GENETIC ANALYSIS OF GRAIN YIELD AND BIOFUEL PRODUCTIVITY IN MAIZE

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ABSTRACT

The study was conducted based on the preliminary research to developed new variety of maize with high yield and sustainable bio-fuel productivity. Twenty-two white dent tropical maize genotypes were planted along with yellow sweet corn, where these maize were allow crossing naturally. As result of the crossing, Filial 1 (F_1) seed were obtained. Out of twenty-two genotypes, only six genotypes manage to produce outcrossing progeny couple with good agronomical traits and greater end yield. Genotypes E1, E5, E9, E11, E12 and E17 were planted along with their (F_1). Throughout the planting season, agronomic characters were evaluated.

Genotype E17 was observed as the highest plant among the parental material as well as F_1s while genotype E1 was observed having the lowest plant height among parental material and F_1s. Days taken to flowering, genotype E9 was observed to have earlier flowering days and F_1 derived from genotype E17 was observed to have late flowering days among the parental material and F_1s. For days taken to maturity, the shortest day taken to reach maturity was observed in genotype E9 while the longest day taken to reach maturity was observed in genotype E1 among parental material and F_1s. Grain filling period, the longest grain filling duration was observed in F_1 derived from genotype E9 and sweet corn while the shortest grain filling duration was observed in genotype E9 among parental material and F_1s. Genotype E12 was observed as the highest grain yielder and the lowest grain yielder were observed in genotype E1 among parental material and F_1s. F_1s derived from genotype E9 was observed as the highest thousand grain yielder while E9 was observed as the lowest thousand grain yielder among the parental material and F_1s. For stover weight, F_1s derived from genotype E 11 was observed to have the highest stover weight while E5 was observed to have the lowest stover weight among the parental material and F_1s.

Genetic analysis was conducted for each maize agronomic character for heritability, Genotypic Coefficient Variance (GCV), Phenotype Coefficient Variance (PCV), and genetic advance (GA). The highest GCV and PCV value was found in trait number of cob whole the lowest GCV and PCV value were observed in number of leaves. The highest GA value was found in plant height while the lowest value was observed in number of leaves. High heritability were observed in days taken to flowering, stem diameter, thousand grain weight, grain filling period, and days taken to maturity. Among yield contributing traits, positive correlations were found in plant height, days taken to flowering, days taken to maturity, number of cob, and leaves length with grain yield.

The stovers were used in pyrolysis process. The pyrolysis process optimized at 550°C, for 45 minutes using size particle of 1.4mm<dp<2.0mm under 2.0 L/min nitrogen flow. Bio-oil was analysed using Gas Chromatography-Mass Spectometer (GC-MS) and Fourier Transform Infrared (FT-IR) to identified and quantified functional group and chemical compound in bio-oil. GC-MS analysis shows bio-oil were dominated by 2,6-dimethoxy phenol and methoxy phenol while FT-IR analysis shows bio-oil have a broad and strong peak indicate the present phenol and alcohol. Other peaks present in FT-IR analysis were alkanes, carboxylic acids, aldehydes and ketones.

ABSTRAK

Kajian ini berdasarkan penyelidikan awal untuk menghasilkan varieti baru jagung yang mempunyai pengeluaran yang tinggi dan mampu untuk menghasilkan 'bio-fuel'. Dua puluh dua jenis jagung tropikal berbiji putih ditanam bersama jagung manis kuning bagi membolehkan kacukan berlaku secara semula jadi dimana generasi 1 (F_1) jagung berjaya dihasilkan. Sebanyak enam varieti jagung ((E1, E5, E9, E11, E12 an E17) yang berjaya dikacukkan, daripada dua puluh dua varieti jagung. Enam varieti ini dipilih berdasarkan karacter agronomi yang baik dan mempunyai hasil akhir tinggi. Enam varieti (F_1) ditanam semula bersama induk masing. Sepanjang musim penanaman, karacter agronomi dinilai.

Varieti E17 diperhatikan mempunyai tinggi pokok maksimum manakala varieti E1 mempunyai tinggi minimum dikalangan varieti induk dan varieti F_1. Varieti E9 mempunyai tempoh masa berbunga paling pendek manakala E17 mempunyai tempoh masa berbunga paling lama dikalangan varieti induk dan varieti F_1. Tempoh minimum diambil untuk mencapai kematangan dilihat pada varieti E9 manakala tempoh maksimum diambil untuk mencapai kematangan dilihat pada varieti E1 dikalangan varieti induk dan varieti F_1. Tempoh isian biji minimum dilihat pada varieti E9 (F_1) manakala tempoh maksimum dilihat pada induk E9. Varieti yang mempunyai hasil akhir yang tertinggi ialah E12 manakala varieti yang mempunyai hasil akir terendah ialah E1 dikalangan varieti induk dan varieti F_1. Varieti E9 (F_1) mempunyai berat 1000 biji maksimum manakala varieti E9 mempunyai berat 1000 biji minimum dikalangan varieti induk dan varieti F_1. Berat batang dan daun maksimum dilihat pada E11 (F_1) manakala, E5 mempunyai berat batang dan daun minimum dikalangan varieti induk dan varieti F_1.

Analisis genetik seperti perwarisan genetic, 'Genotypic Coefficient Variance' (GCV), 'Phenotype Coefficient Variance' (PCV), dan 'Genetic Advance' (GA) yang dijalankan bagi setiap karakter agronomi. Nilai GCV dan PCV tertinggi didapati pada bilangan 'cob' manakala nilai terendah GCV dan PCV terendah dilihat pada bilangan daun. 'Genetic Advance' tertinggi dapat dilihat pada ciri Perwarisan genetik tertinggi didapati pada ciri tempoh masa berbunga, diameter batang, berat 1000 biji, tempoh isian biji dan tempoh masa capai kematangan. Diantara ciri-ciri yang menyumbang kepada hasil akhir tuaian ialah ketinggian pokok, masa berbunga, tempoh masa capai kematangan, bilangan 'cob', dan bilangan daun.

Batang dan daun digunakan semasa ' pyrolysis' proces. 'Pyrolysis' proses dijalankan dalam keadaan optimum pada suhu 550°C, selama45 minit menggunakan saiz zarah 1.4mm<dp<2.0mm dan 2.0 L/min aliran nitrogen. 'Bio-oil' dianalisis menggunakan Gas Chromatography-Mass Spectometer (GC-MS) dan Fourier Transform Infrared (FT-IR) bagi mengenal pasti kumpulan berfungsi dan kompoun kimia di dalam 'bio-oil'. GC-MS analisis menunjukkan 'bio-oil' didominasi oleh 2,6-dimethoxy phenol dan methoxy phenol manakala FT-IR analisis menunjukkan 'bio-oil' mempunyai 'peak' lebar dan teguh. Ini menunjukkan kehadiran phenol dan alkohol. 'Peak' lain yang boleh didapati daripada 'bio-oil' ialah alkane, carboxylic acids, aldehydes and ketones.

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LIST OF ABBREVIATION

^0C	- Degree Celsius
µL	- micro litre
%	- Percentage
% WIS	- Water Insoluble Percentages
ANOVA	- Variance analysis
CHN	- Carbon, Hydrogen, Nitrogen
cm	- Centimeter
dp	- Diameter particle
eV	- Electron volt
F_1	- Filial 1
FT-IR	- Fourier Transform infer red
g	- gram
GA	- Genetic Advance
GC-MS	- Gas Chromatography Mass Spectrometry
GCV	- Genetics coefficient Variance
H_b	- Broad sense heritability
ie.	- In Essence
IR	- Infrared
KF	- Karl Fisher
kBr	- Potassium Bromine
L/min	- Litre per minutes
m	- Meter
Min	- Minute
Mm	- Milimeter
MS	- Mean square
N_2	- Nitrogen

NaOH - Sodium Hydoxide

NiST - National Institute of Standard and Technology

NPK - Nitrogen, Phosphorus, Potassium

PCV - Phenotype Coefficient Variance

PTFE - Polytetrafluoroethylene

SAS - Statistical Analysis Sistem

Wt% - Weight Percentage

TABLE OF CONTENT

1.0 INTRODUCTION

Maize is among three most cultivated crops in the world after rice and wheat (Younas et al., 2002). Maize or *Zea mays L*. are one of the oldest foods known. Maize belongs to tribe Maydeae of grass family *Poacea* (Anonymous, 2010). It has been cultivated for thousand of years. Maize is the only cultivated species in genus Zea which meant food grass in old Greek ("Biology of Maize", 2010). Maize is a versatile crop grown over range of agro climatic zones which can't be matched by other crops. Maize can be grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m, and in areas with 250mm to more than 5000 mm of rainfall per year (Anonymous, 2010). Both tropical maize and sweet corn can be grown successfully in tropical environment. Tropical maize is famous for its high biomass production while sweet corn was popular for the high starch content. Thus, development of hybrid from those two types of maize can be potentially for high biomass and starch production.

Few advance lines had been developed from tropical maize and sweet corn (Golam et al., 2011). These lines were evaluated for agronomical traits as well as the production of high yield. In following chapters will elaborate much about maize in detail, along with the description on the uses, importance, and production of high yielding maize. Besides that, the uses of maize in future which contribute to global sustainability.

1.1 MAIZE HISTORY

Various theories regarding where maize originated from have been debated for centuries by historian and researcher as each theories differs in region and area (Paliwal, 2000c). Due to the evidence found in New Mexico, many maize researchers believe that the true maize originated from there (Cowan et al., 2006). The oldest evidence were found during archaeology excavations in New Mexico, grains and part of maize ears were found buried as deep as 70 metres under the city (Gibson & Benson, 2002; Cowan et al., 2006). This shows the evidence that the present of maize in ancient world as human food that was grown by men and women farmers. Since ancient times, both species from same genus (maize and teosinte) coexist together as there is evidence showing the evolution of both species back to the date (Paliwal, 2000c).

Several historians suggested that, during the new world discovery in 1492, Columbus voyage has brought in the new valuable form of grain to Europe region first through Spain (Gibson & Benson, 2002; Anonymous, 2010). By the end of 1500s, maize was grown widely to other continents in the old world. Throughout the world, maize is introduced mainly by traders. In Africa, Portuguese traders brought in maize as raw material for agriculture as a trade for slave (Desjardins & McCarthy, 2008). The distribution of many varieties of maize is due to the many areas in tropical Africa received maize across Sahara from Arab traders that may contribute to the distribution (Anonymous, 2010).

After 60 years since the first introduction of maize by Spain to Europe, historian reported that maize was spread to Asian continent through silk route (Desjardins & McCarthy, 2008). Maize was first introduced in the north to west Himalayas by Portuguese and Arab traders. It was believed by many scholars that Himalayas has become the second centre of origin for maize. This is due to the type of maize that can only be found in Sikkim (India) and Bhutan regions only (Desjardins & McCarthy,

2008). However in Southeast Asia particularly in China, maize was introduced in early sixteenth century through land maritime route (Paliwal, 2000c). The golden seed were brought in by traders through sea along with their other merchandise.

1.2 MAIZE MORPHOLOGY

After seedling stage, maize plant rapidly grows through its single growing point which is the apical meristem. The stem length varied with varieties. Individual quick ripening varieties mature at a height of only 90 centimetres and certain varieties of popcorn reach a height of only 30-50 centimetres. However, in certain region such as tropical and subtropical average plant height (7-8 ft) can easily grow until 14-15 ft tall (International Maize and Wheat Improvement Center [CIMMYT], 2010). The stems is cylindrical, dense and spongoid inside. Maize usually has 8 to 40 internodes, which normally are short and fairly thick at the base of plant and become longer and thicker higher up the stem (Zsubori et al., 2002).

Maize is monoecious plant. Maize has a unisexual flowers emerged at different parts of the plant (Figure 1). The spikelet separate into two types, male flower and female flower (CIMMYT, 2010). The male inflorescence known as tassel develops from apical growing point at the top of the plant. It can easily be recognized before flowering, as the tassel would emerges from the top leaves of the plant (Anonymous, 2010). These branches bear maize pollen that is yellow in colour which usually is transferred to female part by wind, insect and human. While for the female part, or silk which in the late stage were known as cob or ear consist of modified branch deriving from axillary bud of main stem (Anderson et al., 2004). The leaves are overlap with each others to form the husk cover. These leaves shows great degree of modification with a well developed blade with reduced size or in some cases leaves is entirely

lacking. In different varieties under different temperature, the development of silk are more intensified as the blade turn to full ear bearing branches (Paliwal, 2000e)

Fertilization occurs by means of wind and gravity (Halsey et al., 2005). As were stated before, the structure of maize plant enables them to reproduce based on gravitational manners, where the male inflorescence in the shoot of the plant. Whereas the female inflorescence, the cob settled in lower part of maize stem between the internodes (Paliwal, 2000e). The long pollen tube takes up almost 24 hours to travel down the length of silk to reach the ovule to complete the fertilization (Halsey et al., 2005; Anderson et al., 2004).

Maize root consist of two different roots which provide different function to the plant development. These roots may persist throughout the life of the plant. Total root mass of a maize plant are make up from 52 percent of seminal adventitious roots and 48 percent of nodal roots (Paliwal, 2000e). A set of adventitious roots develops from successive node and goes up between seven and ten nodes during development. An adventitious root grows only below the soil surface which then develops into thick fibrous roots. The adventitious root provides the main anchorage to avoid maize from lodging and supply nutrients and water uptake (Paliwal, 2000e; Anonymous, 2010). Brace roots or pop roots play same role as seminal root which to keep the root from lodging and to keep it upright. It is a part of adventitious root that emerge at two to three nodes above the soil surface. In certain maize type the roots may develop from many nodes. Individual roots may penetrate until 2.5 metres depth (Anonymous, 1994)

Maize is grown for its grain in most country. Maize grain can be divided into several types which are flint type, dent maize, pop maize and sweet corn (Gibson, & Benson, 2002). Flint type which includes in most original landraces has round, hard and smooth kernel. The endosperm mostly made up from corneous starch, with soft starch

in the middle of the grain. Dent maize endosperms are softer, bigger in size, and more flat compared to flint maize. The pop maize kernels are small, have a thick pericarp and vary in shape from round to oblong. When heated the kernel pops and endosperm burst out. As for sweet corn, the kernels are small and shrivel and have sweet taste (Paliwal, 2000b; Pajic, 2011).

1.3 GENOME

Maize has diploid set of ten chromosomes. Other perennial species, *Zea diploperennis* also diploid while *Zea perennis* is tetraploid with 2n = 40. However the wild relative, tripsacum has basic chromosome number of 18 (Paliwal, 2000d). However it is varied throughout the entire species. Many scientist considered maize as true diploid with tha basic ten chromosomes even there is speculation about maize being an amphidiploids between two genera, with each have n = 5 (Paliwal, 2000d). Several theories regarding the ploidy number in maize have been suggested by the scientist. According to Majumdar and Sarkar (1974) the homology in the haploid set is the indication that maize was not a true diploid and had duplication in their genome but Paliwal (2000d) has mention that maize was true diploid.

Transposable element is closely related with maize. This event was first reported by Barbara McClintock (1980), the genetics entity were repositioning from one plant genome into another and resulting in mutation in that particular maize. In her investigation, she found out that the alteration of colour in maize are due to dissociator gene (Ds) under the influence of an activator gene (Ac). This transposable element has given each kernel different colour.

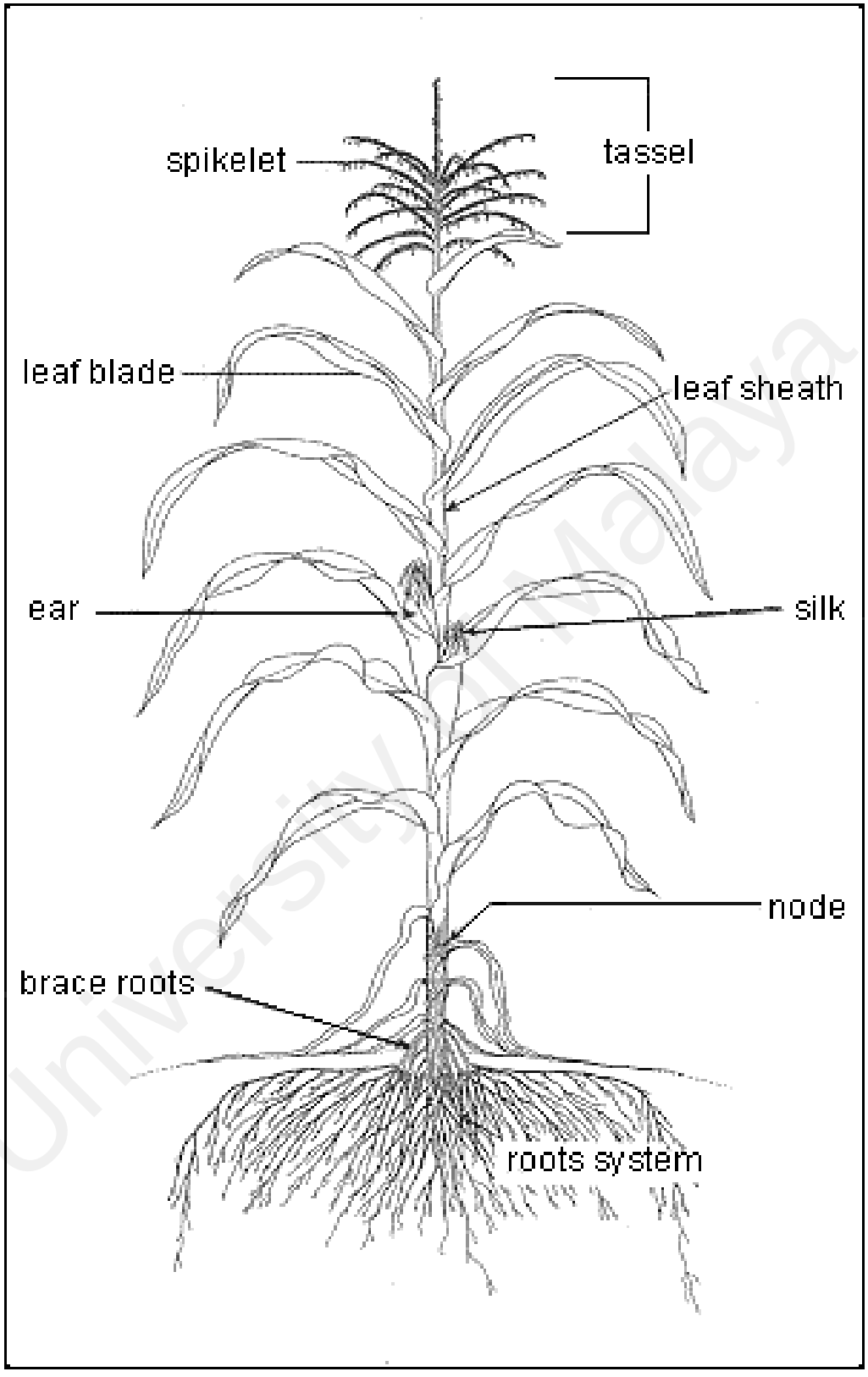


Figure 1. Morphology of maize plant. (Source: http://maizedoctor.cimmyt.org)

1.4 USES OF MAIZE

Well known to world, maize contributes in food chain for decades. Every part of maize employed different purposes, beside grain that being used as food source, the stalk and leaves are used in alternative fuel despite the ordinary used as animal feed. Maize is nutritionally superior to most other cereal especially in protein value. Maize compares very well in nutritive value with rice and wheat (Food and Agriculture Organization of the United States [FAO], 1993; Overend, 1999). Compared to wheat, the protein value are lower which are due to half of protein are made up of zein, which is very low in two essential amino acids, lysine and tryptophan. Various way to prepared maize according to stage of grain development at which it is consumed either as food, drink or to substitute tobacco in cigarette. Different region of world have their own way of preparing corn as their dishes. Green ears with immature grain were parched for immediate consumption or mixed into soup. Juice were also extracted from raw fresh kernels, flavoured, cooked and allowed to cold to become jelly. The kernel also were removed to be used in soup and used as vegetables in various ways. The ground or mashed maize pastes were use as porridge and pudding dishes (Paliwal, 2000f).

Mature dry grain being used as a whole grain, dry milled grain, soaked grain, fermented maize and maize starch. In Africa and Asia whole grain corn usually parched and eaten other than it is boiled or roasted and eaten hot as corn nuts. In most society, corn is at the top food rank source (Anonymous, 2011). African and European society is consumed corn daily in their meal.

Other than maize being used as human consumptions purposes, it also known to provides the cheapest and most valuable fodder for animal (Ahsan & Mehdi, 2000). Maize leaves and stalk were used as poultry food. However in most country, little attention were given to improved the quality of green fodder as the breeders

concentrating on producing better grain yield. Ahsan and Mehdi (2000) also reported the association of dry matter in maize stover were greatly influence by the sink material accumulated for grain yield has affected the weight of dry matter.

1.5 GRAIN YIELD IN MAIZE

In breeding, the main concern was to increase the yield where in maize the same purpose were tried to achieve by plant breeders. Production of yield in maize were greatly influenced by traits contributes to grain such as, plant density, plant height, flowering days, maturation days and grain filling period (Zsubori at al., 2002; Iqbal et al., 2011). In most breeding program, the strategy was based on selection for several traits simultaneously where the evolution of high yielding and well adapted cultivars were combined together (Iqbal et al., 2011). As were reported by Ahmad et al., (2010) among many traits, plant density plays a major role in grain productivity. Plant density influence the growth of maize plant where the sink materials accumulate after photosynthesis process were used for vegetative and reproductive development. This also related to another trait which is the grain filling period and maturation day as extended grain filling period with longer maturation days will lead to higher yield (Richards, 2000; Wang et al., 1999).

