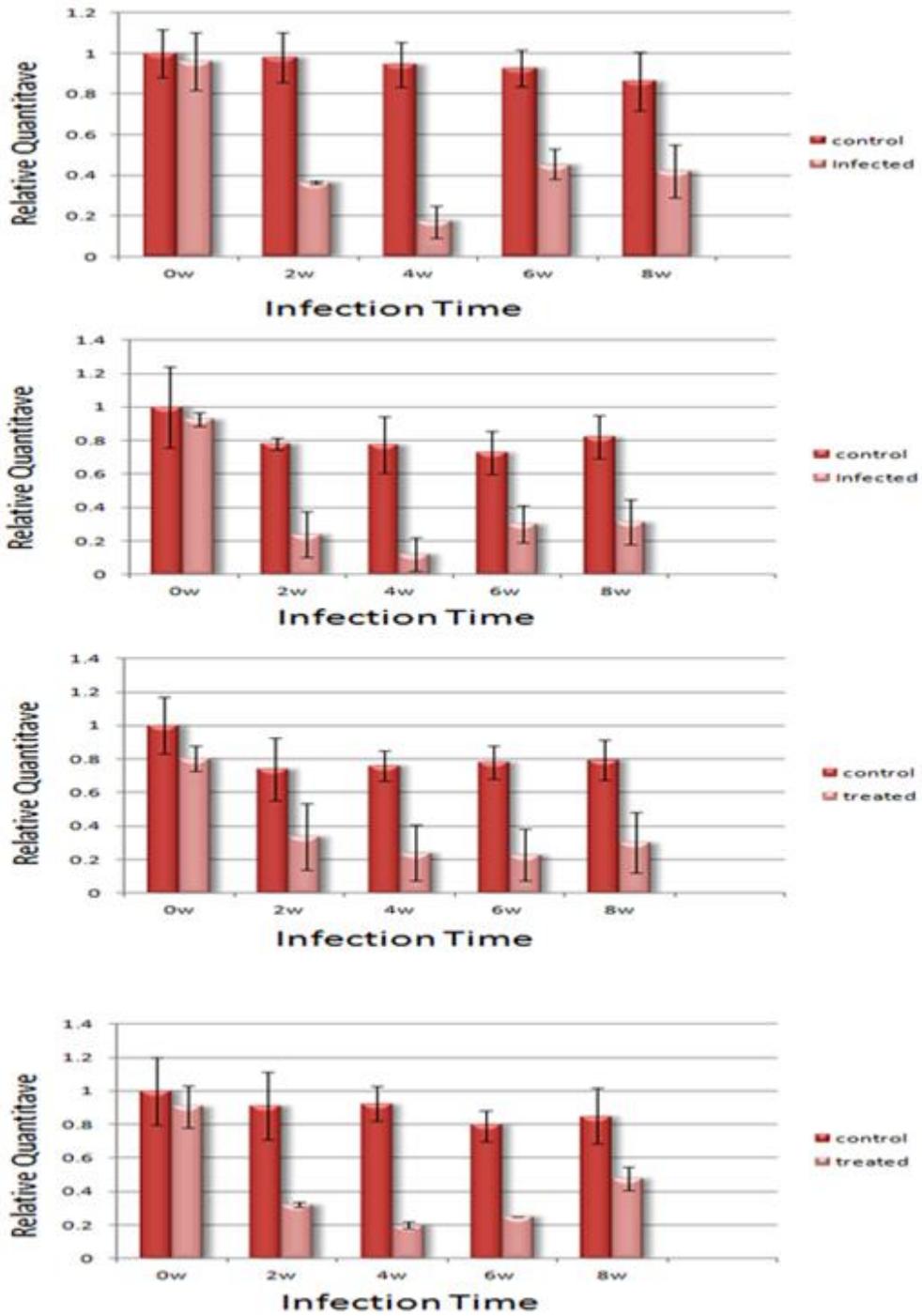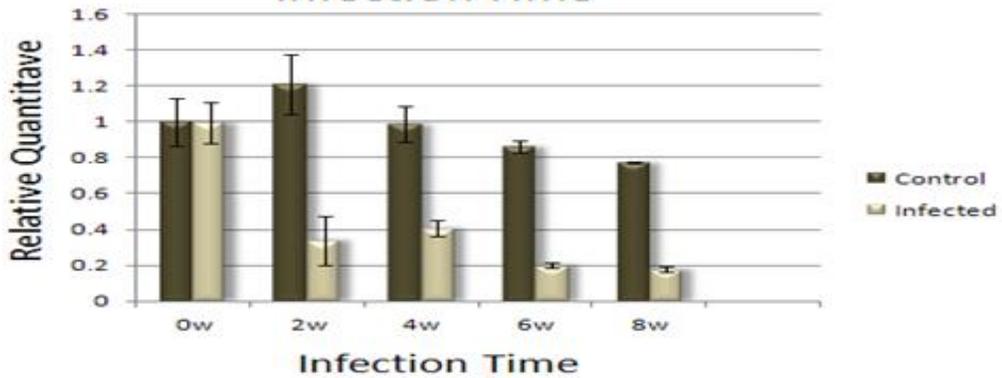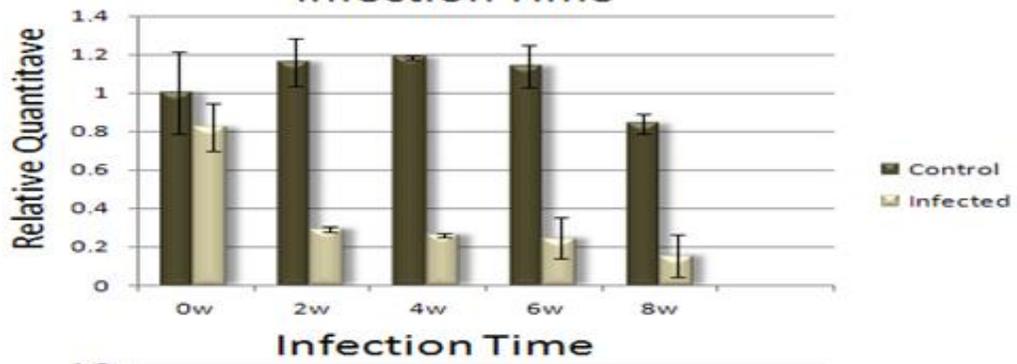