1.6 BIO-OIL

Bio-oil can be obtained from various harvested residue such as rice and wheat straw, stover, palm shell, cotton seedcake (Abnisa et al., 2011). Other than agriculture and forestry leftover, portions of municipal waste, herbaceous and woody crop also can be used as material in production of bio-oil (Fang et al., 2010). Bio-oil was known as the secondary oil. Bio oil were also called pyrolysis liquid, pyrolysis oil, bio-crude-oil,

bio-fuel-oil, wood liquid (Stamatov and Rocha, 2007). Other than that, it was also known as liquid smoke, wood distillates, pyrolytic oil, pyroligneous tar, pyroligneous acid and liquid wood (Abnisa, 2011). It is a complex liquid consist of different size molecules which derived from depolymeration and fragmentation reactions (Abnisa, 2011). The oil has acrid smell with free flowing dark brown colour liquid.

1.7 BIO-FUEL

To meet the demand of world for fuel, bio-fuel that was made from sustainable material is introduced to replace the crude oil that has become the major contributor in energy. Increase in population has a major effect on availabilities of crude oil as the depletion of current crude oil is due to the continuous usage. Bio-fuel consists of solid biomass, liquid fuels such as bio-ethanol and bio-diesel, and biogases (Balat et al., 2008). Material uses in bio-fuel production are biologically biomass which can be found in most agriculture and forestry residue, herbaceous and woody crops or in general, anything that is biologically material (Fang et al., 2010). Alternative fuel has positive effect towards better environment as less major green house gases (GHG) are release to the environment. Green house gases (GHG) contain water vapour, methane, carbon dioxide, nitrogen oxide and ozone which were release by usage of crude oil (Balat et al., 2008). These gases have negative impact towards environment which can be reduced if bio-fuel were used throughout the nation.

Corn has been staple food in various countries throughout the world. The grain and green young corn were use in many dishes. However, the idea of corn residue being use as sustainable transportation fuel has attracted people globally. Residue after harvesting, such as corn stover can be use as biomass source in production of bio-oil which can replace crude oil that already facing depletion throughout the years (Sun &

Cheng, 2002; Dhugga, 2007). There is also no issue of ethical concerning the food resources as it associated with corn stover, the residue left after harvesting but not the grain (Lewis et al., 2010; Lorenz et al., 2009).

1.8 BIO MASS

The components of biomass include cellulose, hemicelluloses, lignin, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash, and other compounds (Demirbas, 2009). The basic structure of all woody biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)_x$, hemicelluloses such as xylan $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3\ (OCH_3)\ 0.9–1.7]_n$ which are foundin trunk, foliage,stem and bark. The proportion of these wood constituents varies between species, and there are distinct differences between hardwoods and softwoods based on the proportion of the basic polymer (Demirbas, 2009).

Lignocellulose that is found in maize stalk and leaves are made of four major components which is cellulose, hemicelluloses, lignin and extractives (Demirbas, 2009). Cellulose and hemicellulose were abundant and common component in hardwoods compared to softwood or straw (Saha, 2003). Softwood have higher lignin compound compared to hardwood while the straw have higher extractives (Ibrahim, 1998). Cellulose is a excellent pure organic polymer, consisting solely of units of anhydroglocose held together in a giant straight chain molecule (Balat et al, 2009). Cellulose is a homopolysaccharide composed of b-D-glucopyranose units linked together by glycosidic bonds (Sun & Cheng et al., 2002; Dhugga, 2007). Lignin is an aromatic polymer synthesised from phenylpropanoid precursors. The basic chemical phenylpropane units of lignin (primarily syringyl, guaiacyl and p-hydroxy phenol) are bonded together by a set of linkages to form a very complex matrix (Balat et al., 2010;

Ibrahim, 1998). Maize were categorizes under straw as the residue containing stalk, leaves and husks (Anonymous, 2011).

1.9 TERMOCHEMICAL PROCESS

Thermochemical conversion of biomass processes can be subdivided into gasification, pyrolysis and direct liquefaction (Goyal et al., 2008). Pyrolysis is the fundamental chemical reaction process that is the precursor of both the gasification and combustion of solid fuels (Overend, 1999). It can be defined as the chemical changes occurring when heat is applied to a material in the absence of oxygen (Overend, 1999; Mullen & Boateng, 2008). It is also thermochemical conversion process which is found to be best suited for conversion of biomass to liquid fuel (Goyal et al., 2006). The products of biomass pyrolysis include water, charcoal, oils or tars and permanent gases including methane, hydrogen, carbon monoxide and carbon dioxide (Bhaskar et al., 2011).

Another type of thermochemical conversion of biomass for biofuel production is gasification. Gasification is one of the most important thermochemical conversion processes as it can produce intermediate synthesis gas, which is known as syngas (Balat et al., 2009). After that, the syngas can be refined and fermented to produce bioethanol. Overend (1999) reported that gasification is an extension of pyrolysis. However the difference between these thermal processes is gasification produce carbon and energy in the gas phase, rather than pyrolysis where it produce char and liquid.

Catalyst can be used to increase the productivity of both processes. In gasification process Ni-supported dolomite, Ni/Dolomite plus Silica binder and Ni-WO_3/Dolomite catalyst were used. After using catalyst to convert the syngas to biofuel, microorganisms which are specifically-designed can be used to ferment the gas (Balat et al., 2009).

Genetic Analysis Of Grain Yield And Biofuel Productivity In Maize (Zea Mays L.)

The whole project were based on the development and screening of maize germplasm in order to produce quality germplasm. Excellent agronomic characters with resistance towards common maize diseases were preferred. This project was continuity from adaptability of tropical maize in Malaysia project which was conducted in year 2010. The main purpose of the project was to evaluate the adaptability of tropical maize in Malaysia environment with high potentiality of grain yield productions. Previously, 22 different genotypes received from International Maize and Wheat Improvement Centre (CYMMYT) was planted along with yellow sweet corn in a field near Genetic and Microbiology Department and the development were continuously evaluated. In the end of the planting season, it is observed that there is hybrid seed due to the cross pollination that occurs between tropical maize which have white dent kernel with yellow sweet corn.

Only few genotypes managed to be cross pollinated which then were screen based on agronomical characters as well as the resistance towards diseases and pest. The F_1s that were selected were (E1, E5, E9, E11, E12 and E16). The F_1s were then planted accordingly with the yellow sweet corn as a top cross. Each genotype was measured and evaluate continuously through out the experiment. After harvesting, the stalk and leaves were dried properly to avoid fungus manifestation. Plant height, flowering days, maturation days, grain filling period, grain weight, 1000 grain weight, and dry matter weight were taken.

Stovers that were collected previously were grind using rapid granulator into smaller size stover particle. These samples were then undergoing pyrolysis process under several treatments to obtained maximum yield for bio oil productivity. The bio-oil were then analysed using FTIR and GC-MS in order to determine the component and functional group present in the bio-oil.

The contributions of agronomic traits towards high yield productivity were evaluated through statistical analysis which includes variance component, Pearson

correlation between traits, mean range test. This will then enable the screening be conducted for superior germplasm. The objectives of the research were successfully achieved as elite germplasm were developed based on screening of yield contributing traits hence the production of bio-oil from maize stover were gained with potentiality to be used as fuel in near future.

This research was conducted based on objectives listed below:

i. Development of yield contributing traits among maize genotype those lead to the high end production of grain through evaluations of correlation between traits as it contributes to elite germplasm and grain yield.
ii. Evaluation of bio-oil productivity in maize by optimizing pyrolysis process and analysis for bio oil (CHN analysis, GC-MS and FTIR).
iii. Incorporation of grain yield with stover quality for bio-oil production.

2.0 LITERATURE REVIEW

2.1 MAIZE

2.1.1 Maize in general

The spread of maize from its origin, Mexico to various part of world start with the discovery of New World by Columbus (Brown & Darrah, 2011). In 1000 AD, the indigenous farmer breeders had improved the maize plant through selection process (Inglett, 1970). This seed then were kept for next planting season. Cultivators choose the largest and desirable ears for this purpose. This will enable them to maintain the purity of the maize lines. Several historians suggested that, during the new world discovery in 1492, Columbus voyage has brought in the new valuable form of grain to Europe region first through Spain. By the end of 1500s, maize was grown widely to other continents in the old world (Elting & Folsom, 1967). Throughout the world, maize is introduced mainly by traders.

The distribution of this crop widely varies within species. Back in 1000 years, the dispersal of maize have been generating from Americas, and subsequently to Europe, Africa, and Asia (Brown & Darrah, 2011). The distribution are divided into 2 era's which is the old world and the new world. During the old world, the distribution have greatly influenced by the cultural, economic, and political impacts of the European. The increase of number in world population at that time also contributes to the crop distribution. Maize or well known as Indian corn in the United States and Great Britain has become one of the major food resource. After 1492, maize rapidly diffused into Europe, Africa, and Asia because it did not directly compete with existing grain crops such as rice, wheat, oats, millet, and barley (Paliwal, 2000). In different part of the world maize has important a role for the consumption and feed of the living organism. Maize provides the world's most cost-effective and highest yield plant

resource currently available for the production of livestock forage, fodder, and feed (Dowswell et al., 1996).

2.1.2 Uses of maize

Maize is proven to have multiple uses which involve the plant and the cob (Nagaraju, 2006). Every part of maize contributed to different purposes. Yet known to the world, beside grain that being used for consumption and industrial use, the stalk and leaves are used alternatively for fuel despite the ordinary used as animal feed.

2.1.2.1 Food grain

Maize is nutritionally superior to most other cereal especially in protein value (Pajic, 2011). Maize has high nutritive value, similar to rice and wheat (Table 1). Compared to wheat, the protein value is lower which is due to half of the total protein are made up of zein, which is very low in two essential amino acids, lysine and tryptophan (Inglett, 1970; Argos, 1982). However this deficiency of protein has been removed with new breed of maize, known as Quality Protein Maize (QPM). QPM is nutritionally the most superior cereal grain (Paliwal, 2000b; Mbuya et al., 2010).

Food	**Energy**	**Protein**	**Fat**	**Calcium**	**Iron**	**Thiamine**	**Riboflavin**	**Niacin**
	(kcal)	***(g)***	***(g)***	***(mg)***	**(mg)**	**(mg)**	**(mg)**	**(mg)**
Maize flour, whole	**353**	**9.3**	**3.8**	**10**	**2.5**	**0.30**	**0.10**	**1.8**
Maize flour, refined	**368**	**9.4**	**1.0**	**3**	**1.3**	**0.26**	**0.08**	**1.0**
Rice, polished	361	6.5	1.0	4	0.5	0.08	0.02	1.5
Rice, parboiled	364	6.7	1.0	7	1.2	0.20	0.08	2.6
Wheat, whole	323	12.6	1.8	36	4.0	0.30	0.07	5.0
Wheat flour, white	341	9.4	1.3	15	1.5	0.10	0.03	0.7
Millet, bulrush	341	10.4	4.0	22	3.0	0.30	0.22	1.7
Sorghum	345	10.7	3.2	26	4.5	0.34	0.15	3.3

Table 1. Content of certain nutrients in 100 g of selected cereals. (Source: http://www.fao.org)

Maize is used in many ways as food source with wide diversity of maize type being prepared. Various ways to prepared maize according to stage of grain development for which it is consumed either as food, drink or to substitute tobacco in cigarette. Different region of world have heir own way of preparing corn as their dishes. In the early stage of ears development, the green ears were pluck before it bears any grain. These green ears are roasted on coal. This method of preparing green ear is well known in Africa, Asia and some parts of America (FAO, 1993). Green ears with immature grain were parched for immediate consumption. Sweet types maize are usually used for this occasion.

Corn also served as main feed mostly to swine, cattle and poultry. The grains are grind to smaller pieces before it is given to the animal. Corn constitutes a major portion of protein (Table 1) in their daily diet (Inglett, 1970). In Philippines for examples, yellow corn is a vital component for feeds of livestock and poultry beside soybean meal (SBM), fish bone and other additives (Costales, 2006).

2.1.1.2 Industry uses

The usage of corn (Figure 2) were not only limited to the grain consumption, where corn can be utilized in industrial field, Jacksons (1992) reported that corn was used in three distinct industries as corn for wet milling, corn for dry milling and corn for alkaline cooking. Corn wet milling involves complex industrial process where, this process will produce various products (Olsen, 2011). Starches were used as major compound in wet milling where it can be fermented to produce alcohol (Lastate, 2010). Other than that, it was also used as adhesive in manufacturing of papers and as filler for pharmaceuticals (Jackson, 1992). Corn dry-milling process involves a simple grinding procedure. The primary products were flour, cornmeal and grit which can be further

used in brewing. Alkaline-cooked corn was used to produce tortilla, tortilla chips corn chips and similar product (Jackson, 1992).

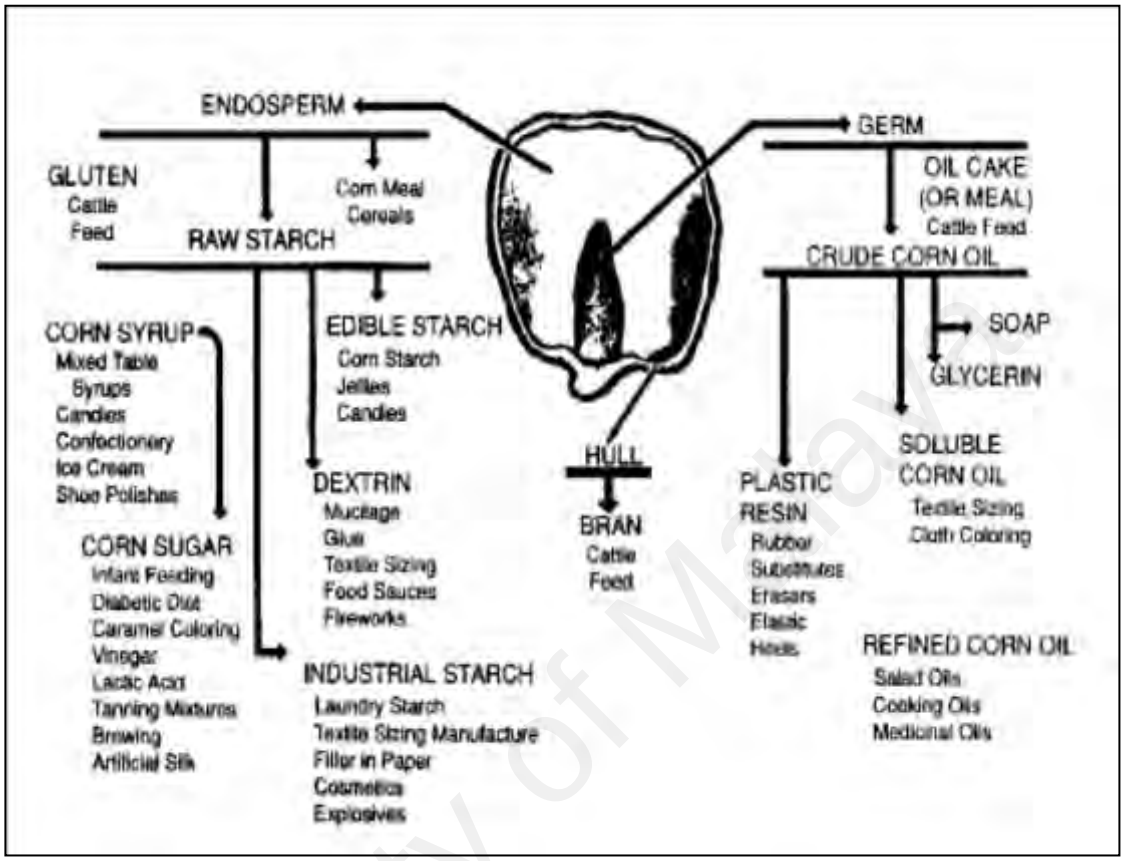


Figure 2. Uses of corn. (Source : Anonymous, 1995)

The non-food industries for corn were extensively developed through out centuries. Chemical can be derived from starches by depolymerisation of polymer. Corn can be converted to alcohols, cyclic and acylic polyols, aldehydes, ketones, acids, esters and ethers (Doane, 1987). He also reported the usage of polymer from starch in production of biodegradable plastics. Corn starch has been evaluated as inert filler in polyvinyl chloride (PVC) plastic (Doane, 1987). Yet this idea was against by many as the corn plastic might not be green after all (Wilson, 2012). Encapsulated herbicides which used starch as a matrix in encapsulation were proven to be environmental friendly effect with less volatility and decomposition by light (Doane, 1987).

2.1.2.3 Bio-fuel

The world oil reserved was facing depletion in the next 40 years due to the reduction in crude oil and the increasing usage rate (Dhugga, 2007). Fuel generated from non-food plant materials significantly reduced in the uses of petroleum for automobile transportation or industrial used (Figure 3) such as corn wet milling (Mosier, 2006). Conversion technologies for producing bio-fuel from biomass were still in progression in many countries. These technologies were divided into two broad pathways: chemical decomposition and biological digestion (Balat, 2006). Biological digestion involving the usage of microbial digestion in fermentation processes while chemical decomposition, applying heat to breakdown the biomass component into smaller monomer (Osburn L. & OsburnJ., 1993). The main products of biomass conversion were solid biomass, liquid fuels such as bio-ethanol and biodiesel, and biogases. Fuel produces from this conversion were called second generation oil which used biological materials such as agriculture and forestry residue, straw, herbaceous and woody crops (Mosier, 2006). The corn grain and green young corn were use as food through out the world (Ahsan & Mehdi, 2000). However, the public have not realise that corn residue (stalk and leaves) were capable of producing sustainable alternative fuel which can replace crude oil (Dhugga, 2007).

Many issues may arise with the usage of consumable agriculture material. However, there are no ethical issues concerning the food resources as it associated with corn stover, the residue left after harvesting but not the grain (Lewis et al., 2010). Environmental benefits gains from the usage of bio-fuel as it can reduce the release of carbon monoxide as much as 25 percent if 10 percent ethanol were blend with the fuel. It is also low in reactivity and high oxygen content which reduced the ozone pollutions (Gadisser, 2011). However, different concern was express by Schienke (2007), where the emission of green house gas from the uses of bio-fuel may have a significant

negative impact on the environment and ecosystem. The same opinion was also shared by Thompson (2008) where the technologies that utilize agriculture as a manufacturing system for non food product without mentioning the severity of harm that may arise in future. He also indicated that the usage of non-consumable plant matter will not be adequate for the system, which then will encourage farmers to change from growing corn to switchgrass for example and that will disturb the food supplies. The situation worsens when the availability of land for food production is limited.

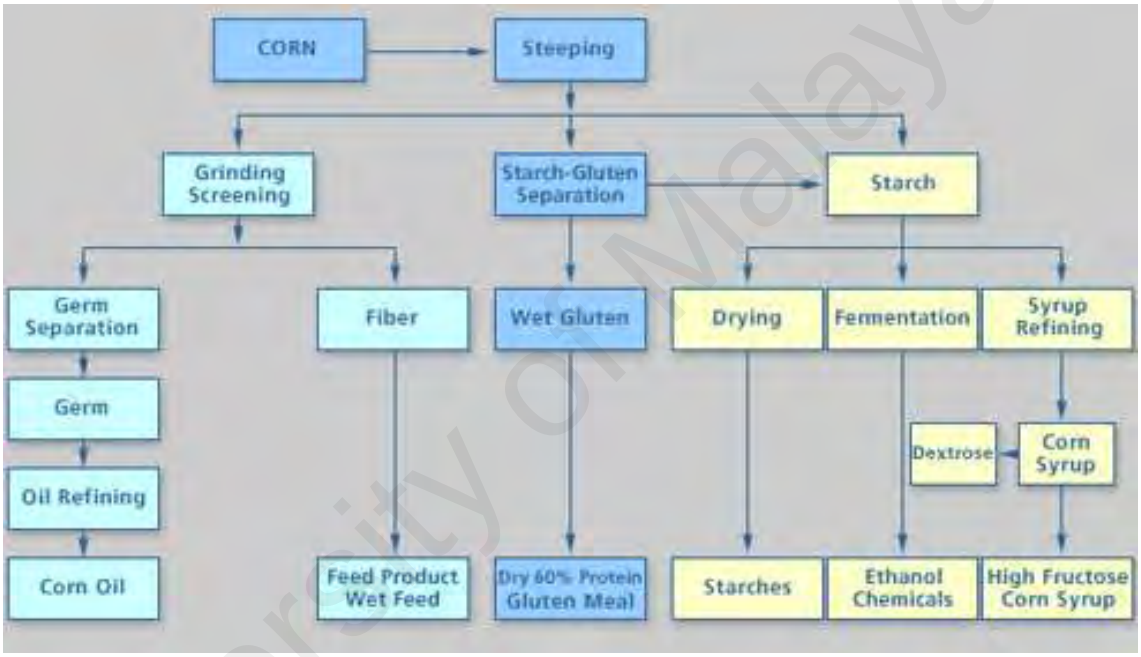


Figure 3. Corn wet milling for ethanol production. (Source: Anonymous, 2005-2012)

2.2 GENETICS ANALYSIS OF GRAIN AND YIELD CONTRIBUTING CHARACTERS IN MAIZE

2.2.1 Grain yield of maize

Grain production is the key to feed the increasing human population. The yield of grain crop has been rising since the advent of the agriculture revolution and it made it possible to develop the urban culture today to support the diminishing number of farmers. In maize, the estimation of yield was helpful in making crop management decisions such as when to harvest a field or in making grain marketing decisions (Lee & Herbek, 2005). According to them, the estimation of corn yield in large scale planting can be made using the following equation:

[1]

Lee and Herbek (2010) also added that the procedures to estimate corn yield are targeted at determining or estimating each of the terms in this equation. All of the procedures to estimate corn yield require counting kernels per ear. Simpler methods for estimating yield include making assumptions about ears per acre and kernels per bushel. The procedures outlined here range from the very simple but not very accurate to the more complicated but more accurate methods of estimating corn yields. However, in smaller scale planting, the yield of corn can be calculated by the number of ears. These then multiply by number of row on an ear. This will give out the rough estimation of yield.

Water availability, insects, weeds, diseases, and other factors can affect seed fill and final yield. As the corn plant approaches black layer or maturity, environmental stresses have less impact on final yield. The exceptions to this are when a catastrophic stress causes severe yield losses, such as a heavy rain that knocks down corn. Since

environmental stresses have less impact on final yield as the corn matures, yield estimates made on corn that is closer to maturity should be more accurate than yield estimates made on corn in the early stages of seed development. Other than the environment effect on grain yield, many other factors can influence yield. Suggested by Elmore and Roeth (2000) yield was influence by kernel weight after the black layer formation. This is due to the stability of the kernel weight after black layer formation and enables the grain to dry properly to be measured.

2.2.2 Grain yield and its influencing factors

Yields were closely related with genetic makeup and environment. Despite the relationship of grain yield with environment, another factor that capable in enhancing the yield productivity is the trait. In earlier finding by Zsubori et al., (2002) the positive association of height with grain yield was found. Other researcher shares the same finding with Zsubori et al., (2002). Iqbal et al., (2011) observed higher plants have potentiality to produce better grain yield compared to the shorter plant. Male flowering and female flowering days have significant and positive association with grain yield (Anderson, 2004). Anderson (2004) mention that the synchronized time for tassel and silk will result in increasing of grain yield as the percentage for each silk to be pollinated by shredded pollen is higher. The synchronization of tasseling time and silking time will result in reduction of kernel abortion as endosperm development was continued after fertilization occurred.

Other than that, grain filling period also plays major role in yield. Badu-Apraku et al., (1983) suggested that the decreasing in grain yield was almost entirely determined by short grain filling period. The association of grain yield with grain filling period were greatly influence by the mobilization and assimilation of photosynthesis product. During photosynthesis process, carbohydrates stored in vegetative part of the

plant which were assimilating then being remobilized to kernels. The capacity of kernels to used up assimilates store in stem were proportional with the duration of grain filling period (Badu-Apraku et al., 1983). Different physiological maturity stages in corn have varied impact on its end yield. Minimum stage of maturity that a plant must reach to gain the highest yield was when the kernel developed at least into dent stage where at this point 65-75% of potential grains were made and the development are more stable (Ojo et al., 2006; Wang et al., 1999).

2.2.3 Genetic analysis

The characters that were shown by each individual were controlled by interaction of genes coupled with environmental effect and supplementary effect. The genetic parameters were elaborated extensively by Rao and Bhatia (2011) where it is used in breeding plans. It is a crucial step to know the relationship between heritability and environmental effect on certain traits. In general, phenotypic variance was partly influence by genotypic variance and environment variance. Heritability in general represents the ratios of genotypic variance and phenotypic variance. It also can be generates from the observation of phenotypic likeliness among relatives (Rao & Bhatia, 2010). The role of heritability is very prominent in predicting breeding value of an individual.

Genetic correlation usually shows the interaction of two or more traits simultaneously. Correlations usually were express based on heritability and environmental causes. A common genetic analysis was ANOVA, which involves the calculation of variance component of particular traits. Significant difference for parameters can be calculated through variance analysis. Numerous software can be used

to determine the variance component such as SAS, Statistica, and SPSS (Rao & Bhatia, 2010).

2.3 BIO-OIL PRODUCTION FROM MAIZE STOVER

2.3.1 Influencing factors of bio-oil production from maize stover

2.3.1.1 Biomass

Biomass is anything that is alive (Goyal et al., 2008). It is also anything that was alive a short time ago. Trees, crops, garbage and animal waste are all biomass (Goyal et al., 2008; Wu, 2011; United Nation Environmental Program [UNEP], 2009). Waste from agriculture can also serve as biomass sources (Figure 4). Biomass conversion may be conducted on two broad pathways: chemical decomposition and biological digestion (Osburn L. & Osburn J., 1993). The conversion technologies for utilizing biomass can be separated into four basic categories which are direct combustion processes, thermochemical processes, biochemical processes and agrochemical processes (Demirbas, 2001). But, from these four processes, there are two main processes in which the biomass can be converted to useful products: thermochemical processes and biochemical processes (Goyal *et al*., 2006; Osburn L. & Osburn J., 1993). Thermochemical process involves the applying high temperature to breakdown the biomass following by sudden cooling while biochemical process involving the breaking of biomass into its component by means of microorganism reaction (Osburn L. & Osburn J., 1993).

According to Balat et al., in their 2009 research, the components of biomass include cellulose, hemicelluloses, lignin, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash, and other compounds (Yokoyama, 2007a). The basic structure of all woody biomass consists of three basic polymers: cellulose

$(C6H10O5)_x$, hemicelluloses such as xylan $(C5H8O4)_m$, and lignin $[C9H10O3(OCH3)0.9–1.7]_n$ in trunk, foliage, stem and bark. The proportion of these wood constituents varies between species, and there are distinct differences between hardwoods and softwoods (Balat et al., 2009).

Hardwoods or deciduous woods have a higher proportion of cellulose, hemicelluloses, and extractives than softwoods, but softwoods have a higher proportion of lignin. In general, hardwoods contain about 42–44% cellulose, 28–31% lignin, 27–29% hemicelluloses, and 2–5% extractives while softwoods contain about 45–47% cellulose, 20–42% lignin, 30–35% hemicelluloses, and 5-8% extractives (Diffen, 2011). Maize is known to be categorized as straw which has high proportion of lignin and cellulose material.

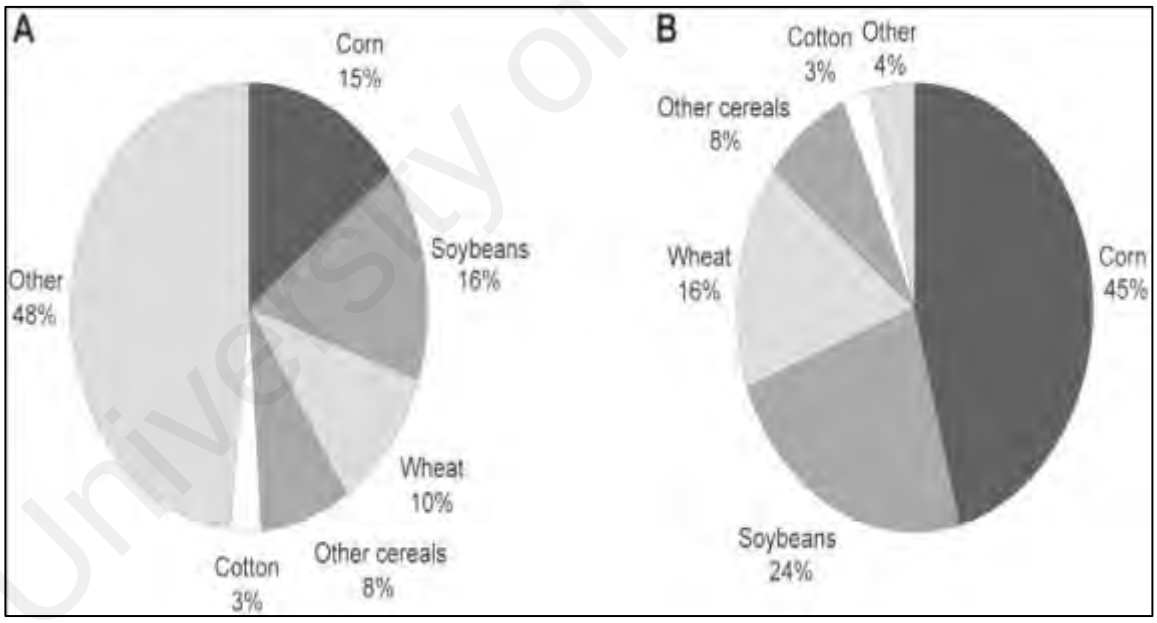


Figure 4. (A) Agricultural area under different crops and (B) residue produced by each crop or crop group. (Source; Dhugga, 2007).

2.3.1.2 Lignocellulose material

Lignocellulosic (Figure 5) that is found in maize stalk and leaves are made of lignin, hemicellulose and cellulose (Dashtban et al., 2009; Howard et al., 2003). Cellulose is an abundant component in plant and woods (Zhang et al., 2007). Cellulose fibres provide wood's strength and comprise at least 40–50 wt. % of dry wood (Balat, 2006). Some lignocellulosic materials can have more cellulose than wood (Goyal et al, 2006).

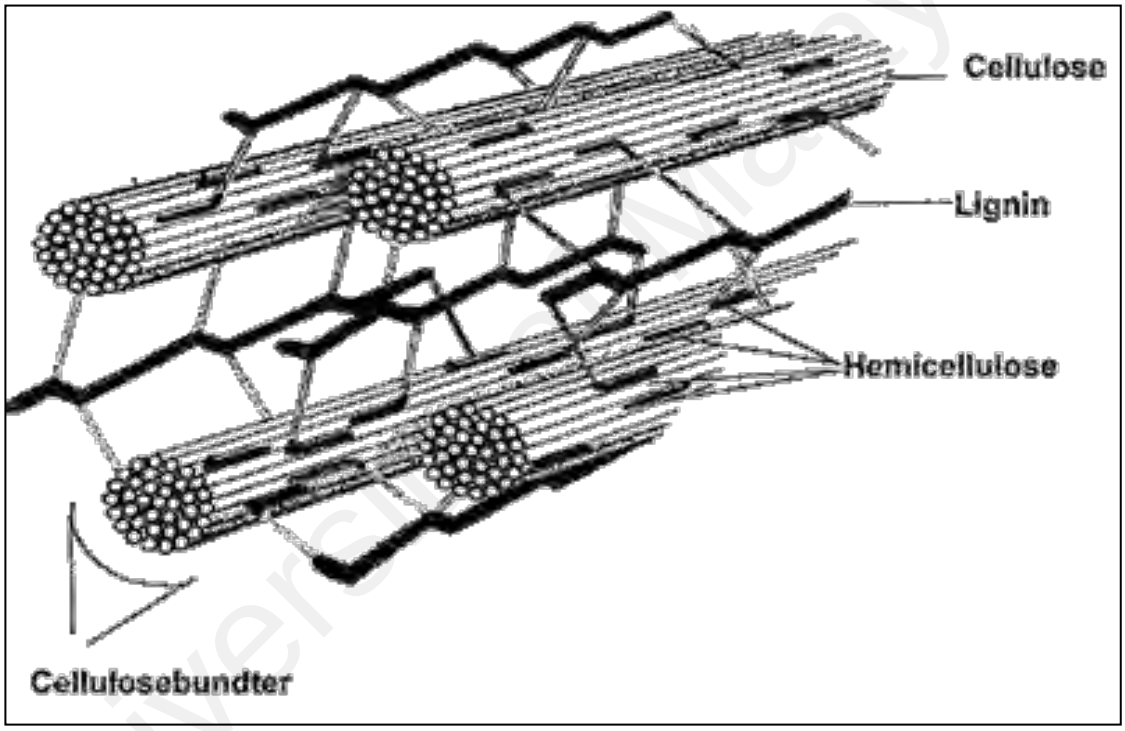


Figure 5. Lignocellulosic structure. Source: (http://www.life.ku.dk/forskning.aspx)

Cellulose is an excellent pure organic polymer, consisting solely of units of anhydroglocose held together in a giant straight chain molecule (Howard et al., 2003). Cellulose is a homopolysaccharide composed of b-D-glucopyranose units linked together by glycosidic bonds (Balat et al., 2008; O'Sullivan, 1997).The basic repeating unit of the cellulose polymer consists of two glucose anhydride units (Figure 6), called a cellobiose unit (Perez and Makie, 2001). Cellulose must be hydrolyzed to glucose

before fermentation to bio-ethanol. By forming intramolecular and intermolecular hydrogen bonds between OH groups within the same cellulose chain and the surrounding cellulose chains, the chains tend to be arranged parallel and form a crystalline super molecular structure (Balat et al., 2009). Then, bundles of linear cellulose chains (in the longitudinal direction) form a microfibril which is oriented in the cell wall structure. Cellulose is insoluble in most solvents and has a low accessibility to acid and enzymatic hydrolysis (Demirbas, 2009).

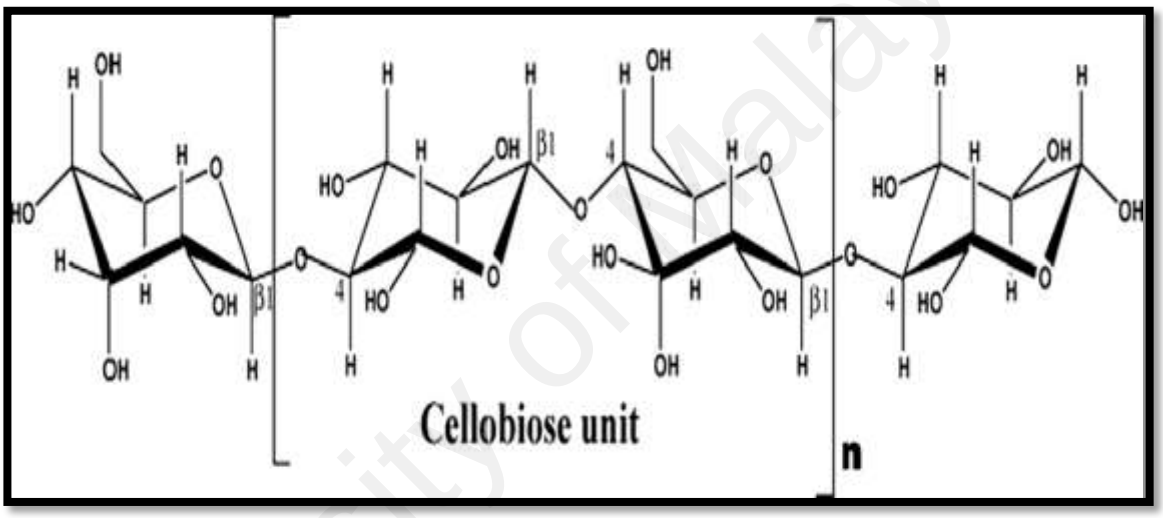


Figure 6. Chemical structure of cellulose. (Source: Balat et al., 2009)

Lignin is an aromatic polymer synthesised from phenyl propanoid precursors. The basic chemical phenyl propane units of lignin consist of primarily syringyl, guaiacyl and p-hydroxy phenol (Figure 7) are bonded together by a set of linkages to form a very complex matrix (Demirbas et al., 2011; Ralph, 1999). This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl and carbonyl, which impart a high polarity to the lignin macromolecules (Demirbas et al, 2011). Lignin provides additional rigidity and compressive strength as well as rendering the walls hydrophobic and water impermeable (Nasar-Abbas et al., 2008). Softwoods generally contain more lignin than hardwoods. Lignin contents on a dry basis in both

softwoods and hardwoods generally range from 20% to 40% by weight and from 10% to 40% by weight in various herbaceous species, such as bagasse, corn, peanut shells, rice hulls and straws (Goyal et al., 2009; Anonymous, 2011).

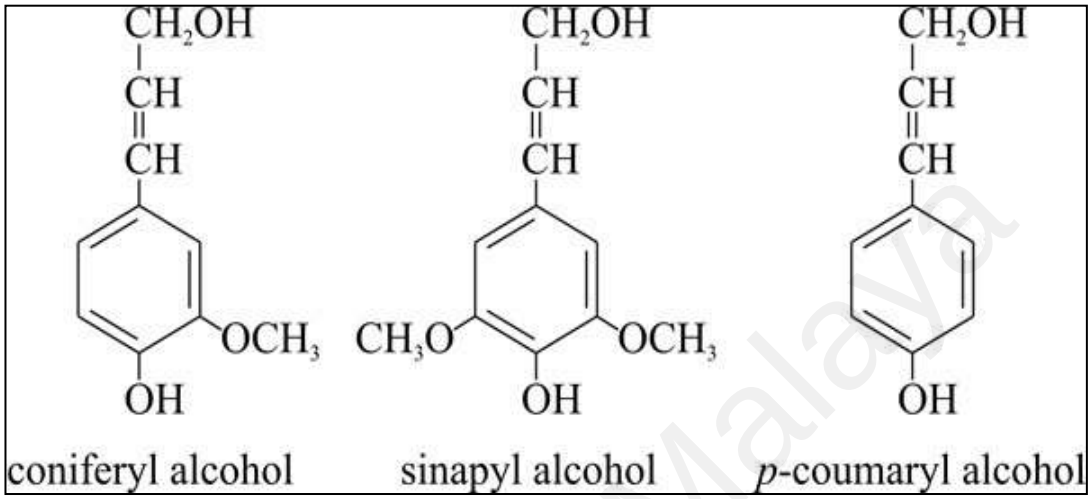


Figure 7. Building unit of lignin. (Source: Moore et al., 2011)

2.3.2 Production of bio-oil from maize stover using thermochemical process

Thermochemical conversion (TCC) technologies can be subdivided into gasification, pyrolysis and direct liquefaction (Demirbas, 2001; Midgett, 2008; Zhang, 2010). Instead of these three processes, there are other different thermochemical conversion processes which were known as combustion and hydrogenation. However, gasification and pyrolysis are the two basic approaches in the thermochemical conversion of biomass (Goyal *et al*., 2008). As mentioned before, through the thermochemical conversion, biomass can also be turned to useful product like bio-fuel.

2.3.2.1 Pyrolysis

Pyrolysis is the fundamental chemical reaction process that is the precursor of both the gasification and combustion of solid fuels. It is simply defined as the chemical changes occurring when heat is applied to a material in the absence of oxygen (Bhaskar et al., 2011; Keleş *et* al., 2011). It is also thermochemical conversion process which is found to be best suited for conversion of biomass to liquid fuel (Goyal et al., 2008). The products of biomass pyrolysis include water, charcoal, oils or tars and permanent gases including methane, hydrogen, carbon monoxide and carbon dioxide (Xu et al., 2011).

There are four types of pyrolysis: slow pyrolysis, fast pyrolysis, flash pyrolysis and catalytic biomass pyrolysis. In slow pyrolysis process, biomass is pyrolysed at slow heating rates (5-7 K/min). This leads to less liquid and gaseous product but more of char production. Compared to the fast pyrolysis, rapid heating rate is involved but not as fast as flash pyrolysis. Heating rate for flash pyrolisis is somewhere about 300 -500^{0}C/min. Generally, the goal of fast pyrolysis is to produce liquid fuel from lignocellulosic biomass that can substitute for fuel oil in any application (Overend, 1999). These are the main reason why fast pyrolysis requires very high heating and heat transfer rate. These

essential features lead to the main products of fast pyrolysis which is bio oil (Bhaskar et al., 2011).

Flash pyrolysis is the process in which the reaction time is of only several seconds or even less. Because of the heating rate is very high, it requires special reactor configuration in which biomass residence times are only of few seconds (Goyal et al., 2008). Any types of biomass can be pyrolysed through flash pyrolysis. Therefore, the particle size of the particular biomass should be fairly small approximately 105-250 μm (Goyal *et al*., 2008). Liquids obtained from biomass by slow, fast or flash pyrolysis process could not be directly used as transportation fuel. These oils need to be refined as they have high oxygen and water content (Goyal et al., 2008). They are also found to be less stable and less miscible in conventional fuels. Thus, that is why catalytic biomass pyrolysis is introduced as it can improve the quality of the oil produced (Goyal et al., 2008). In catalytic biomass pyrolysis, the oil obtained does not require costly pre-up gradation techniques involving condensation and re-evaporation (Goyal *et al*., 2008).

There are various catalysts such as zeolites and basic material was introduced with the biomass feedstock. The catalysts can be divided to several groups that are homogenous, heterogenous and enzymatic catalysts. Catalysts which exist in the same phase as the reactant and product are known as homogenous catalysts, and those in different phases is called heterogenous catalysts. These heterogenous catalysts can be separated easily from the reactants and products, thus can be used again whereas the homogenous is difficult to be separated and used again (Huber et al., 2006).