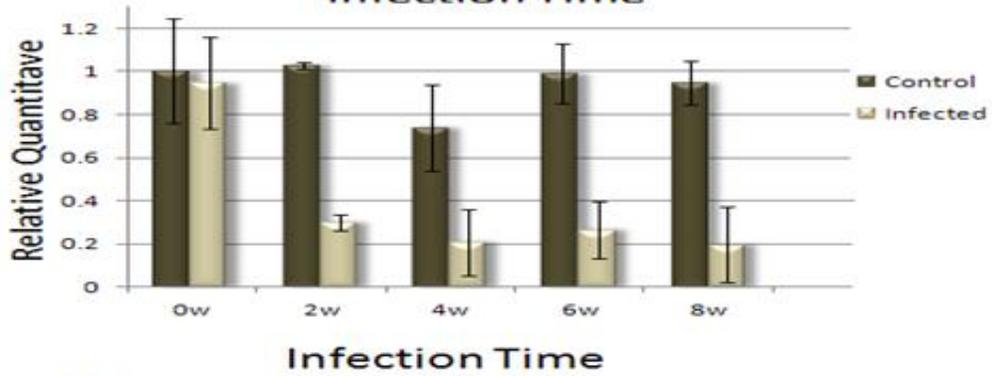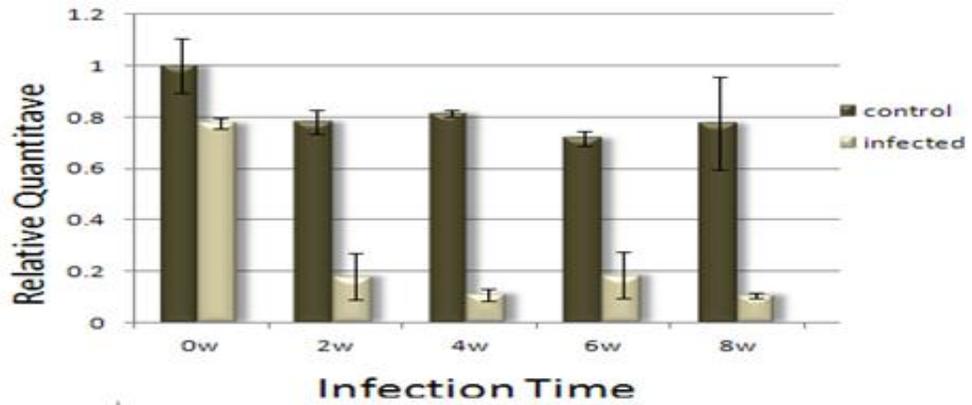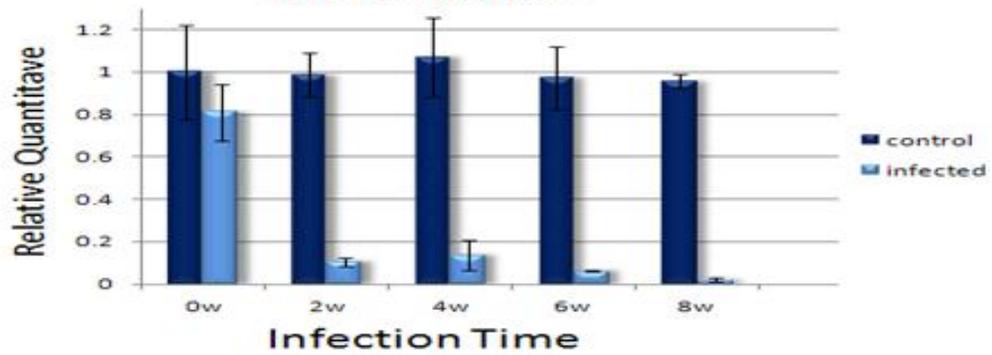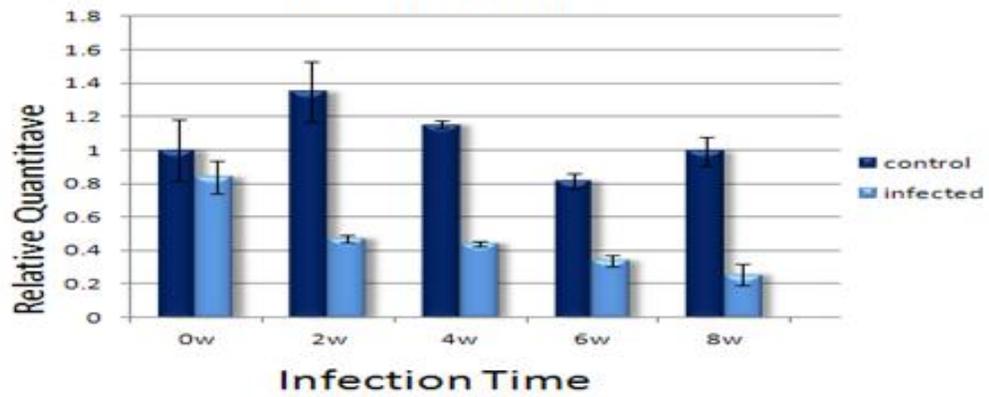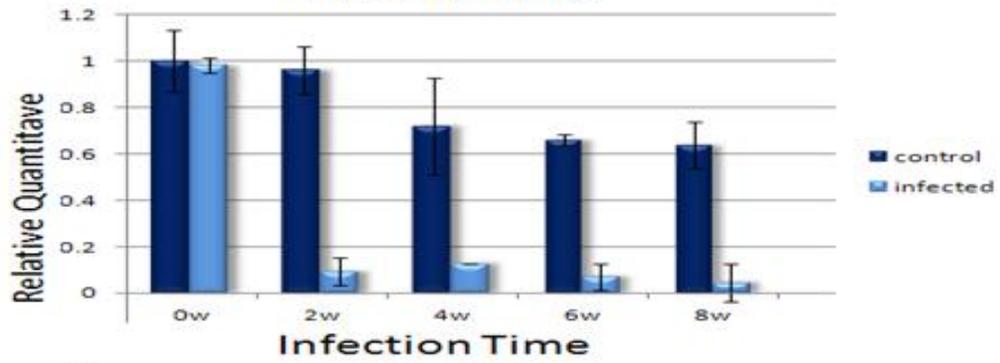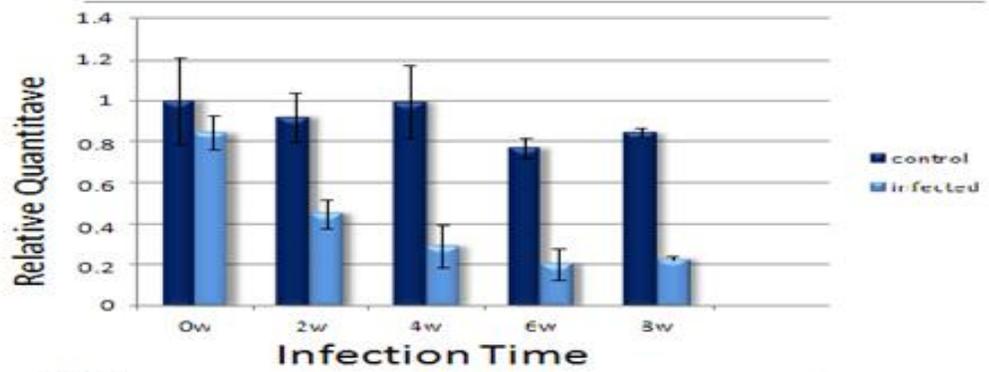
Statistical analysis for genomic investigation