2.3.2.2 Gasification

Another type of thermochemical conversion of biomass for biofuel production is gasification. Gasification is one of the most important thermochemical conversion processes as it can produce intermediate synthesis gas, which is known as syngas (Rosillo-Calle, 2006). After that, the syngas can be condensed and fermented to produce bioethanol compared to pyrolysis, where the pyrolysis main product is bio oil or also known as pyrolysis oil. Overend (1999) reported that gasification is an extension of pyrolysis, however with different end product optimization to give the highest yield of carbon and energy in the gas phase, rather than to produce char or a liquid (Overend, 1999). Gasification is also performed at high temperature as well as the pyrolysis process, in order to optimize gas production (Demirbas, 2010). Kumar *et al*. (2009) discovered that the main steps involved in the gasification process (Figure 8) starts from upstream processing, gasification and downstream processing. The flow of the steps is as follow:

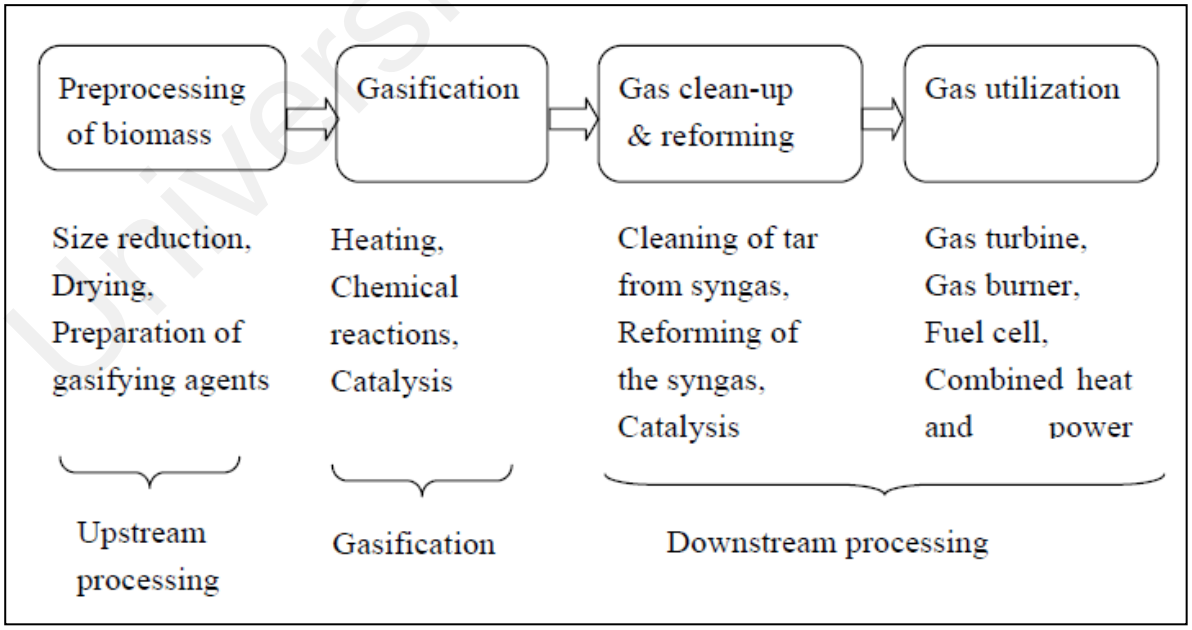


Figure 8. Processes involved in biomass gasification. (Source: Energies, Vol. 2)

Based on Figure 8, the upstream processing includes processing of biomass to make it suitable for gasification to take place. Size reduction is needed to obtain appropriate particle sizes, like grinding or chopping the biomass into smaller pieces (Kumar et al., 2009). This is due to the smaller particles have larger surface areas per unit mass and larger pore sizes which facilitate faster rates of heat transfer and gasification (Kumar et al., 2009). Drying is also needed to achieve appropriate moisture so that the process can work efficiently (Kumar et al., 2009). For example, moisture of corn grain must achieve below than 14 percent for its better performance in the gasification process (Kumar at al., 2009).

As mentioned before, gasification takes place at high temperature in the presence of an oxidizing agent. In this process, heat is supplied to the gasifier either directly or indirectly which raises the gasification temperature of 600-1000 ^{o}C (Kumar et al., 2009). Oxidizing agents are typically air, steam, nitrogen, carbon dioxide, oxygen or a combination of these. Thus, in the presence of an oxidizing agent at high temperature, the large polymeric molecules of biomass decompose into lighter molecules and eventually to permanent gases (carbon dioxide, hydrogen, methane and lighter hydrocarbons), ash, tar, char and minor contaminants (Kumar et al., 2009).

Synthesis gas or syngas is one of the products of the gasification process, which consist primarily a mixture of carbon monoxide, hydrogen and carbon dioxide (Demirbas, 2009). There are several products in producing liquid biofuels by various catalytic processes at various stages of development and commercialization via gasification such as Fischer-Tropsh liquids, biomethanol, higher alcohols, mixed alcohols and also hydrogen. Syngas is a major building block in production of fuels and chemicals and may be combusted for heat and power (Klasson et al., 1992). But, in order to get liquid bio-fuel, the syngas has to undergo a fermentation process. Fermentation of syngas is an attractive process to produce ethanol as a transportation

fuel because the biological catalyst are more specific resulting in less side product (Kumar *et al.*, 2009). In terms of production of ethanol, catalytic processes are used to convert syngas component into a variety of fuels and chemicals (Younesi et al., 2005). Instead of producing ethanol, the catalytic processes are also convert the syngas component into hydrogen, methane, methanol, acetic acid and many more (Younesi *et al*., 2005).

Microorganisms are used as suitable biocatalysts to convert syngas into chemicals and fuels (Younesi et al., 2005). Recently, an anaerobic bacterium, *Clostridium ljungdahlii* is found to be the best biocatalyst in the production of ethanol from syngas which is successfully carried out in batch bioreactor. Datar et al., (2004) demonstrated using the producer gas, generated from gasification of switchgrass, to produce ethanol by fermentation (Kumar et al., 2009). The producer gas was utilized in a 4-L bioreactor using a novel clostridial bacterium (Datar et al., 2004). The novel bacterium utilized is a clostridial species and is referred to as P7 (Datar et al., 2004).

2.3.2.3 Liquefaction

Research on liquefaction has been widely studied since early 70's using various feedstock such as wood and municipal solid waste (MSW). Hydrothermal (direct) liquefaction or HTL, was use to produce oil. Hydrothermal liquefaction is pyrolysis in hot compressed water of around 300°C and 10MPa. Similar as pyrolysis, liquefaction process (Figure 9) converted biomass was converted into gas, liquid and solid (Yokoyama, 2011). As the name suggested, HTL proceed in the present of water whereby drying of feedstock is not needed. This condition is suitable for high moisture content biomass, such as aquatic biomass, food processing waste, animal manure, wood, garbage, and organic sludge (Yokoyama, 2011; Zhang, 2010). Crop residues and wood primarily contain lignocellulose, while animal and food processing waste contains lipids, protein, and usually small amounts of lignocellulose (except ruminant animal manure) which were essential in conversion process (Zhang, 2010).

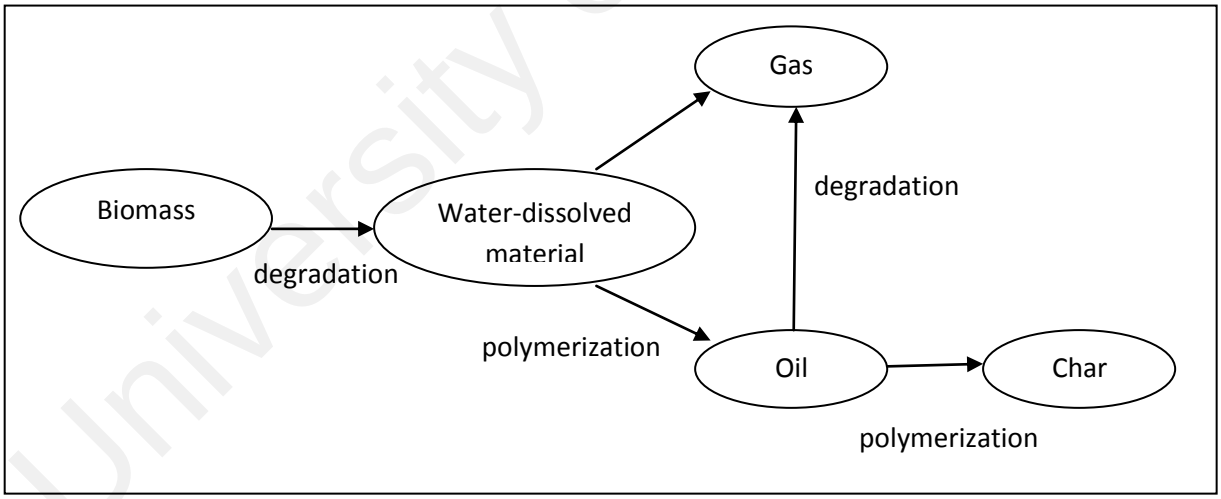


Figure 9. Simple reaction scheme of liquefaction. (Source: Asian biomass handbook, 2011)

Like any other thermochemical, catalyst can be used in liquefaction process. At present researcher been using alkali salts, such as sodium carbonate and potassium carbonate as catalysts for hydrolysis of biomass such as cellulose and hemicellulose into smaller fragments, then the broken down fragments are degraded into smaller

compounds by dehydration, dehydrogenation, deoxygenation and decarboxylation which can lead to these compounds further converted into new compounds such as crude oil through condensation, cyclization and polymerization (Demirbas, 2004; Wu, 2011).

Liquefaction processes were slightly different for each feedstock. Midgett (2008) was describing the whole process with regards to studies conducted by Kanich (1984) and Minawa (1998). The materials were first dried and were ground to powder form and the waste separated into different oil and water slurries to be treated separately. Temperatures ranged from 295-450°C with pressures up to 14 MPa using retention times of 20-90 minutes (Demirbas, 2009). The slurry feedstock was injected into reactor through a pressurized injector and the oil product was extracted by pentane and toluene (Demirbas, 2010). While, Zhang (2010) reported that for cellulose as feedstock, using CO and H_2 as initial gas, at 2 hours, 350 ° C, 1500 psi initial pressure, a much higher benzene - soluble oil yield was obtained when using CO rather than using H_2 as initial process gas. Using CO as the process gas was more effective as it is reduced in oxygen content in oil. Residue and oil have similar elemental composition but different structures, making them appear differently. Water-soluble fraction resulting from hydrolysis of the cellulose is believed to be a precursor of the oil because it can be converted to oil by recycling the aqueous solution through the process with subsequent charges of refuse (Zhang, 2010).

3.0 METHODOLOGY

3.1 LOCATION

Field experiment was conducted from May 2010 to December 2011, at experimental field in Genetics and Microbiology Department, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, whereas the laboratory studies were conducted from October 2010 to March 2011, at Department of Chemical Engineering, Faculty of Engineering, University of Malaya, Kuala Lumpur.

3.2 MATERIALS

3.2.1 Field experiment

This study was a part of a research project adaptability of tropical maize and sweet corn cultivation in Malaysia conducted in Division of Genetics and Molecular Biology, Institute of Biological Sciences, University of Malaya. In the previous study, 22 tropical maize genotypes were planted along with yellow sweet corn inbred lines. The small distance between these two genotypes enable natural crossing as well as manual crossing among the selected genotype. Based on agronomic performance yield contributing traits, F_1 hybrids from 6 different crosses were chosen. The crosses as were listed below.

i. E1 ♀ x Sweet corn ♂ [E1 (F1)]

ii. E5 ♀ x Sweet corn ♂ [E5 (F1)]

iii. E9 ♀ x Sweet corn ♂ [E9 (F1)]

iv. E11 ♀ x Sweet corn ♂ [E11 (F1)]

v. E12 ♀ x Sweet corn ♂ [E12 (F1)]

vi. E17 ♀ x Sweet corn ♂ [E17 (F1)]

Along with these crosses, the female parents for each genotype were planted. The parents were listed below.

i. E1

ii. E5

iii. E9

iv. E11

v. E12

vi. E17

The hybrid maize and female parent were used as sole sample for genetic analysis and evaluation of yield contributing traits as well as biomass in bio-oil production.

3.2.2 Bio-oil production

Stover (leaves and stalk) were collected from six F_1 hybrids from six different crosses prior harvesting and labelled. The stover were cut into smaller pieces and dried under in oven at 50°C for a period of 72 hours – 120 hours to ensure the moisture is below 12%.

3.3 METHODS

3.3.1 Experimental design and plot arrangement

The materials comprised six entries generated from crossing of parental inbred lines i.e. tropical maize and yellow sweet corn. There were six different types of F_1 hybrid maize genotypes ie. twelve individuals of each types were planted. The F_1 developed from different crosses of tropical maize and yellow sweet colour variety, where E1, E5, E9, E11, E12, and E17 (9ILWH1201/ILWH1203) were used as parental material and yellow coloured corn (unknown pedigree, received from IITA). The plot for one entry comprised five rows. The row was 3 m long with 75 cm spacing between each rows, spacing between hills within each row is 50 cm. Each hill was pointed with a depth of 5 cm approximately.

3.3.2 Cultivation practices

Before planting, the soil was ploughed to a depth of about 15 cm. Planting was done manually, where four seeds were sown per hill and later thinned to just two plants per hill, after 10 to 14 days after planting. Both NPK (15:15:15) and urea fertilizers with ratios of 2:1 were used during planting. Again, urea was top dressed at 15 days, 30 days, and 45 days after planting. In this experiment, weeds and pest were controlled manually which was done when necessary. The whole experiment was conducted mostly under rainfed condition.

3.4 LABORATORY STUDIES

3.4.1 Sample Preparation

At harvest (approximately 3 months), stovers which consisted of stalks and leaves were collected from each genotype, followed by removal of the leaves from the stalks. Then, they were dried under hot sun for about seven days or using oven for three days at 50°C. The stovers were then cut into smaller pieces approximately 10 cm before they were grounded using rapid granulator to smaller particle size (≈ 0.1 mm – 4.0 mm). Each sample was oven dried again at 100^{o}C to remove any left moisture. After the samples were dried, the laboratory sieve test was conducted; where each sample was sieved using different sizes sieve (1.4 mm, 1.7 mm, 2.0 mm and 3.0 mm) in order to separate the different size of particle which was used later in pyrolysis process.

Pyrolysis was conducted using reactor with manual temperature control equipped with a condenser. First, 80 g of maize stover sample was place inside the stainless steel tubular reactor with a length of 127 cm and an internal diameter of 2.4 cm. The reactor was then sealed by placing the K- type thermocouples on the reactor head and the bolts were tightened. The reactor was heated externally by electric vertical furnace, where the temperature was control by the thermocouple. Then, nitrogen hose was connected to the reactor in order to channel the nitrogen gas into the reactor. When the condenser showed temperature at 1^{o}C, the reactor controller (heater) was turned on and the temperature set point was specified. The connection need to be checked continuously to ensure no leakage while experiments were conducted. Once the reactor reaches the desirable temperature, the reactor is left according to residence time needed. When the reaction was completed, the heater was shut down. The reactor was allowed to cool down using a big stand fan. Cooled down temperature for reactor was 50°C - 60°C. Bio-oil was then collected from the vessel below the condenser and from the hose

which is connected to the reactor. Bolts were removed to collect the char left inside the reactor. Both bio-oil and char were weighed and labelled.

Four parameters were studied in order to obtain optimize condition for maize pyrolysis. These parameters are temperature, particle size, and residence time and nitrogen volume. Varying temperatures of 300°C, 350°C, 400°C, 450°C, 500°C, 550°C, 600°C and 650°C were used, residence times ranging from 15 min, 30 min, 45 min, 60 min and 75 minutes, nitrogen volume starting at 0.5 L/min, 1.0 L/min, 2.0 L/min and 3.0 L/min. Particle size of 0.5 mm< dp <1.0 mm, 1.0 mm< dp <1.4 mm, 1.4mm < dp < 2.0 mm, 2.0 mm < dp < 3.0 mm.

The first series of experiment were carried out to determine the effect of the temperature on pyrolysis yields with constant particles size of 1.4 mm < dp < 2.0 mm, 2L/min of N_2 flow rate and 45 min reaction time and the temperatures were maintained at 350°C, 400°C, 450°C, 500°C, 550°C, 600°C, 650°C and 700°C. At the end of each experiment, the condensable liquid and the solid char was removed from inside the reactor were collected and weighed. The product yield is calculated as follows:

[2]

The second series of experiment were conducted to investigate the effect of particle size on yields. This series of experiment, final pyrolisis process condition were kept constant at temperature 550°C, 2L/min of N_2 flow rate and 45 min reaction time. Particle size used are of 0.5 mm < dp < 1.0 mm, 1.0 mm < dp < 1.4 mm, 1.4 mm < dp < 2.0 mm, 2.0 mm < dp < 3.0 mm. At the end of each experiment, the condensable liquid and the solid char was removed from inside the reactor were collected and weighed. The product yield was calculated using formula [2].

The third set was carried out to study the effect of reaction time on pyrolysis yield. The time varies at 15 min, 30 min, 45 min, 60 min, 75 min with constant condition at 550°C, 2L/min of N_2 flow rate, 1.4 mm < dp < 2.0 mm. At the end of each experiment, the condensable liquid and the solid char were removed from inside the reactor were collected and weighed. The product yield was calculated using formula [2].

The last series of experiment was to investigate the gas flow rates at 0.5 L/min, 1.0 L/min, 2.0 L/min, 3.0 L/min with the constant of 550°C of temperature, 1.4 mm < dp < 2 mm particle size, and 45 min reaction time. At the end of each experiment, the condensable liquid and the solid char were removed from inside the reactor were collected and weighed. The product yield was calculated using formula [2].

3.5 DATA COLLECTION

Data were collected during plantation season and post-harvest. The characters including flowering days for both male and female parts of each entry (days), maturation days for each entry (days), grain filling period (days), plant height (cm), number of leaves per genotype, leaves length (cm), number of ears per genotype, stem diameter (cm), total grain weight (g) and 1000-grain weight (g). For laboratory studies, 80g of sample were used for each experiment. Data were collected during the reaction and after the experiment has finished including time of heater started and stopped (min), time of reaching desired temperature (min), time of gas product released (min), oil weight (g), char weight (g), oil percentage (%), char percentage (%) and produced gas percentage (%).

3.6 STATISTICAL ANALYSIS

PROC GLM in SAS/STAT (SAS Institute, 2004) was used for variance analysis (ANOVA) for each traits evaluated during and after planting season. The direct and indirect effects of measured traits on grain yield was determined using standardized partial regression coefficient which suggested by (Manal, 2011) using STATISTICA (StatSoft Inc.) version 10. Analysis was conducted on F_1 hybrid plant for each replicate.

Variance component were calculated including genotypic variance, phenotypic variance, heritability (H_b), genotypic coefficient variance (GCV), phenotypic coefficient variance (PCV), and genetic advance (GA). Traits to traits correlation were calculated as partially Pearson correlation. As reported by Singh (2005); Roa and Bhatia (2010), the equation for variances as follows:

[3]

$$\textit{Genotypic variance } (\sigma_g^2) = (\textit{Ms due to genotype} - \textit{Ms due to error}) / \textit{replication}$$

[4]

$$\textit{Phenotypic variance } (\sigma_p^2) = \textit{Genotypic variance} + \textit{Ms due to error}$$

[5]

$$\textit{Heritability } (H_b) = \textit{Ratios of Genotypic variance by Phenotypic variance}$$

[6]

$$\textit{Genetic advance } (GA) = (k)\,(\sqrt{\sigma_p})\,(H_b)$$

**Where k is Selection differential, $\sqrt{\sigma_p}$ is Phenotypic standard deviation and H_b is Heritability*

[7]

$$GCV = (\sqrt{\sigma_g^2} / X) \times 100$$

[8]

$$PCV = (\sqrt{\sigma_p^2} / X) \times 100$$

**Where X is the grand mean respectively to character under consideration, $\sqrt{\sigma_g^2}$ is the Genotypic standard deviation and $\sqrt{\sigma_p^2}$ is Phenotypic standard deviation.*

3.7 OIL CHARACTERIZATION

3.7.1 Fractionation of bio oil

2 ml bio oil obtain from previous pyrolysis experiment (Temperature of 550°C, residence time of 45 min, 2L/min nitrogen flow and particle size of 2 mm) were centrifuge at 1300 rpm for 10 minutes. The oil is separated into 2 layers. The upper layer part was called light oil while the bottom part is heavy oil. Light oil was used later in oil analysis.

3.7.2 CHN Analysis

1.3µL of light oil were pipette into aluminium vessels (CHN liquid vessel). Approximately 1.5µg (1.3 µL) of sample was inserted into each vessel. Once the oil is weight, the vessels were seals and placed inside the CHN analyzer. Each element was calculated as percentage.

3.7.3 FT-IR Spectrometer

1.3 µL light bio oil from fractionation process were place on one of KBr plates using Pasteur pipette. The second plate was place on top of the first plate and makes a quarter turn to obtain even film. The plates were place into the sample holder and the spectrum was runned. After obtain the IR spectrum, the plates was rinse thoroughly using acetone or methylene chloride for several time to prevent future contamination.

3.7.4 Gas Chromatography/ mass spectrometer (GC/MS)

Liquid extraction was conducted on approximately 5 ml of bio-oil sample mixed with 5 ml dichloromethane in a separating funnel. The organic solvent was transferred into a vial, any traces of water was removed with anhydrous sodium sulphate and then subjected to GC-MS analysis.

GC-MS analysis was performed using an Agilent Technologies 6890N gas chromatograph equipped with a 5975 inert mass selective detector (70 eV direct inlet) on fused silica capillary column HP-5ms (30.0 m × 25 mm ID × 0.25 μm film thickness). The carrier gas was helium (99.999%) at a flow rate of 1 mL per minutes and split ratio was 1:20. The column temperature was programmed initially at 30°C and was kept isothermally for 30 minutes, then increased 3°C per minute to 260°C. The temperature of injector port, detector and interface of mass spectrometry were programmed at 300°C.

The total ion chromatogram obtained was integrated by Chemstation Software and the compounds were identified by comparison with published mass spectra database (NIST 02 Mass Spectral Library).