In genomic analysis student *t-test* were performed to validate the significant in the expression analysis for the three genes (PGIP, LTP.PR10) using the real–time PCR comparing the infected and control oil palm root.

2DE t-test (Image master platinum 7)

Two sample *t-tests* is to analyse differences in protein expression between classes of gels .the idea is to draw conclusion about the significance of the protein expression changes by extrapolating information information from the data collected. For example ,when you have two samples (clasess) with different means (that is ,fiffrent mean from the spot values of a particular match),you might want to know whether the data were sampled from population with different means or the populations have the same means with the observed differences being coincidence of random sampling.

The two-sample *t-test* is generally used to determine whether the mean of a variable differs between two populations.

The *t-test* statics is based on the difference between the mean values (x) of the two classes, normalized by the standard deviation (s). For this test, the number of degree of freedom equlaes the total sample size (n1+n2) minus 2. The t ratio is calculated as follows:

$$- \quad -$$

...ertain threshold, chosen according to a confidence level and the sample size, you can conclude that the difference between the two populations is statically significant.

If the data for the sample to be analysed come from populations whose distribution violate the assumption of normality ,then nonparametric test like the Mann-Whitney or Kolmogorov-Smirnov test can provide a better analysis.

Statistical test report

The statistical Tests report contains, for each selected match, the desired statistical values, which quantify the differences between the means of two classes. These values should be considered, as qualitative indications of the variations in protein expression between two populations, and only rarely (in deal cases) should be used to calculate probabilities and draw quantitative conclusions. In addition, one should always check the results by visual inspection of the spots, since the conclusion may be erroneous due to inaccuracies in the detection or matching steps.

ume of contribution of oil palm root protein (continued)

red: represent the down-regulated protein, blue: represent the up-regulated one

No.	Sample name.	Percentage Volume (%vol)				
1	7		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.124806	0.038556	0.050547	0.058619
		Infected	0.073944	0.013065	0.022671	0.017813
		Fold-change	1.64	2.96	2.295	3.29
2	8		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.081787	0.108032	0.141129	0.069828
		infected	0.043371	0.058249	0.07957	0.042348
		Fold-change	1.88	1.86	1.772	1.725
3	12		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.083137	0.029813	0.225737	0.081258
		infected	0.046058	0.013842	0.095077	0.029697
		Fold-change	1.806	2.10	2.36	2.74
4	14		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.119681	0.204461	0.111993	0.097753
		infected	0.059	0.082365	0.079903	0.048611
		Fold-change	2.01	2.47	1.389	2.009
5	22		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.098453	0.018736	0.101763	0.142673
		infected	0.052496	0.005574	0.042559	0.055718
		Fold-change	1.88	3.4	2.404	2.58
6	23		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.11996	0.185286	0.18729	0.061287
		infected	0.052059	0.111323	0.125906	0.047765
		Fold-change	2.30	1.666	1.558	1.2788
7	28		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.06306	0.023919	0.018405	0.019202
		infected	0.006049	0.009972	0.003112	0.008325
		Fold-change	10.43	2.414	5.9125	2.313
8	30		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.036333	0.046653	0.035065	0.038833
		infected	0.023225	0.028329	0.015395	0.017373
		Fold-change	1.56	1.64	2.287	2.24