3.7.5 pH

Acid concentration was determined using titration of bio oil with 0.1 standardized NaOH solution using PHS-25C precision pH meters.

3.7.6 Karl Fisher titration (KF)

Water content of bio oil were determined using Karl Fisher (KF) titration using 3:1 methanol:chloroform as solvent. Mixed warm water and bio oil in a 2:1 ratio to determined the water insolubles, the filtration of sample through a 0.45μm PTFE filter

followed by determination of water content of aqueous layer by KF. Water insolubles were calculated according to the equation reported by Mullen et al (2008).

[8]

*Where, A is weight dry oil (g), B is water content in oil (g), C is water added (g) and D is the water content by KF after water addition and filtration. Water soluble is then determined by difference.

3.7.7 Viscosity

The dynamic viscosity was measured with SYP-IA viscosmeter.

4.0 RESULTS

4.1 AGRONOMIC CHARACTER EVALUATION

Evaluation of agronomic traits were done using several parameters such as plant height, female and male flowering days, grain filling period, maturation days, stem diameter, number of cob per plant, number of leaves, leaves length, total grain weight, 1000 grain weight, bio oil and stover weight. The data collected from pre and post harvesting, were compute using SAS 9.2 software and presented in Table 2 and Table 3.

4.1.1 Plant height

Considering plant height, the mean for twelve genotypes were 170.48 cm with standard deviation value of 13.51. Least Significance Difference (LSD) value at 0.05 significance level was 22.689 and the Coefficient Variance (CV) for plant height was 7.92. Variance ratios (F value) within genotype were 0.92, while Variance ratios (F value) among genotypes were 23.09. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest plant height was observed in genotype E17 while the lowest plant height was observed in genotype hybrid between E1 and sweet corn.

4.1.2 Flowering days

For flowering days data, group mean for days to tasseling days was 58.86 cm with standard deviation value of 1.07. Least Significance Difference (LSD) value at 0.05 significance level was 1.81 and the Coefficient Variance (CV) for days to tasseling was 1.82. Variance ratios (F value) within genotype were 0.10, while Variance ratios (F value) among genotypes were 133.72. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3.

Group mean for days to silking was 60.56 with standard deviation value of 1.127. Least Significance Difference (LSD) value at 0.05 significance level was 1.91 and the Coefficient Variance (CV) was 1.86. Variance ratios (F value) within genotype were 0.28, while Variance ratios (F value) among genotypes were 88.05. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The longest flowering days for male and female flower was observed in genotype E17 while the lowest flowering days for male was observed in genotype E1 and female flower was observed in genotype hybrid between E9 and sweet corn.

4.1.3 Grain filing period

Grain filling period was obtained by deducting female flowering days from maturation days for each entry. Considering grain filling period, the mean group was 25.36 with standard deviation value of 0.91. Least Significance Difference (LSD) value at 0.05 significance level was 1.54 and the Coefficient Variance (CV) was 3.59. Variance ratios (F value) within genotype were 3.04, while Variance ratios (F value) among genotypes were 15.21. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The longest grain filling period was observed in genotype E9 while the lowest grain filing period was observed in genotype hybrid between E9 and sweet corn.

4.1.4 Days to maturity

Days to maturity are counted from the plant start germination until it is ready to be harvested. Mean for days to maturity was 85.94 considering standard deviation values of 2.48. Least Significance Difference (LSD) value at 0.05 significance level was 4.20 and the Coefficient Variance (CV) was 2.88. Variance ratios (F value) within genotype were 0.98, while Variance ratios (F value) among genotypes were 24.43.

Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The longest days taken to maturity was observed in genotype E17 while the lowest days taken to maturity were observed in genotype hybrid between E9 and sweet corn.

4.1.5 Stem diameter

Measurements for stem diameter were taken after the first nodes for each genotype. Mean for stem diameter was 7.81 cm considering standad deviation value of 0.06. Least Significance Difference (LSD) value at 0.05 significance level was 0.11 and the Coefficient Variance (CV) was 0.89. Variance ratios (F value) within genotype were 0.80, while Variance ratios (F value) among genotypes were 1184.78. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The largest stem diameter was observed in genotype hybrid between E11 and sweet corn while the lowest stem diameter was observed in genotype E1.

4.1.6 Number of cob per genotype

Considering number of cob per genotype, the group mean was 2.92cm with standard deviation of 0.51. Least Significance Difference (LSD) value at 0.05 significance level was 0.87 and the Coefficient Variance (CV) was 17.65. Variance ratios (F value) within genotype were 2.83, while Variance ratios (F value) among genotypes were 3.91. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest number of cob per plant was observed in genotype E17, hybrid between E12 and E17 with sweet corn while the lowest number of cob per genotype was observed in genotype E1, E9, hybrid between E1, E9, E11 with sweet corn.

4.1.7 Number of leaves per plant

Group mean calculate for number of leaves per plant was 11.47 with standard deviation of 0.66. Least Significance Difference (LSD) value at 0.05 significance level was 1.12 and the Coefficient Variance (CV) was 5.76. Variance ratios (F value) within genotype were 1.21, while Variance ratios (F value) among genotypes were 13.38. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest number of leaves per plant was observed in genotype E11, E12, E17 hybrid between E12, E17 with sweet corn while the lowest stem diameter was observed in hybrid genotype E5 and E11 with sweet corn.

4.1.8 Leaves length

Considering leave length, the mean obtain was 92.96 with standard deviation of 3.42. Least Significance Difference (LSD) value at 0.05 significance level was 5.78 and the Coefficient Variance (CV) was 3.67. Variance ratios (F value) within genotype were 0.16, while Variance ratios (F value) among genotypes were 8.45. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest leaves length was observed in genotype hybrid between E11 with sweet corn while the lowest leaves length was observed in genotype E9 with sweet corn.

4.1.9 Grain yield

Group mean calculate for total grain weight was 280.49 with standard deviation of 23.88. Least Significance Difference (LSD) value at 0.05 significance level was 39.27 and the Coefficient Variance (CV) was 8.27. Variance ratios (F value) within genotype were 2.25, while Variance ratios (F value) among genotypes were 6.29. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT)

at 0.05 significance level as were shown in Table 3. The highest grain yield was observed in genotype hybrid between E12 and sweet corn while the lowest stem diameter was observed in genotype E1 with sweet corn.

4.1.10 Thousand grain weight

Group mean calculate thousand grain weight was 278.51 with standard deviation of 9.24. Least Significance Difference (LSD) value at 0.05 significance level was 15.64 and the Coefficient Variance (CV) was 3.32. Variance ratios (F value) within genotype were 2.15, while Variance ratios (F value) among genotypes were 38.06. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest thousand grain weight was observed in genotype E9 while the lowest thousand grain weight was observed in genotype hybrid genotype E12 sweet corn.

4.1.11 Productivity of bio-oil

Productivity of biol oil have group mean of 36.47 with standard deviation of 1.58. Least Significance Difference (LSD) value at 0.05 significance level was 2.68 and the Coefficient Variance (CV) was 4.33. Variance ratios (F value) within genotype were 0.47, while Variance ratios (F value) among genotypes were 72.50. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest bio-oil production was observed in genotype E12 while the lowest bio-oil production was observed in genotype E9.

4.1.12 Stover weight

Mean calculated for stover weight was 255.14 with standard deviation of 14.58. Least Significance Difference (LSD) value at 0.05 significance level was 24.69 and the

Coefficient Variance (CV) was 5.72. Variance ratios (F value) within genotype were 1.65, while Variance ratios (F value) among genotypes were 124.01. Mean range between each genotype were group using Duncan's Multiple Range Test (DMRT) at 0.05 significance level as were shown in Table 3. The highest stover weight was observed in genotype E9 while the lowest stover weight was observed in genotype hybrid genotype E5 sweet corn.

4.2 GENETIC ANALYSIS OF AGRONOMIC TRAITS

Analysis of variances comprises genotypic and phenotypic variance through SAS PROC (9.2 version), genotypic coefficient of correlation (GCV) and phenotypic coefficient of correlation (PCV), heritability, and genetic advance was calculated according to methods of Singh (2005); Rao and Bhatia (2010).

4.2.1 Plant height

Genotypic variance for height was 200.2 while phenotypic variance was 382.6. Broad sense heritability was 0.52 while genetic advance calculated at 5 % intensity (k = 2.06), was 394.1. GCV value considering height was 8.30 and PCV value was 1.47.

4.2.2 Flowering days

Genotypic variance for days to tasseling was 42.67 while phenotypic variance was 43.81. Broad sense heritability was 0.97 while genetic advance calculated at 5 % intensity (k = 2.06), was 87.5. GCV value considering days to tasseling was 11.10 and PCV value was 11.25.

Genotypic variance for for days to silking was 31.1 while phenotypic variance was 32.37. Broad sense heritability was 0.96 while genetic advance calculated at 5 % intensity (k = 2.06), was 64.0. GCV value considering days to silking was 9.21 and PCV value was 9.37.

4.2.3 Grain filing period

Genotypic variance for grain filling period was 3.42 while phenotypic variance was 4.25. Broad sense heritability was 0.82 while genetic advance calculated at 5 % intensity (k = 2.06), was 7.00. GCV value considering grain filling period was 7.40 and PCV value was 8.13.

4.2.4 Days to maturity

Considering days to maturity, genotypic variance was 40.63 while phenotypic variance was 4.25. Broad sense heritability was 0.87 while genetic advance calculated at 5 % intensity (k = 2.06), was 83.60. GCV value was 7.42 and PCV value was 7.96.

4.2.5 Stem diameter

Genotypic variance for stem diameter was 1.50 while phenotypic variance was 1.51. Broad sense heritability was 0.98 while genetic advance calculated at 5 % intensity (k = 2.06), was 4.71. GCV value considering stem diameter was 16.15 and PCV value was 16.21.

4.2.6 Number of cob per genotype

Genotypic variance for number cob per genotype was 0.24 while phenotypic variance was 0.51. Broad sense heritability was 0.48 while genetic advance calculated at 5 % intensity (k = 2.06), was 0.50. GCV value considering number of cob was16.77 and PCV value was 24.46.

4.2.7 Number of leaves per plant

Genotypic variance for number of leaves per plant was 0.22 while phenotypic variance was 0.66. Broad sense heritability was 0.33 while genetic advance calculated at 5 % intensity (k = 2.06), was 0.05. GCV value considering number of leaves trait was 4.09 and PCV value was 7.08.

4.2.8 Leaves length

Genotypic variance for leaves length was 24.01 while phenotypic variance was 35.68. Broad sense heritability was 0.67 while genetic advance calculated at 5 % intensity (k = 2.06), was 49.46. GCV value considering leaves length was 5.27 and PCV value was 6.43.

4.2.9 Grain yield

Considering grain yield, the genotypic variance was 150.79 while phenotypic variance was 236.13. Broad sense heritability was 0.64 while genetic advance calculated at 5 % intensity (k = 2.06), was 310.80. GCV value for grain yield was 4.377 and PCV value was 5.43.

4.2.10 Thousand grain weight

Considering thousand grain yield, the genotypic variance was 897.19 while phenotypic variance was 982.5. Broad sense heritability was 0.91 while genetic advance calculated at 5 % intensity (k = 2.06), was 1847.80. GCV value considering thousand grain yields was 10.75 and PCV value was 11.25.

4.2.11 Productivity of bio-oil

Genotypic variance for bio-oil productivity was 50.31 while phenotypic variance was 52.80. Broad sense heritability was 0.95 while genetic advance calculated at 5 % intensity (k = 2.06), was 0.95. GCV value considering bio-oil was 19.45 and PCV value was 19.92.

4.2.12 Stover weight

Genotypic variance for stover weight was 109.60 while phenotypic variance was 322.19. Broad sense heritability was 0.34 while genetic advance calculated at 5 % intensity (k = 2.06), was 225.78. GCV value considering stover weight was 4.10 and PCV value was 7.04.

Table 2. Data entries for height, flowering period, grain filling period, maturation period, stem diameter, number of cob per genotype, number of leaves, leaves length, grain yield, thousand grain weight, bio oil and stover weight.

Genotype	Height (cm)	Flowering		Grain filling Period	Mature days	Stem Diameter (cm)	Number cob	Number leaves	Leaves Length (cm)	Grain yield (g)	1000 grain Weight (g)	Bio oil (%)	Stover Weight (g)
		male	female										
E1 (F_1)	118.7	52	55	27	82	9.3	2	9	95.9	188.4	263.3	32.9	173.0
E5 (F_1)	132.0	51	55	25	80	6.2	3	10	88.4	230.0	315.1	42.0	144.7
E9 (F_1)	134.8	52	53	21	74	7.6	2	12	83	246.9	231.3	31.2	163.3
E11 (F_1)	142.7	51	55	26	81	10.3	2	10	86.5	230.8	261.6	38.5	153.0
E12 (F_1)	156.3	55	56	24	80	7.3	4	11	104.5	365.7	251.3	35.3	211.3
E17 (F_1)	158.0	58	59	24	83	8.5	4	13	100.2	300.8	244.7	33.5	183.3
E1	166.9	43	65	27	94	6.1	2	12	91.6	288.8	294.5	33.5	318.2
E5	215.6	59	61	27	88	6.8	3	12	92.5	300.6	297.4	26.4	326.3
E9	183.8	62	62	29	88	6.0	2	12	93.7	251.5	342.9	24.3	414.7
E11	200.5	68	67	25	92	8.4	3	13	94.0	273.8	291.9	45.7	350.7
E12	193.0	68	67	25	93	7.3	3	13	92.4	336.9	252.0	49.4	323.7
E17	239.6	72	72	25	97	7.1	4	13	93.0	352.7	296.2	44.4	330.0
Mean	170.47	58.86	60.56	25.36	85.94	7.58	2.92	11.47	92.96	280.49	278.51	36.47	255.14
Standard deviation	13.51	1.07	1.13	0.91	2.48	0.07	0.51	0.66	3.42	23.88	9.24	1.58	14.58

Table 3. Agronomic traits genetic analysis

Genotype	Height (cm)	Flowering		Grain filling period	Days to maturity	Stem Diameter (cm)	Number cob	Number leaves	Leaves Length (cm)	Grain yield (g)	1000 grain Weight (g)	Bio oil (%)	Stover Weight (g)
		tassel	silk										
F value	20.20	0.10*	0.28**	3.04**	0.98**	0.80**	2.83**	1.21**	0.16*	2.25**	2.15**	0.47**	1.65**
LSD	22.69	1.81	1.91	1.54	4.19	0.11	0.87	1.12	5.78	39.27	15.64	2.68	24.69
CV%	7.92	1.82	1.86	3.59	2.89	0.89	17.65	5.76	3.67	8.27	3.32	4.33	14.58
Genotypic variance	200.2	42.67	31.1	3.42	40.63	1.50	0.24	0.22	24.01	150.79	897.19	50.31	109.60
Phenotypic variance	382.6	43.81	32.37	4.25	46.78	1.51	0.51	0.66	35.68	236.13	982.5	52.80	322.19
GCV	8.30	11.10	9.21	7.40	7.42	16.16	16.77	4.09	5.27	4.38	10.75	19.45	4.10
PCV	11.47	11.25	9.37	8.13	7.96	16.21	24.46	7.08	6.43	5.48	11.25	19.92	7.04
Heritability	0.52	0.97	0.96	0.80	0.87	0.98	0.48	0.33	0.67	0.64	0.91	0.95	0.34
Genetic advance, GA	394.1	87.5	64.0	7.00	83.60	4.71	0.50	0.05	49.46	310.80	1847.8	103.3	225.78

LSD = Least Significance Difference, CV= coefficient variance, GCV = Genotypic coefficient variance, PCV = Phenotypic coefficient variance

* = Significance difference at P = 0.05 (95%) , ** = Significance difference at P = 0.01 (99%)

Table 4. Duncan's Multiple Range Test (DMRT) of variation of physiology traits of maize in each genotype.

Genotype	HE	FM	FF	GF	MD	SD	NC	NL	LL	GY	TGW	BIO	STV
E1 (F_1)	118.7h	55.0fg	55.0fg	26.7bc	81.7c	9.3b	2.3b	8.7d	95.9bc	188.4f	263.3d	32.9ef	173.0gf
E5(F_1)	132.0gh	55.0fg	55.0fg	25.0def	80.0c	6.2i	2.3b	9.7d	88.4def	230.6e	315.1b	41.9c	144.7h
E9(F_1)	134.8fgh	53.0g	53.0g	20.7g	73.7c	7.6e	2.3b	12.0abc	83.0f	246.9de	231.3f	31.2f	163.3gfh
E11(F_1)	142.8efgh	55.0fg	55.0fg	26.0bc	81.0c	10.3a	2.3b	9.7d	86.5ef	230.8e	261.7d	38.5d	153.0gh
E12(F_1)	156.5efg	56.0f	56.0fg	24.3ef	80.3c	7.3f	3.7a	11.3c	104.5a	365.7a	251.3ef	35.3e	211.3e
E17(F_1)	158.0ef	58.7e	58.7e	23.7f	82.7c	8.5c	3.7a	12.7ab	100.2ab	300.8bc	244.1f	33.9ef	183.3f
E1	166.9ed	59.7d	64.3c	26.8bc	94.3a	6.1ij	2.3b	12.3abc	91.3cde	288.8cd	294.5c	33.5ef	284.7d
E5	215.6b	58.7d	61.0d	27.0b	88.0b	6.8h	3.3ab	11.7bc	92.5cde	300.6bc	297.4c	26.4g	326.3bc
E9	183.8cd	61.7c	62.3d	29.0a	88.0b	6.0j	2.3b	11.7bc	92.7cd	250.5de	342.9a	24.4g	417.7a
E11	200.5bc	67.7b	67.0b	25.3cde	92.3ab	8.4d	12.7ab	1.7ab	93.9cd	273.8cd	291.9c	45.7b	350.7b
E12	193.0bc	67.7b	67.3b	25.3cde	92.7a	7.3f	3.3ab	13.0a	92.6cde	336.4ab	251.9de	49.4a	323.3c
E17	242.9a	72.0a	72.0a	23.7f	96.7a	7.1g	3.7a	12.3abc	92.9cde	352.7ab	296.2c	44.9b	330.0bc

HE= Plant height, FM= Days to tasseling, FF= Days to silking, GF= Grain filling period, MD= Days to maturity, SD= Stem diameter, NC= Number of cob/plant NL= Number of leaves, LL= Leaves length, GY= Grain yield, TGW= Thousand grain weight, BIO= Bio -oil, STV= Stover weight

Means with same letter are not significantly different.

Table 5. The Phenotypic Correlation among the traits

TRAITS	HE	FM	FF	GF	MD	SD	NC	NL	LL	GY	TGW	BIO	STV
HE	1.000000												
FM	0.826664	1.000000											
FF	0.843252	0.957000	1.000000										
GF	0.244065	0.172652	0.251708	1.000000									
MD	0.767719	0.834581	0.916848	0.450926	1.000000								
SD	-0.334258	-0.282195	-0.321058	-0.164985	-0.311854	1.000000							
NC	0.477716	0.411628	0.310256	-0.107253	0.173284	-0.073902	1.000000						
NL	0.570905	0.666824	0.607245	-0.182565	0.457186	-0.354334	0.331172	1.000000					
LL	0.078265	0.185820	0.113532	0.175022	0.133454	0.001046	0.460293	0.108380	1.000000				
GY	0.621935	0.570449	0.523994	-0.136719	0.399672	-0.318016	0.729272	0.577138	0.460582	1.000000			
TGW	0.334518	0.272354	0.348565	0.625436	0.418787	-0.554342	-0.140162	-0.074459	-0.027816	-0.089645	1.000000		
BIO	0.214279	0.444870	0.442084	-0.239446	0.343340	0.169899	0.227045	0.191890	-0.065456	0.256170	-0.171886	1.000000	
STV	0.728143	0.799270	0.796556	0.498381	0.775915	-0.493464	0.117132	0.562416	0.129541	0.369990	0.556533	0.011027	1.000000

HE= Plant height, FM= Days to tasseling, FF= Days to silking, GF= Grain filling period, MD= Days to maturity, SD= Stem diameter, NC= Number of cob/plant NL= Number of leaves, LL= Leaves length, GY= Grain yield, TGW= Thousand grain weight, BIO= Bio -oil, STV= Stover weight

Red = significant at $P<0.05$; Black = non significant

4.3 OPTIMIZATION OF BIO-OIL

Temperature, particle size, reaction time and nitrogen flow rate effect on productivity of bio oil were recorded. For each parameter, the experiment was conducted for three replicates. The optimization details were compute into Figure 10, Figure 11, Figure 12 and Figure 13.

4.3.1 Effect of temperature

Pyrolisis were conducted on 80g grounded stover using constant parameter which is particle size ranging from 0.5mm<dp<4.0mm, nitrogen flow rate of 2 L/min and reaction time of 60 min. The highest production of oil was at temperature of 550°C with 45.1 wt%. The lowest oil production was at 300°C with 33.3 wt %. Figure 10 shown the detail of the temperature effect.

4.3.2 Effect of particle size

Pyrolisis were conducted on 80g grounded stover using constant parameter which is temperature of 550°C, nitrogen flow rate of 2 L/min and reaction time of 60 min. The highest production of oil using 1.4mm<dp<2.0mm with oil weight of 41.3 wt%. The lowest oil production was at 1.0mm<dp<1.4mm with oil weight of 28.1wt %. Figure 11 shown the detail of the particle size effect.

4.3.3 Effect of reaction time

Pyrolisis were conducted on 80g grounded stover using constant parameter such as temperature of 550°C, particle size ranging from 0.5mm<dp<4.0mm and nitrogen flow rate of 2 L/min. The highest production of oil taken using 45 min reaction time with oil weight of 43.6 wt%. The lowest oil production was at 15 min reaction time with oil weight of 22.1 wt %. Figure 12 shown the detail of the reaction time effect.

4.3.4 Effect of Nitrogen flow rate

Pyrolisis were conducted on 80g grounded stover using constant parameter which is temperature of 550°C, particle size ranging from 0.5mm<dp<4.0mm, and reaction time of 60 min. The highest production of oil was using 2 L/min nitrogen flow rate with oil weight of 45 wt%. The lowest oil production was using 0.5 L/min nitrogen flow with oil weight of 33.3 wt %. Figure 13 shown the detail of the nitrogen flow rate effect.

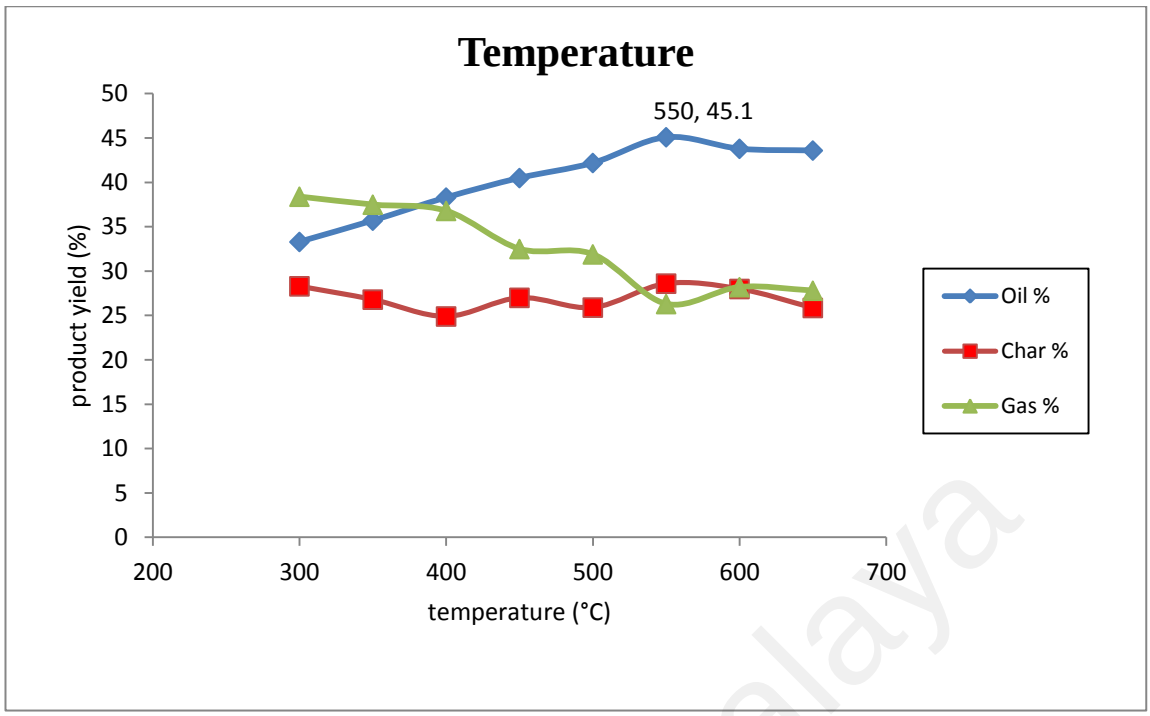


Figure 10. Effect of temperature on yield products. Constant; Particle size: 0.5mm<dp<4.0mm, N_2 flow rate: 2 L/min, Reaction time: 60 min.

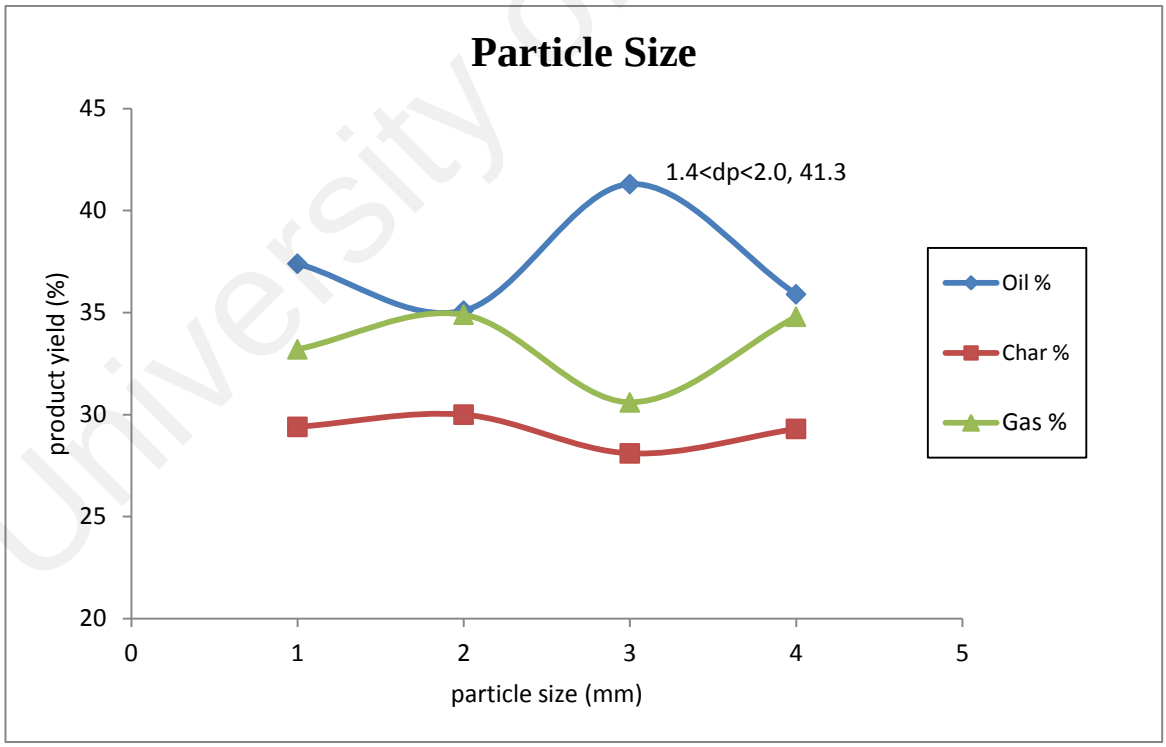


Figure 11. Effect of particle size on yield products. Constant; Temperature: 550°C, N_2 flow rate: 2 L/min, Reaction time: 60 min.

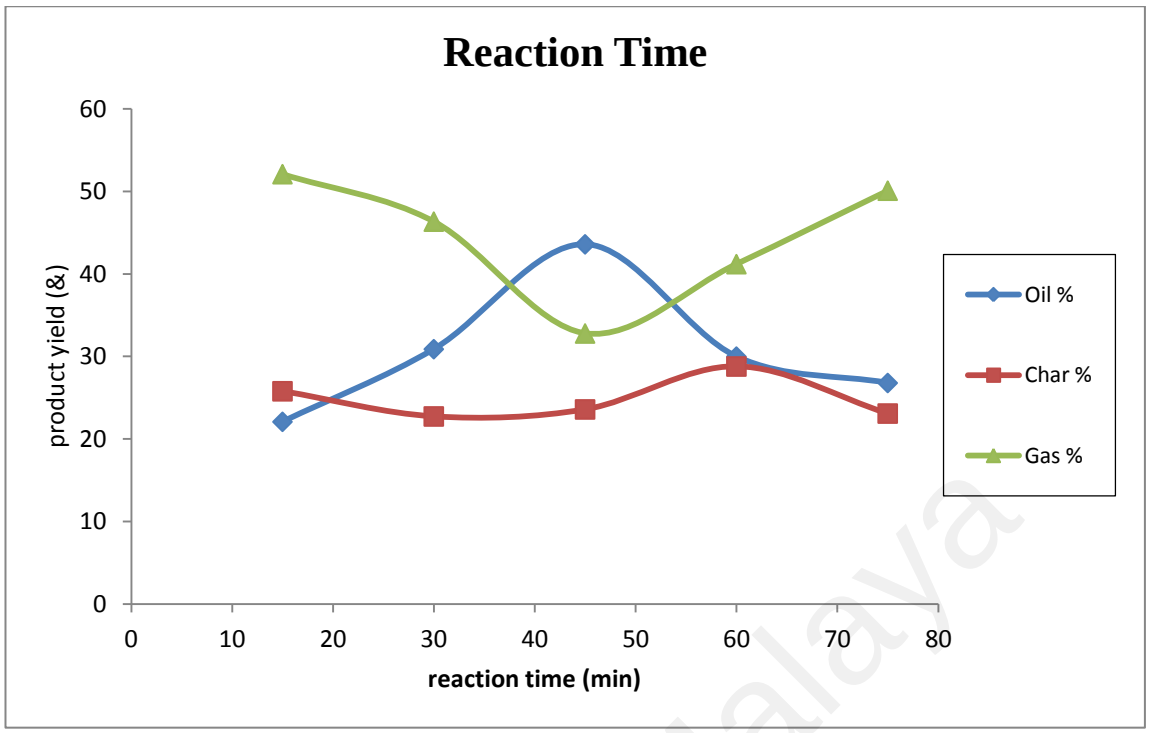


Figure 12. Effect of reaction time on yield products. Constant; Temperature: 550°C, particle size: 0.5mm<dp<4.0mm, N_2 flow rate: 2 L/min.

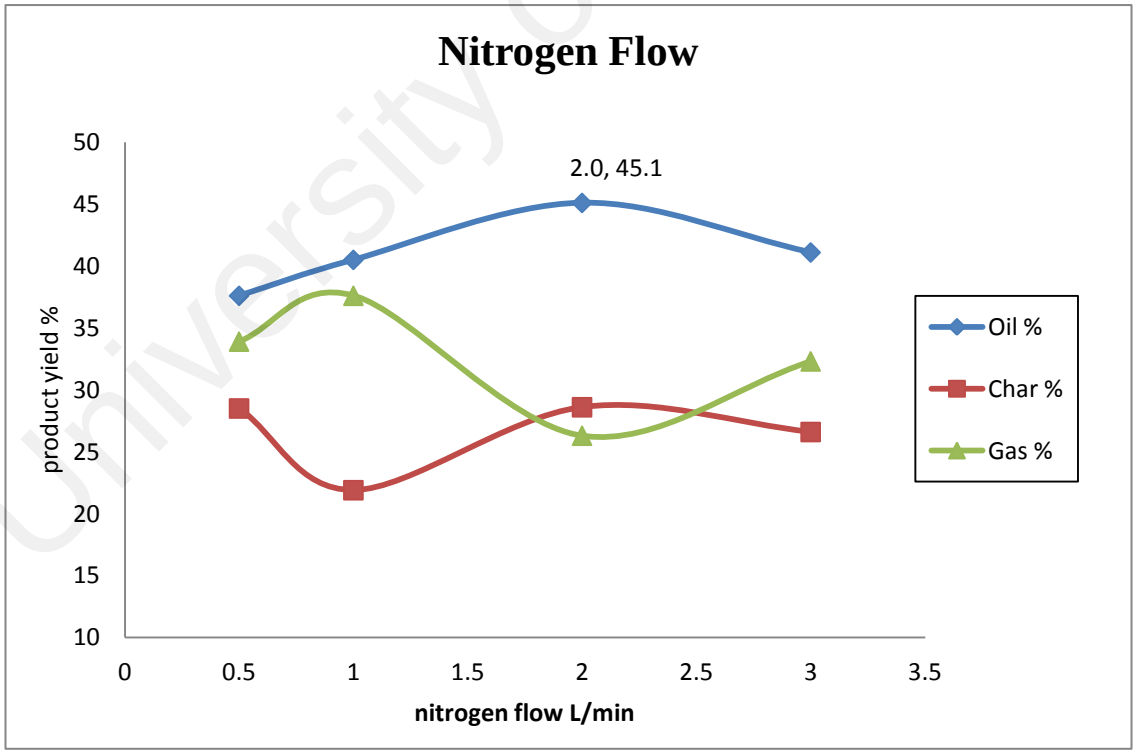


Figure 13. Effect of N2 flow rate on yield products. Constant; Temperature: 550°C, Particle size: 0.5mm<dp<4.0mm, Reaction time: 60min.

4.4 IDENTIFICATION AND QUANTIFICATION OF BIO-OIL

4.4.1 Elemental analysis of bio-oil

Element present in bio-oil were listed in Table 6 for each entry. The highest percentage of carbon was observed in hybrid genotype E12 with sweet corn while the lowest percentage of carbon was observed in genotype E11. The highest percentage of hydrogen was observed in hybrid genotype E5 with sweet corn while the lowest percentage of hydrogen was observed in genotype E17. The highest percentage of nitrogen was observed in genotype E11 while the lowest percentage of nitrogen was observed in genotype E9.

Table 6. Identification of Carbon, Hydrogen and Nitrogen component in bio-oil using CHN analysis

No	Genotype	Carbon, C (%)	Hydrogen, H (%)	Nitrogen, N (%)
1	E1 (F_1)	76.65	9.40	13.94
2	E5 (F_1)	69.68	14.0	16.31
3	E9 (F_1)	75.00	11.01	13.99
4	E11 (F_1)	71.76	11.70	16.50
5	E12 (F_1)	85.54	8.16	6.29
6	E17 (F_1)	82.27	8.40	6.34
7	E1	81.13	8.52	10.35
8	E5	73.00	8.11	18.86
9	E9	82.10	13.14	4.76
10	E11	67.30	12.13	20.57
11	E12	72.99	8.56	18.46
12	E17	83.89	2.26	13.99

4.4.2 Physical properties of bio-oil

Viscosity, Karl Fisher titration, total base number (TBN) and total acid number (TAN) for bio-oil were concluded in Table 7.

Table 7. Physical properties of bio-oil.

Properties	Value	Unit
Viscosity at 40°C	1.192	cP
Density at 24°C	1032.90	kg/m^3
Water content	46.00	wt%
TBN	18.55	mg/KOH
TAN	-0.38	mg/KOH

4.4.3 Functional group composition of bio-oil

Absorbance peak for each genotypes from FT-IR analysis were compute in Figure 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25. The peak for functional group was summarized in Table 8.

Table 8. Absorbance peak of functional group for bio-oil obtain from parent material

Genotype	Groups	Absorbance peak (cm^{-1})	Class of compound
E1	O-H	3337.02 cm^{-1} 1230.66 cm^{-1}	Alcohols and phenol
	C-H	2960.04 cm^{-1} 2854.75 cm^{-1} 1455.03 cm^{-1} 1371.11 cm^{-1}	Alkanes
	C-H	1609.79 cm^{-1} 1595.76 cm^{-1} 1514.00 cm^{-1}	Aromatic group
	C-H (in plane)	1030.50 cm^{-1}	
	C-H (oop)	815.02 cm^{-1} 752.47 cm^{-1}	
	C-O	1702.86 cm^{-1}	Aldehydes and ketones groups
E5	O-H	3302.91cm^{-1} 1231.24cm^{-1}	Alcohols and phenol
	C-H	2962.00 cm^{-1} 2930.33 cm^{-1} 1452.63 cm^{-1} 1372.69 cm^{-1}	Alkanes

	C-H	1606.92 cm^{-1} 1595.11 cm^{-1} 1513.83 cm^{-1}	Aromatic group
	C-H (in plane)	1031.30 cm^{-1}	
	C-H (oop)	831.76 cm^{-1} 814.33 cm^{-1} 735.08 cm^{-1}	
	C-O	1701.36 cm^{-1}	Aldehydes and ketones groups
E9	O-H	3302.91 cm^{-1} 1231.24cm^{-1}	Alcohols and phenol
	C-H	2962.00 cm^{-1} 2930.33 cm^{-1} 1452.63 cm^{-1} 1372.69 cm^{-1}	Alkanes
	C-H	1606.92 cm^{-1} 1595.11 cm^{-}	Aromatic group
	C-H (in plane)	1031.30 cm^{-1}	
	C-H (oop)	831.76 cm^{-1} 814.33 cm^{-1} 735.08 cm^{-1}	
	C-O	1701.36 cm^{-1}	Aldehydes and ketones groups
E11	O-H	3325.13 cm^{-1} 1229.52 cm^{-1}	Alcohols and phenol
	C-H	2960.25 cm^{-}1 2855.33 cm^{-1} 1454.20 cm^{-1} 1370.67 cm^{-1}	Alkanes
	C-H	1608.88 cm^{-1} 1595.74 cm^{-1} 1514.02 cm^{-1}	Aromatic group
	C-H (in plane)	1029.36 cm^{-1}	
	C-H (oop)	832.11 cm^{-1} 814.61 cm^{-1} 752.73 cm^{-1}	
	C-O	1702.06 cm^{-1}	Aldehydes and ketones groups
E12	O-H	3334.16 1231.25 cm^{-1}	Alcohols and phenol
	C-H	2960.00 cm^{-1} 2927.65 cm^{-1} 1452.92 cm^{-1} 1370.17 cm^{-1}	Alkanes
	C-H	1606.99 cm^{-1} 1595.74 cm^{-1} 1514.12 cm^{-1}	Aromatic group
	C-H (in plane)	105.80 cm^{-1}	
	C-H (oop)	831.84 cm^{-1} 814.45 cm^{-1} 752.93 cm^{-1}	
	C-O	1702.41 cm^{-1}	Aldehydes and ketones groups

E17	O-H	3337.02 cm^{-1} 1230.66 cm^{-1}	Alcohols and phenol
	C-H	2960.04 cm^{-1} 2854.75 cm^{-1} 1455.03 cm^{-1} 1371.11 cm^{-1}	Alkanes
	C-H	1609.79 cm^{-1} 1595.76 cm^{-1} 1514.00 cm^{-1}	Aromatic group
	C-H (in plane)	1030.50 cm^{-1}	
	C-H (oop)	815.02 cm^{-1} 752.47 cm^{-1}	
	C-O	1702.86 cm^{-1}	Aldehydes and ketones groups

Table 9. Absorbance peak of functional group for bio-oil obtain from F_1 material

Genotype	Groups	Absorbance peak (cm^{-1})	Class of compound
E1 (F_1)	O-H	3334.93 cm^{-1} 1230.26 cm^{-1}	Alcohols and phenol
	C-H	2960.44 cm^{-1} 2855.27 cm^{-1} 1454.52 cm^{-1} 1370.78 cm^{-1}	Alkanes
	C-H	1608.32 cm^{-1} 1595.74 cm^{-1} 1514.06 cm^{-1}	Aromatic group
	C-H (in plane)	1030.52 cm^{-1}	
	C-H (oop)	832.44 cm^{-1} 814.73 cm^{-1} 752.75 cm^{-1}	
	C-O	1703.14 cm^{-1}	Aldehydes and ketones groups
E5 (F_1)	O-H	3314.68cm^{-1} 1228.75cm^{-1}	Alcohols and phenol
	C-H	2960.31 cm^{-1} 2855.44 cm^{-1} 1453.32 cm^{-1} 1372.19 cm^{-1}	Alkanes
	C-H	1606.16 cm^{-1} 1595.21 cm^{-1} 1514.07 cm^{-1}	Aromatic group
	C-H (in plane)	1030.70 cm^{-1}	
	C-H (oop)	831.93 cm^{-1} 814.39 cm^{-1} 752.30 cm^{-1}	
	C-O	1700.37 cm^{-1}	Aldehydes and ketones groups
E9 (F_1)	O-H	3334.93 cm^{-1} 1230.26cm^{-1} 1514.06 cm^{-1}	Alcohols and phenol
	C-H	2960.44 cm^{-1}	

		2855.27 cm^{-1} 1454.52 cm^{-1} 1370.78 cm^{-1}	Alkanes
	C-H	1608.32 cm^{-1} 1595.74 cm^{-1} 1514.06 cm^{-1}	Aromatic group
	C-H (in plane)	1030.52 cm^{-1}	
	C-H (oop)	832.44 cm^{-1} 814.73 cm^{-1} 752.75 cm^{-1}	
	C-O	1703.14 cm^{-1}	Aldehydes and ketones groups
E11 (F_1)	O-H	3303.45 cm^{-1} 1228.41 cm^{-1}	Alcohols and phenol
	C-H	2961.27 cm-1 2928.00 cm^{-1} 1453.30 cm^{-1} 1373.82 cm^{-1}	Alkanes
	C-H	1608.61 cm^{-1} 1595.16 cm^{-1} 1514.03 cm^{-1}	Aromatic group
	C-H (in plane)	1026.07 cm^{-1}	
	C-H (oop)	831.59 cm^{-1} 814.12 cm^{-1} 751.75 cm^{-1}	
	C-O	1699.10 cm^{-1}	Aldehydes and ketones groups
E12 (F_1)	O-H	3313.95 cm^{-1} 1229.93 cm^{-1}	Alcohols and phenol
	C-H	2960.75 cm^{-1} 2927.58 cm^{-1} 1453.78 cm^{-1} 1373.04 cm^{-1}	Alkanes
	C-H	1608.48 cm^{-1} 1595.21 cm^{-1} 1514.09 cm^{-1}	Aromatic group
	C-H (in plane)	1030.13 cm^{-1}	
	C-H (oop)	831.80 cm^{-1} 814.73 cm^{-1} 752.44 cm^{-1}	
	C-O	1700.52 cm^{-1}	Aldehydes and ketones groups
E17 (F_1)	O-H	3298.55 cm^{-1} 1229.49 cm^{-1}	Alcohols and phenol
	C-H	2961.76 cm^{-1} 2928.40 cm^{-1} 1452.39 cm^{-1} 1374.26 cm^{-1}	Alkanes