			(2weeks)	(4weeks)	(6weeks)	(8weeks)
			0.058909	0.034203	0.011247	0.019187
		infected	0.068805	0.004372	0.045458	0.032869
		Fold-change	+1.17	+1.24	+2.66	+3.04
10	47		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.016262	0.006549	0.084272	0.005222
		infected	0.0433	0.0452	0.128876	0.027651
		Fold-change	2.67	6.95	1.523	3.734
11	48		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.063946	0.072032	0.091069	0.146042
		infected	0.015393	0.053163	0.054474	0.036077
		Fold-change	4.151	1.355	1.67	4.047
12	49		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.033773	0.02345	0.017691	0.020932
		infected	0.005588	0.01291	0.009433	0.010873
		Fold-change	6.039	1.782	1.955	2.09
13	58		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.12309	0.177277	0.30367	0.125793
		infected	0.098356	0.101112	0.247318	0.072335
		Fold-change	1.25	1.75	1.226	1.7361
14	9		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.053202	0.103341	0.151725	0.063669
		infected	0.147824	0.225479	0.271314	0.139075
		Fold-change	2.77	2.1844	1.788	2.18
15	18		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.046075	0.054401	0.059461	0.045666
		infected	0.087717	0.109719	0.147565	0.068236
		Fold-change	1.906	2.01	2.474	1.5111
16	25		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.047082	0.043955	0.046612	0.041978
		infected	0.096637	0.070027	0.092458	0.071061
		Fold-change	2.055	1.59	1.97	1.707
17	32		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.057479	0.060702	0.090503	0.049368
		infected	0.134309	0.126467	0.166099	0.082038
		Fold-change	2.33	2.1	1.83	1.673

			(2weeks)	(4weeks)	(6weeks)	(8weeks)
			0.063947	0.105066	0.049852	0.049434
		infected	0.10199	0.1700	0.1654	0.1204
		Fold-change	1.603	1.61	3.36	2.448
19	44		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.048484	0.10368	0.06908	0.018621
		infected	0.098222	0.157642	0.112449	0.058014
		Fold-change	2.024	1.52	1.623	3.11
20	60		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.044051	0.034775	0.018926	0.034706
		infected	0.084833	0.08422	0.052216	0.076897
		Fold-change	1.909	2.47	2.76	2.213
21	61		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.068488	0.068491	0.043641	0.016083
		infected	0.133522	0.115548	0.085791	0.061684
		Fold-change	1.96	1.698	1.97	3.85
22	2		(2weeks)	(4weeks)	(6weeks)	(8weeks)
		Control	0.051706	0.140106	0.126621	0.05613
		infected	0.02372	0.058565	0.102953	0.030719
		Fold-change	2.1814	2.8	1.23	1.824

Appendix P1: *EgPGIP*

5'GTTGAACGGGCCTCTTCTCCAACCTGCAGCTGCAGGACGACGACGACGAGGTGTACCTCCGGCTGTCGCAC
AACAACCTGTCCGGGAGCGTCCCGGCGGACCTGCTGGCGGCGGCCGAACCTGGCGCTGGTGGACCTGTCGCGG
AACGCGCTGACCGGCGACGCGTCGGCGGTGTTCCGGCGGGCAAGGTACGTGGACAAGGCGGCAATCACTAGTG
AATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTA
TAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAA
TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAA3'