	C-H	1606.47 cm^{-1} 1595.03 cm^{-1} 1514.05 cm^{-1}	Aromatic group
	C-H (in plane)	1028.43 cm^{-1}	
	C-H (oop)	814.09 cm^{-1} 752.67 cm^{-1}	
	C-O	1699.34 cm^{-1}	Aldehydes and ketones groups

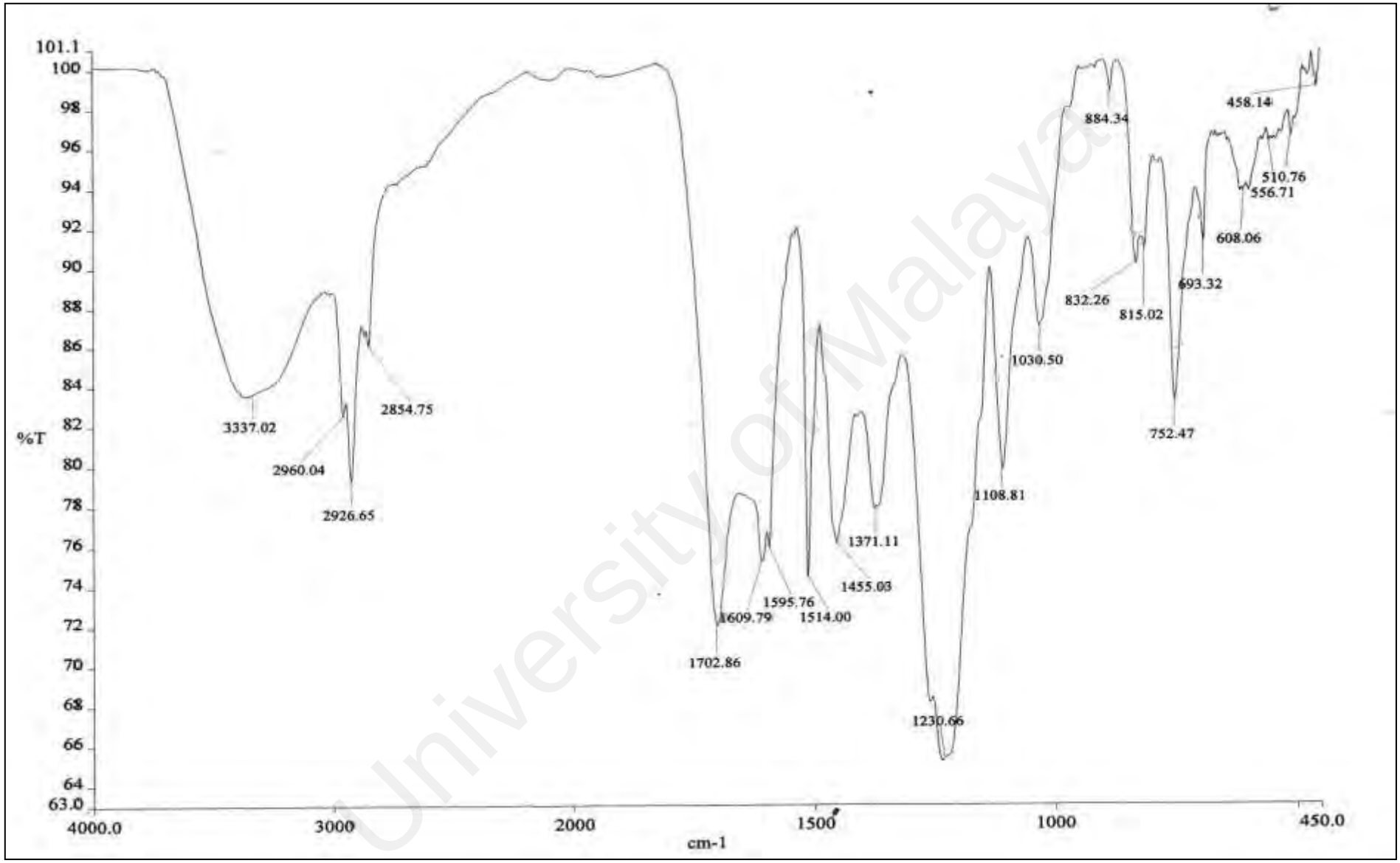


Figure 14. FT-IR graph for genotype E1

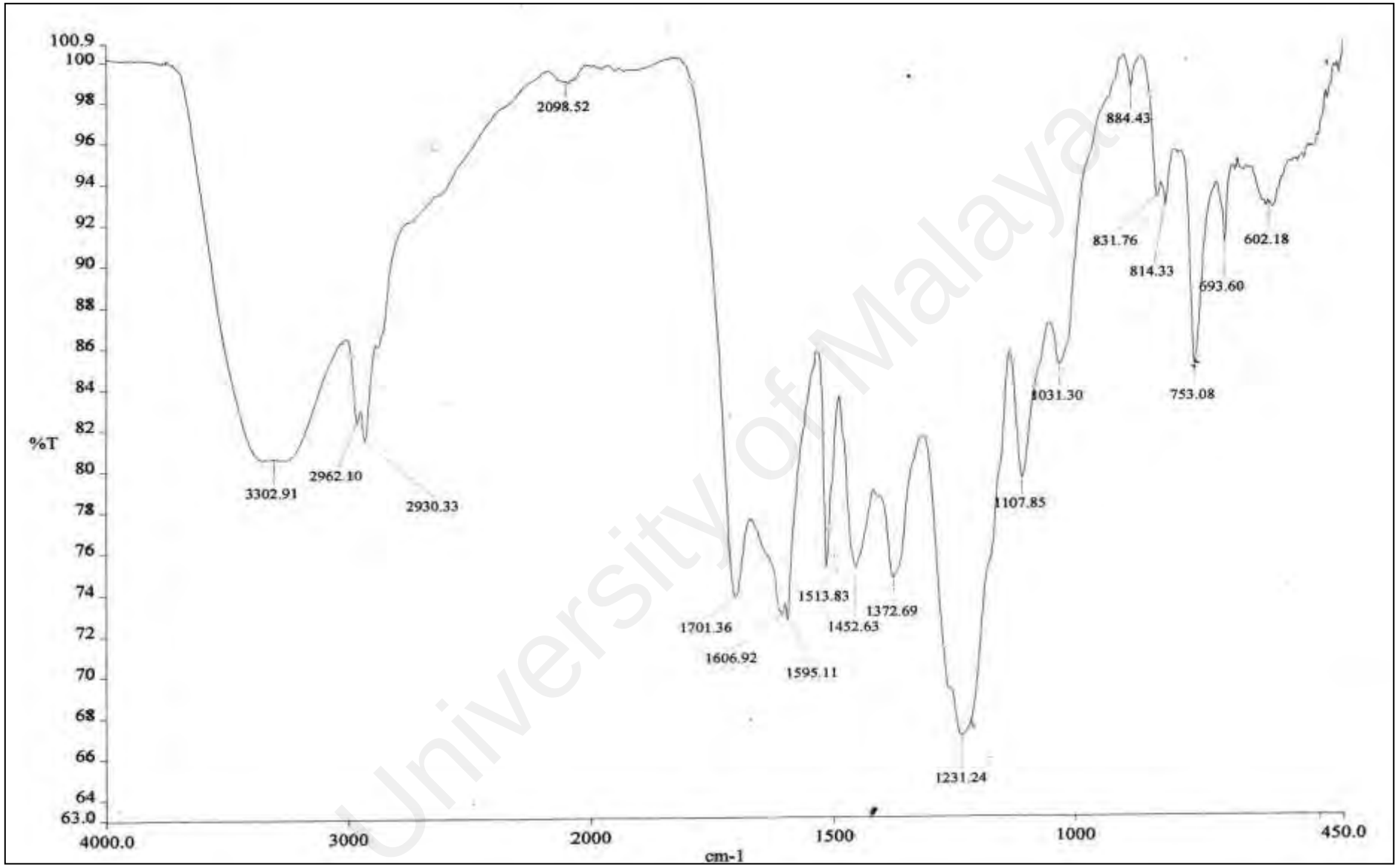


Figure 15. FT-IR graph for genotype E5

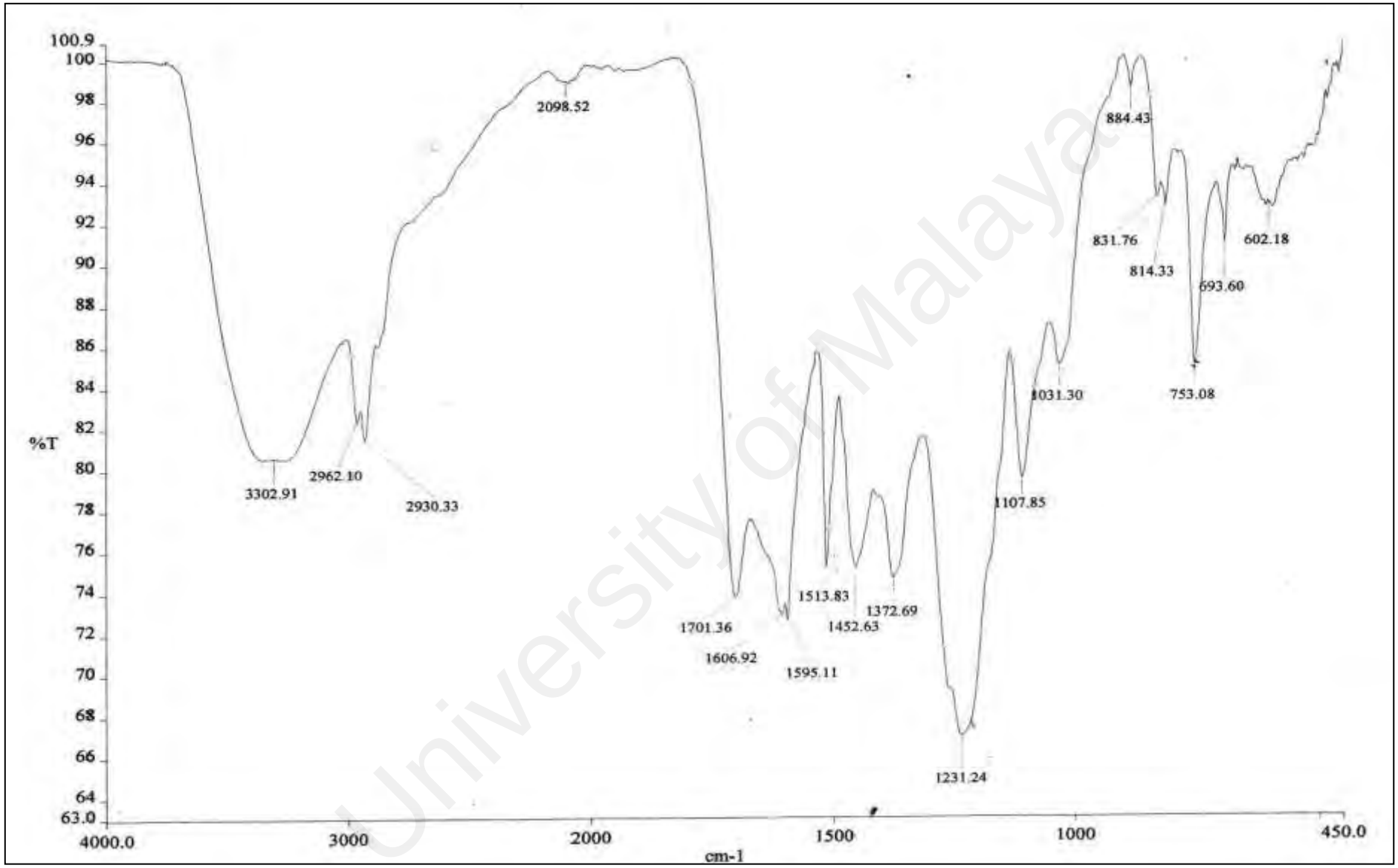


Figure 16. FT-IR graph for genotype E9

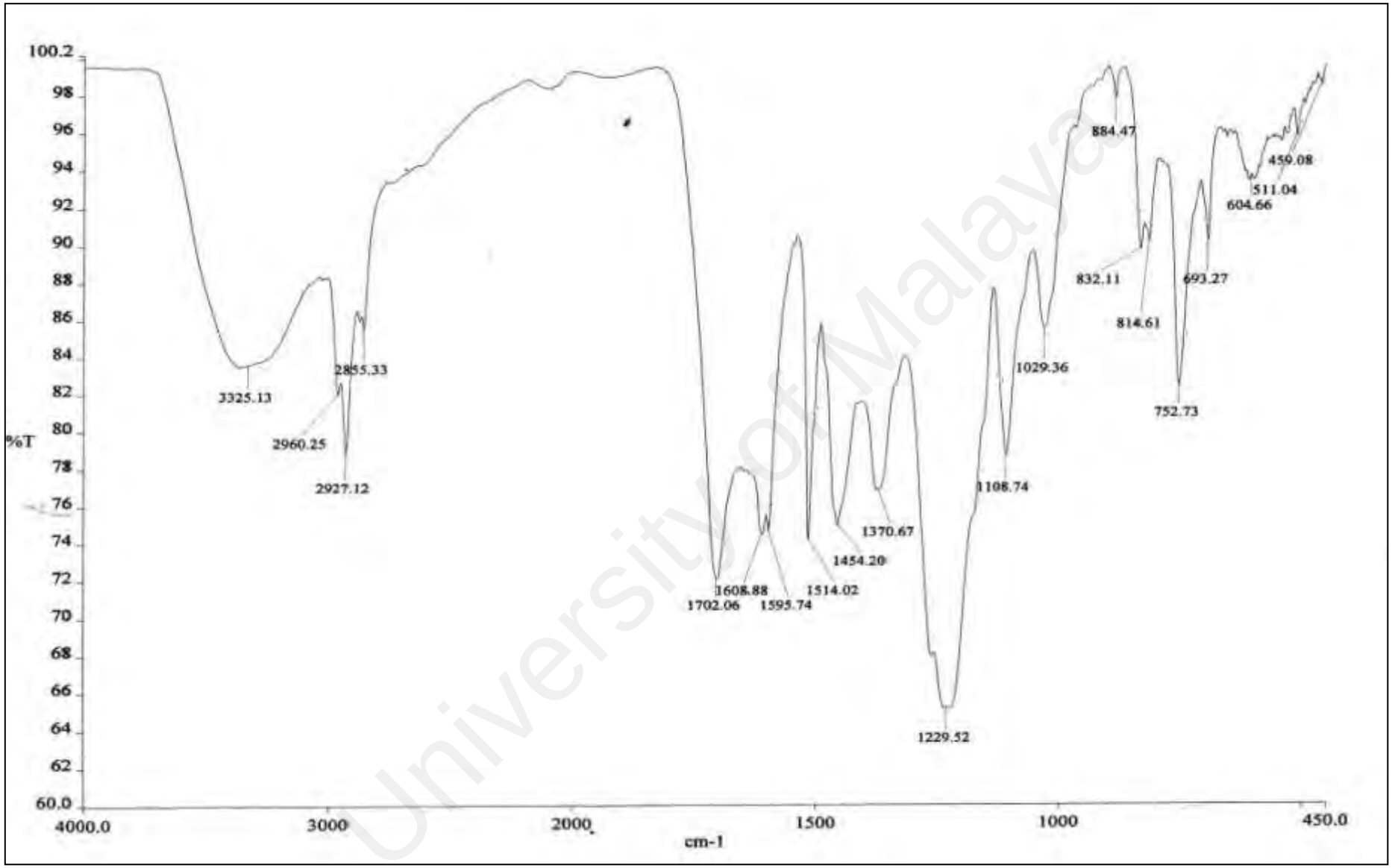


Figure 17. FT-IR graph for genotype E11

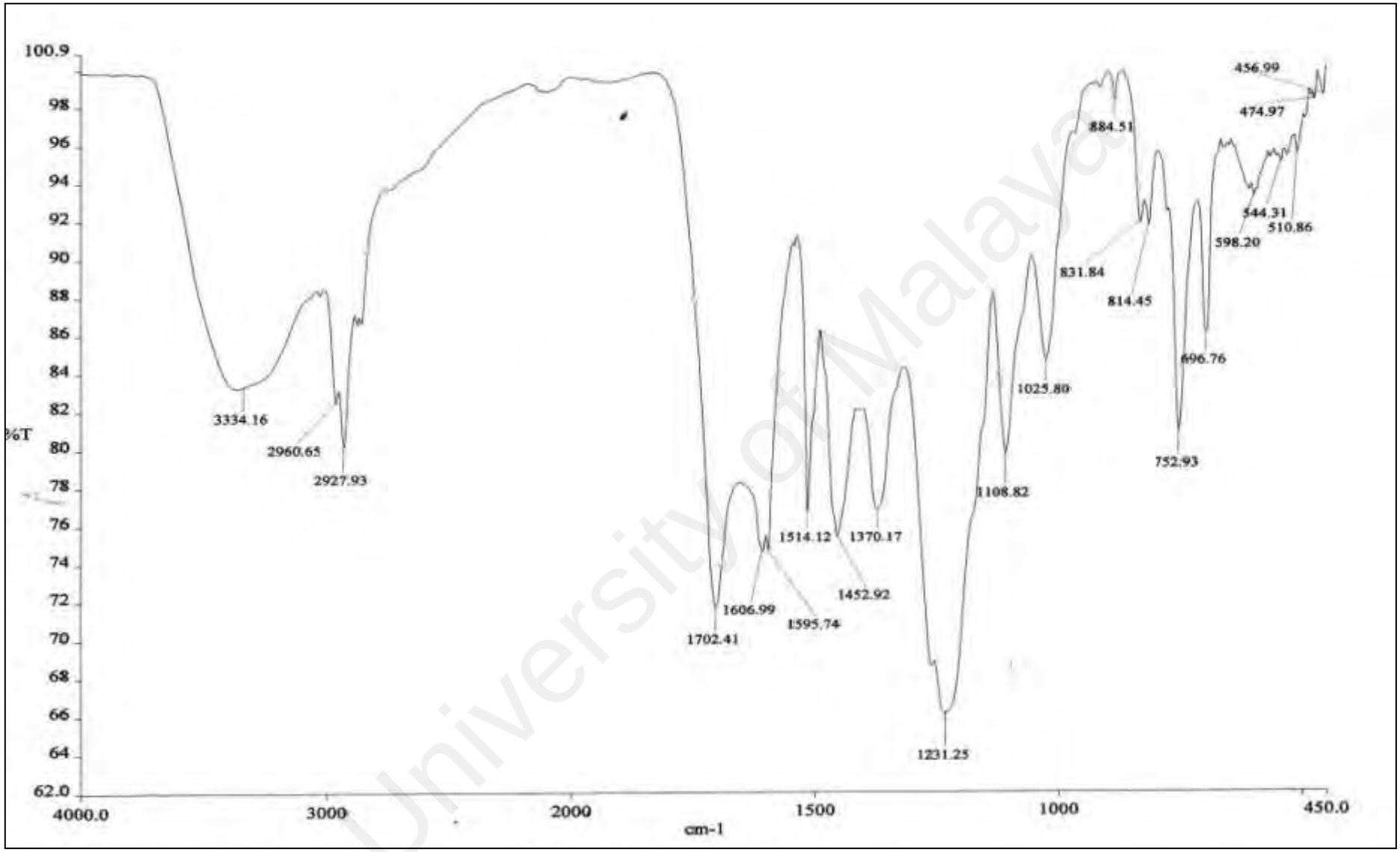