Appendix P2: *EgLTP*

5'ACCCCGACTGGCCCGTGACAGTTGGTGTTGGTCGCCCTGGTGGCAGCTCTGCTCCTCGCCGCCCCGCACGC
CGCCGTGGCCATCACCTGCGGCCAGGTCAACTCCGCCGTCGGGCCCTGCCTGACCTACGCCCGCGGCGGCGCC
GGGCCGTCGGCGGCCTGCTGCAGCGGCGTGAGGAGCCTTTTTGCTGCAGCAAGCACGACCGCAACACGTCATC
CAGGTCCGCCGAAAACGAGCTACTATGGCAGTCGCTCGCATACGCG3'

Appendix P3: *EgPR10*

5'TGGCTCTGTTTGCAAGTTGACCACCAGCTTCCACACCAAGGGTATGGCCTCCACCGACAGCTGGACCCACG
AGATCGAATCGCCGGTGGCCGCAGCGCGCCTCTTCCGCGCCGGCGTCATGGACTGGCACACACTGGCCCCTAA
GCTCGCACCGCACATCGTCGCCAGCGCCCACCCTGTTGAGGGCGAAGGCGGCATCGGCAGCGTCAGGCAGTTC
AACTTCACCTCAGCCATGCCCTTCAGCCTCATGAAAGAGAGGCTCG3'

publication

Publication

-**Jameel R Al-Obaidi**, Yusmin Yusoff, Rofina Yasmin Othman (2010) Identification of a Partial Oil Palm Polygalacturonase-Inhibiting Protein (EgPGIP) Gene and its Expression during Basal Stem Rot Infection Caused by Ganoderma boninense. African Journal of Biotechnology 9(46) pp7788-7897 (*ISI-Cited Publication*)

Proceeding

- **Jameel R Al-Obaidi**, Yusmin Yusoff, Rofina Yasmin Othman (2009) "Differential expression levels of an oil palm Polygalacturonase-inhibiting protein (*EgPGIP*) gene during Basal Stem Infection caused by *Ganoderma boninense*. MSMBB scientific meeting arrange by University of Malaya Conference. Saujana kuala lumpur.

-**Jameel R Al-Obaidi**, Yusmin Yusoff, Rofina Yasmin Othman Comparison of the defense related gene Lipid Transfer Protein (EgLTP) gene between the oil palm root cell infected with Ganoderma boninense and its control During the Basal stem Rot infection9th Malaysia Genetic Congress (9th MGC 2011) Faculty of Resource Science and Technology Universiti Malaysia Sarawak, Sarawak, MALAYSIA.

-**Jameel R Al-Obaidi**, Yusmin Mohd-Yusuf. Onn H. Hashim. Rofina Yasmin Othman (2011). Root proteomics analysis for Oil palm Elaeis guineensis during the infection with Ganoderma bonniness causing basal stem rot infection by two-dimensional gel electrophoresis and LC-MS/MS..PP-2-04, the 16th biological studies graduate congress (16th BSGC), National University Of Singapore, Singapore.

- Maswida M. Kamal, HasnainHussain, ZainabNgaini, **Jameel R. Al-Obaidi**,YusminMohd-Yusuf. Analysis of protein extracted from trunking and non-trunkingsago palm (Metroxylonsagu), International Conference on Humanities, Social Sciences, Science & Technology 27 – 28 June 2011, Manchester, United Kingdom.

-**Jameel R Al-Obaidi**, Yusmin Yusoff, Rofina Yasmin Othman 2009 "Expression analysis of Oil Palm Pathogen related protein PR10 in response to the BSR disease cause by the fungal *Ganoderma boninense.*BSGC Conference arrange by Chulalongkorn University –THAILAND –Bangkok.

-**Jameel R Al-Obaidi**, Yusmin Yusoff, Rofina Yasmin Othman (2008). Molecular study of Oil Palm Lipid transfer protein LTP in response to the BSR disease. *PIPOC* Conference arranged by Malaysian palm oil board –MALAYSIA –Kuala Lumpur presenting poster "

Identification of a partial oil palm polygalacturonase-inhibiting protein (EgPGIP) gene and its expression during basal stem rot infection caused by ganoderma boninense

Al-Obaidi, J.R.[ab] , Mohd-Yusuf, Y.[b] , Chin-Chong, T.[c] , Mhd-Noh, N.[c] , Othman, R.Y.[ab]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

[b] Centre for Research in Biotechnology for Agriculture, University Of Malaya, 50603 Kuala Lumpur, Malaysia

[c] Plant Protection Unit-Sime Darby Sdn Bhd, Sime Darby Sdn. Bhd, Km 10, Jalan Banting Kelanang, P.O. box 207, 42100 Banting, Selangor, Malaysia

▼ View references (38)

Abstract

Basal stem rot disease (BSR) is a common and serious fungal disease of the oil palm caused by Ganoderma boninense. This fungal disease infects thousands of hectares of plantings in Southeast Asia every year causing not only yield but also tree losses. A natural plant self defence mechanism against fungal infection is the production of fungal resistance protein. A fungal resistance gene that has been reported previously in other monocotyledonous plants such as rice and barley is polygalacturonase-inhibiting protein (PGIP) gene, a plant defence cell wall glycoprotein that has been shown to inhibit the activity of fungal endopolygalacturonase (endo-PGs) and modulate their activity and has the potential to be developed as a disease or resistance biomarker for the oil palm. The identification and isolation of this gene in oil palm allowed for the study of its differential expression during the fungal infection. The oil palm PGIP gene (EgPGIP) has between 60-100% similarities with the database sequence of PGIP from other monocotyledons. Interestingly, we found that the expression of EgPGIP gene measured using Real-Time PCR showed that the expression level of EgPGIP in infected oil palm was temporally down regulated. The results suggest that, down regulation of the EgPGIP is related to the establishment of infection by G. boninense. © 2010 Academic Journals.

Author keywords

Basal stem root; Ganoderma infection; Oil palm; Polygalacturonase-inhibiting protein

Indexed Keywords

EMTREE drug terms: polygalacturonase inhibiting protein; unclassified drug; vegetable protein

EMTREE medical terms: Arecaceae; article; controlled study; DNA sequence; down regulation; Elaies guineensis; fungal plant disease; Ganoderma; Ganoderma boninense; gene expression regulation; gene function; gene isolation; nonhuman; nucleotide sequence; plant gene; plant genetics; protein analysis; quantitative analysis; real time polymerase chain reaction; sequence alignment; sequence homology; stem rot

Species Index: Elaeis; Ganoderma; Ganoderma boninense; Hordeum; Liliopsida

Molecular Sequence Numbers: GENBANK, AY550991 (referenced)

Certificate of Participation

We hereby extend our heartiest acknowledgement to

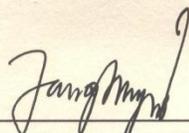## Mr. Jameel Rabee Jameel Alobaidi

for participating as

ORAL PAPER PRESENTER

in the

18th Scientific Meeting of the
Malaysian Society for Molecular Biology and Biotechnology
(MSMBB)

at
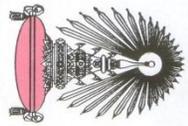
The Saujana Kuala Lumpur
18th – 20th August 2009

Assoc. Prof. Dr. Fong Mun Yik
President
MSMBB
(2007-2009)

Jointly organised by:

MSMBB

UMBIO TECHNOLOGY & PRODUCT CLUSTER

262

Certificate of Participation

Jameel Rabee Alobaidi

(Poster Presenter)

has participated in

The 14th Biological Sciences Graduate Congress 2009

10-12 December 2009
Faculty of Science, Chulalongkorn University,
Bangkok, Thailand

Assist. Prof. Dr. Sukkaneste Tungasmita
Associate Dean
Faculty of Science
Chulalongkorn University

Prof. Dr. Supot Hannongbua
Dean
Faculty of Science
Chulalongkorn University

NUS
National University
of Singapore

16th BSGC
Singapore
2011

Certificate of Participation

This is to certify that

Jameel Rabee Al-Obaidi

(Poster Presenter)

has participated in

The 16th Biological Sciences Graduate Congress

12th – 14th December 2011

Department of Biological Sciences, Faculty of Science
National University of Singapore

Professor Andrew Wee
Dean, Faculty of Science
National University of Singapore