Figure 18. FT-IR graph for genotype E12

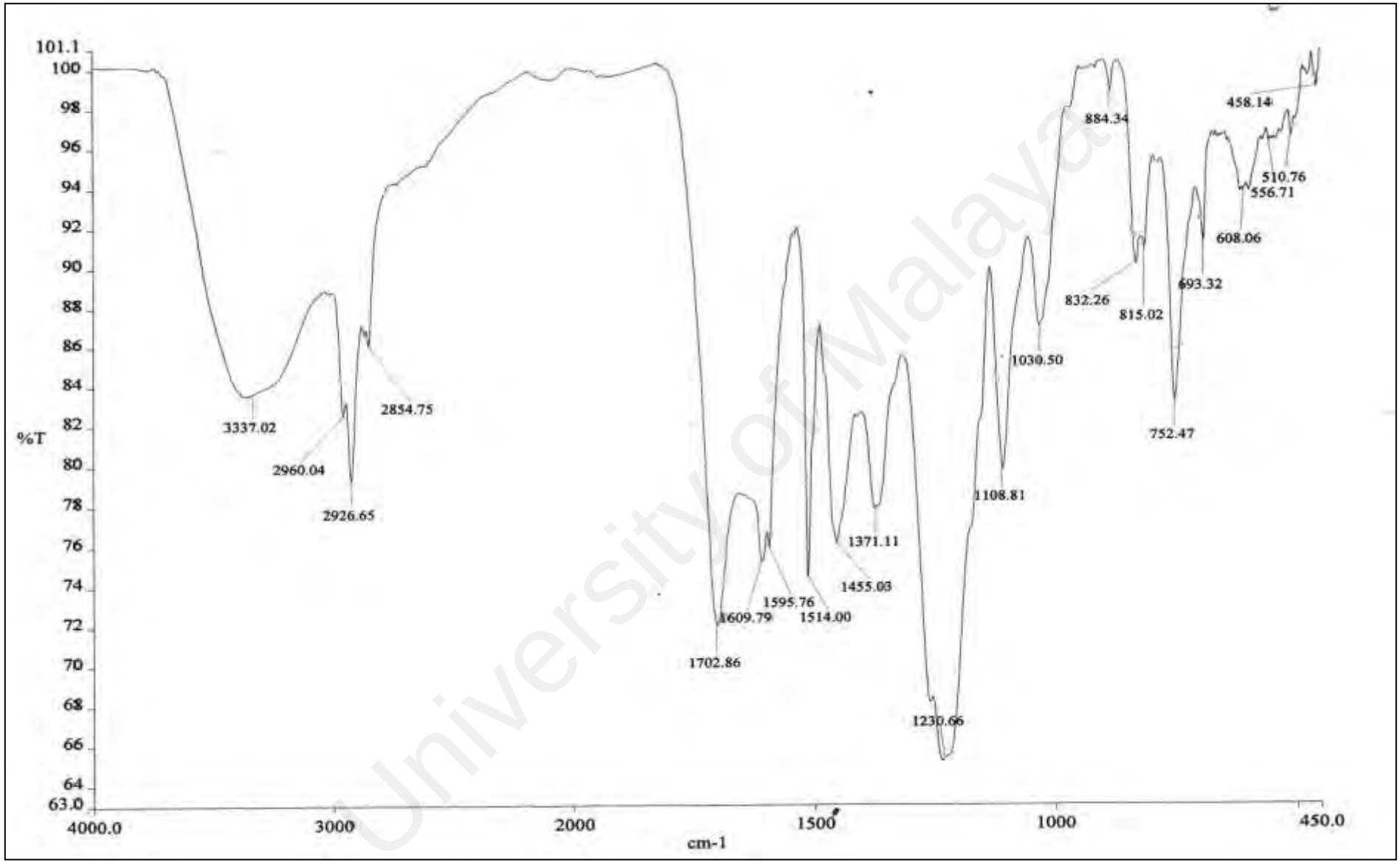


Figure 19. FT-IR graph for genotype E17

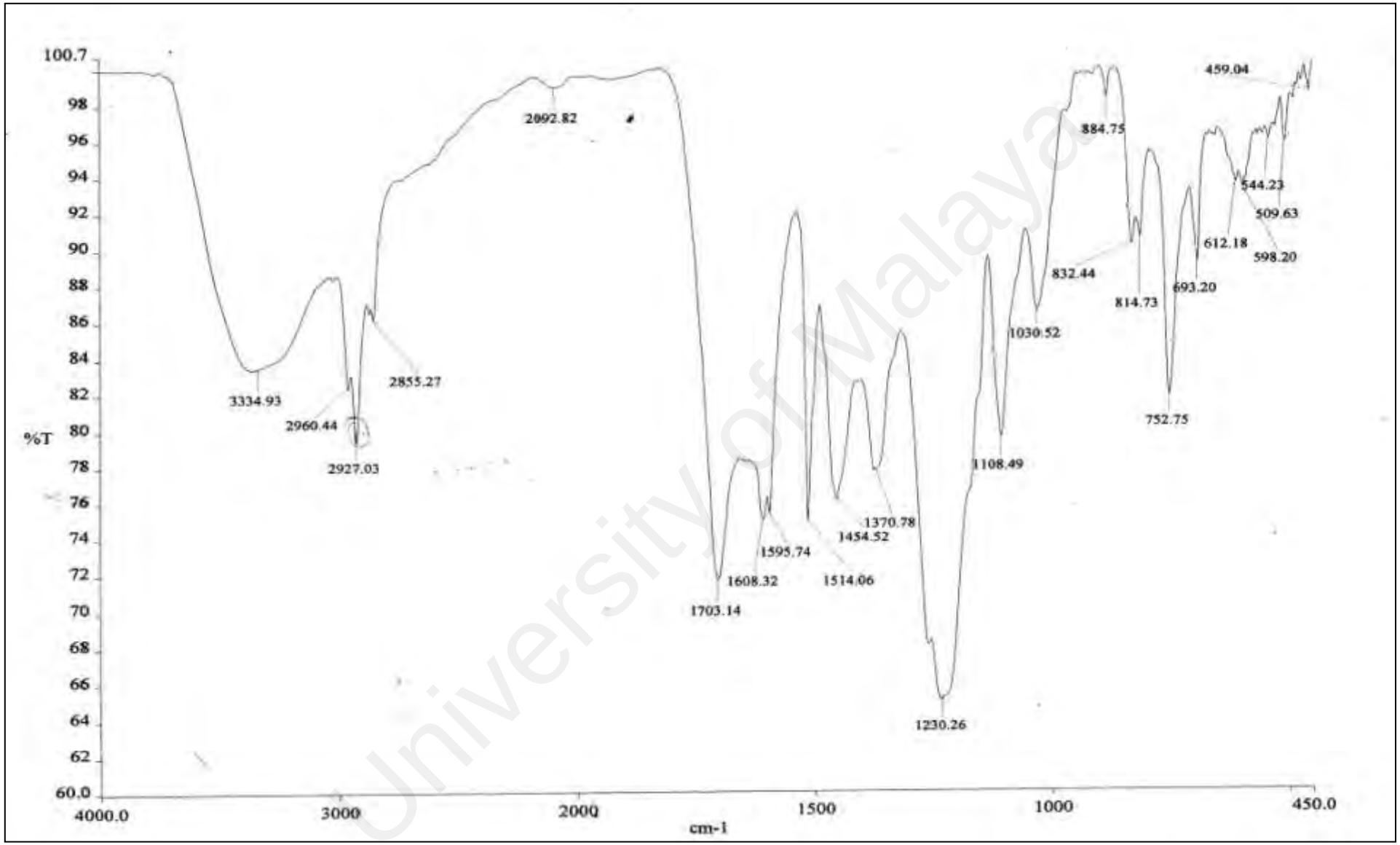


Figure 20. FT-IR graph for genotype E1 (F1)

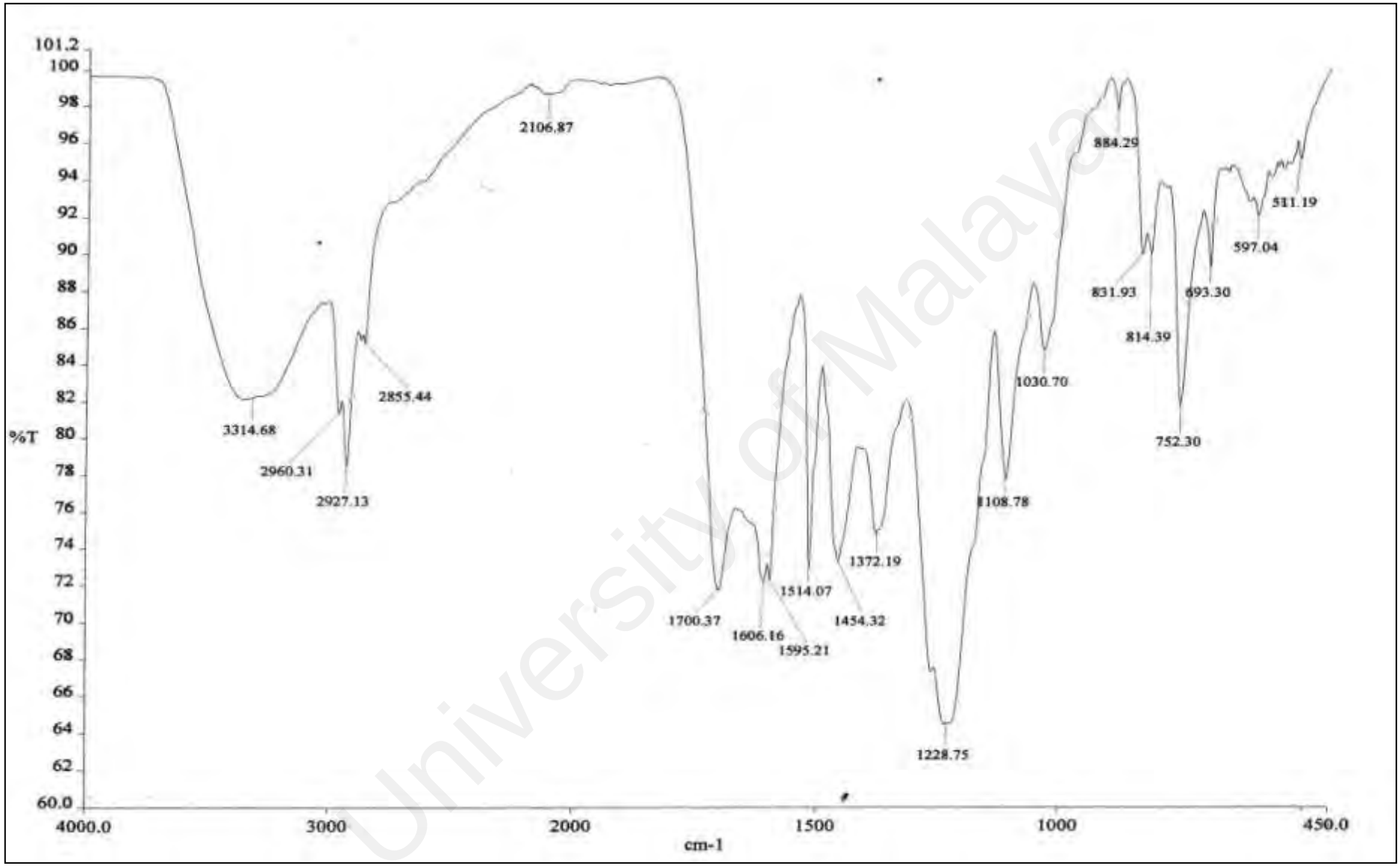


Figure 21. FT-IR graph for genotype E5 (F1)

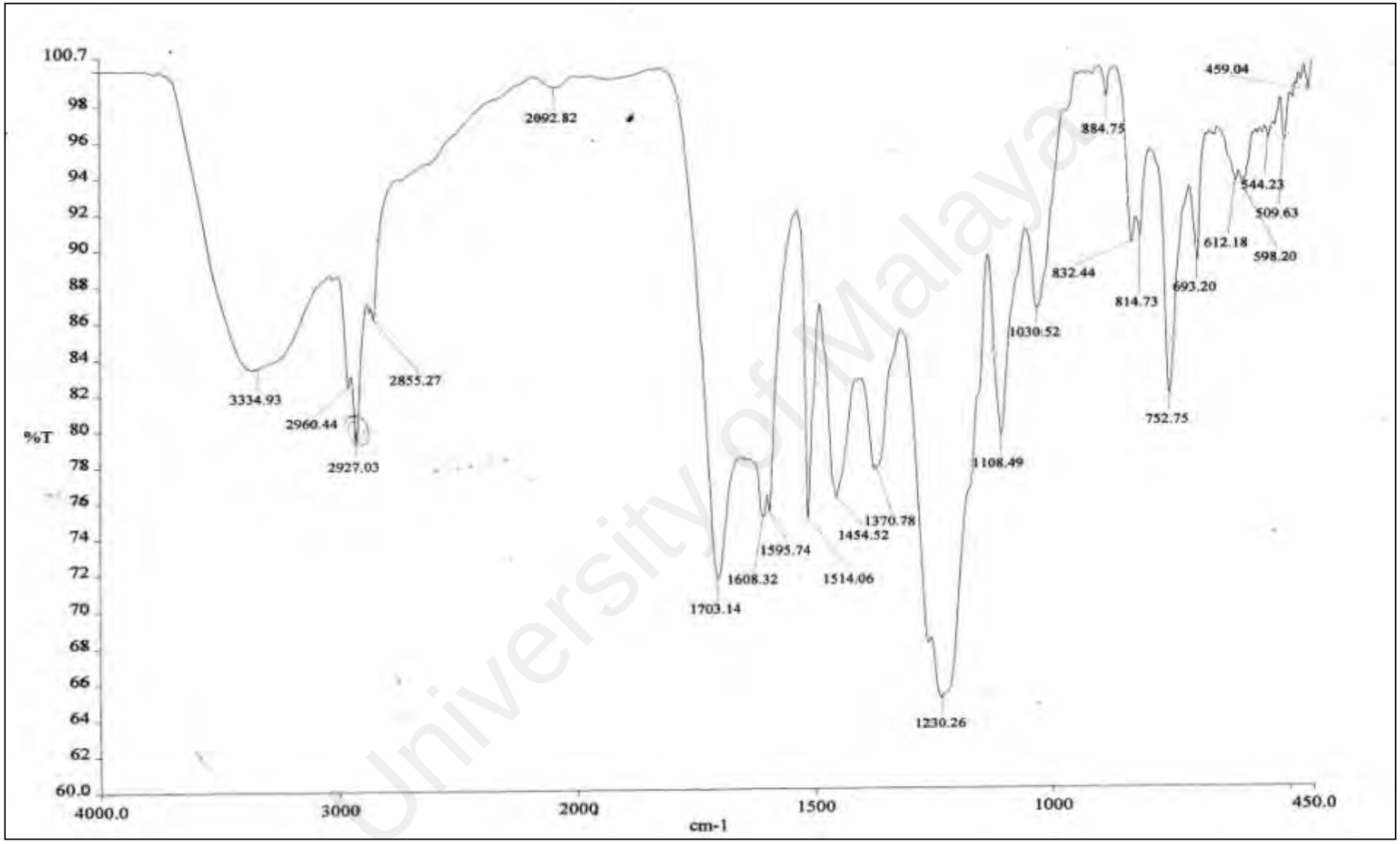


Figure 22. FT-IR graph for genotype E9 (F1)

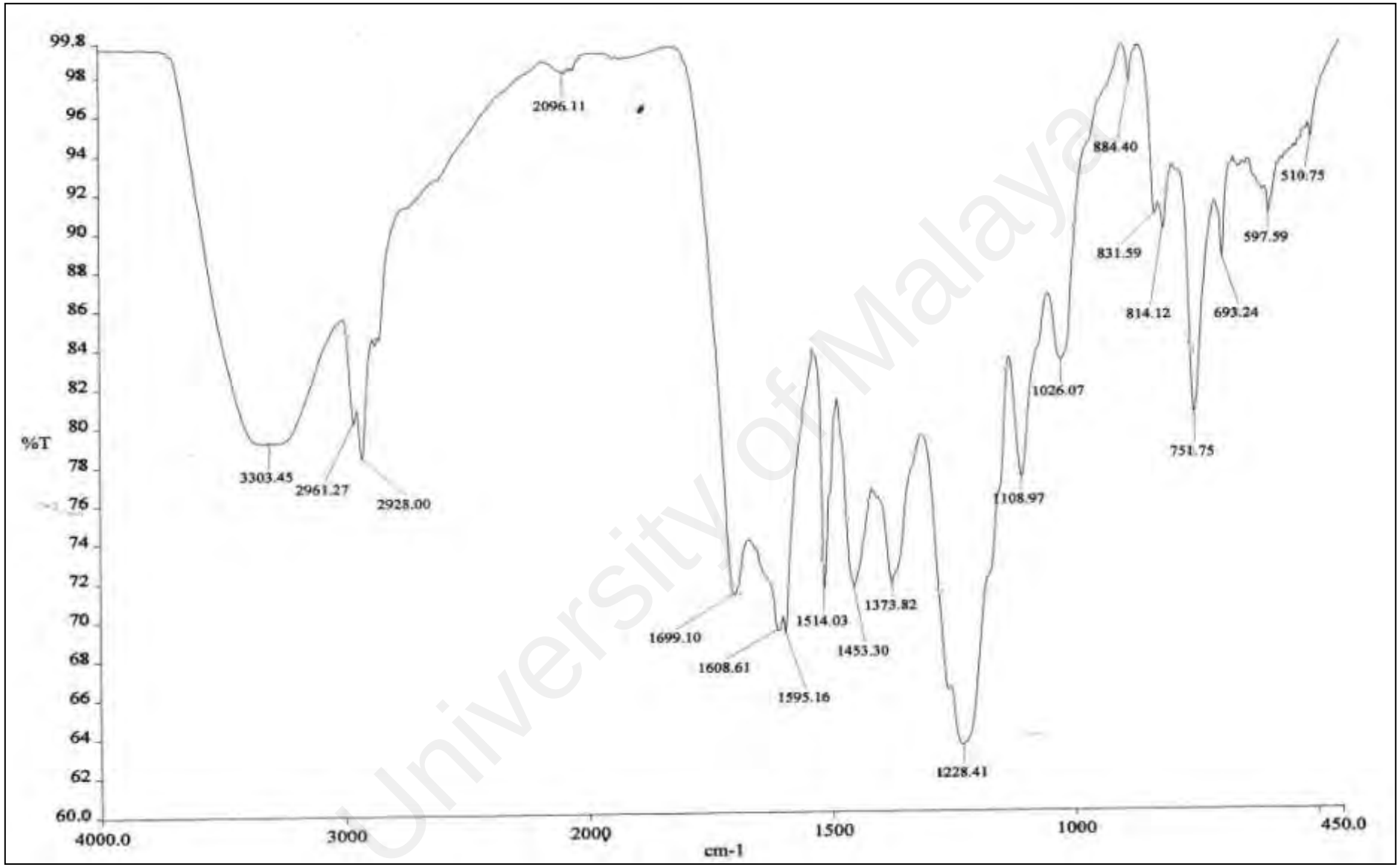


Figure 23. FT-IR graph for genotype E11 (F1)

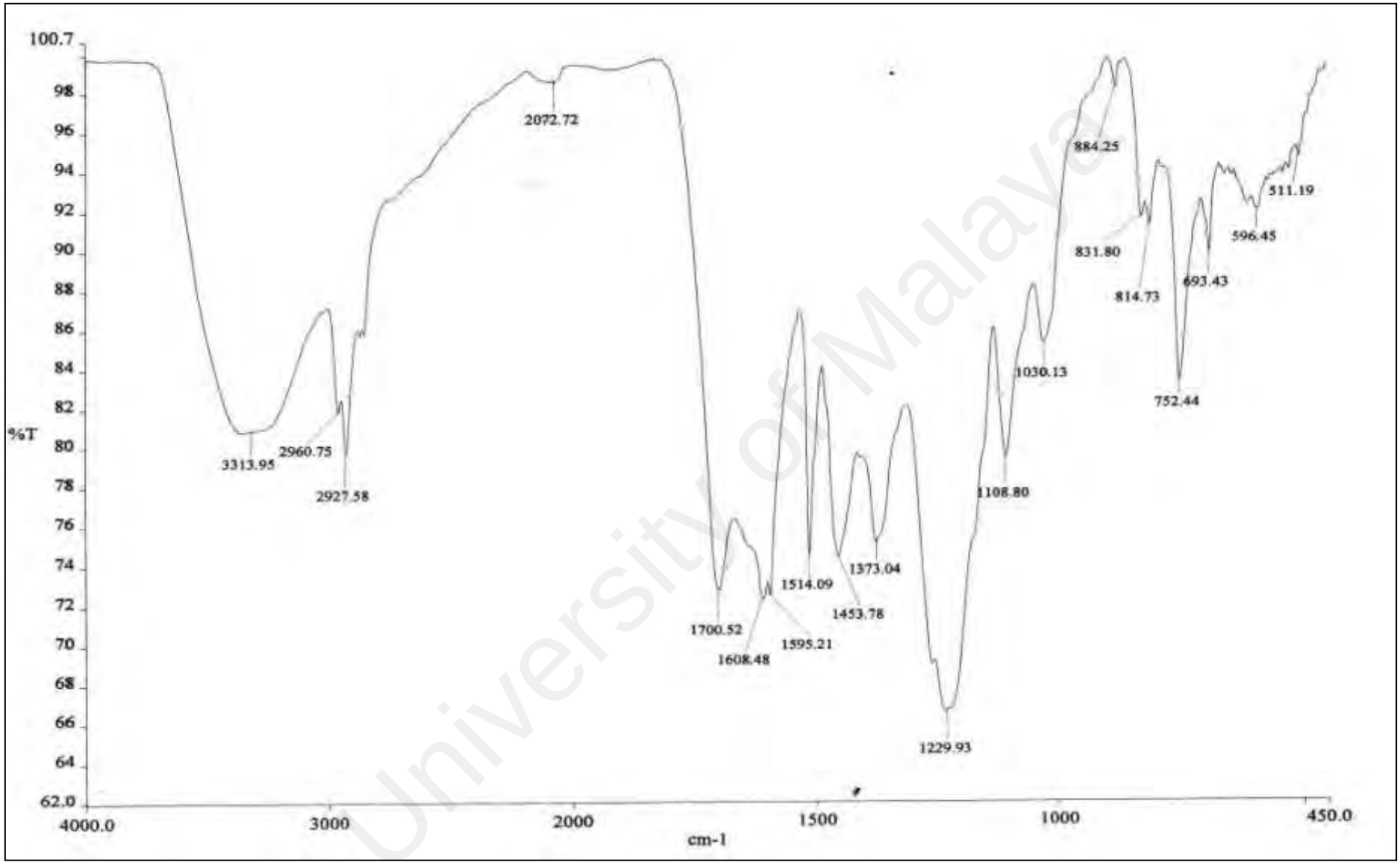


Figure 24. FT-IR graph for genotype E12 (F1)

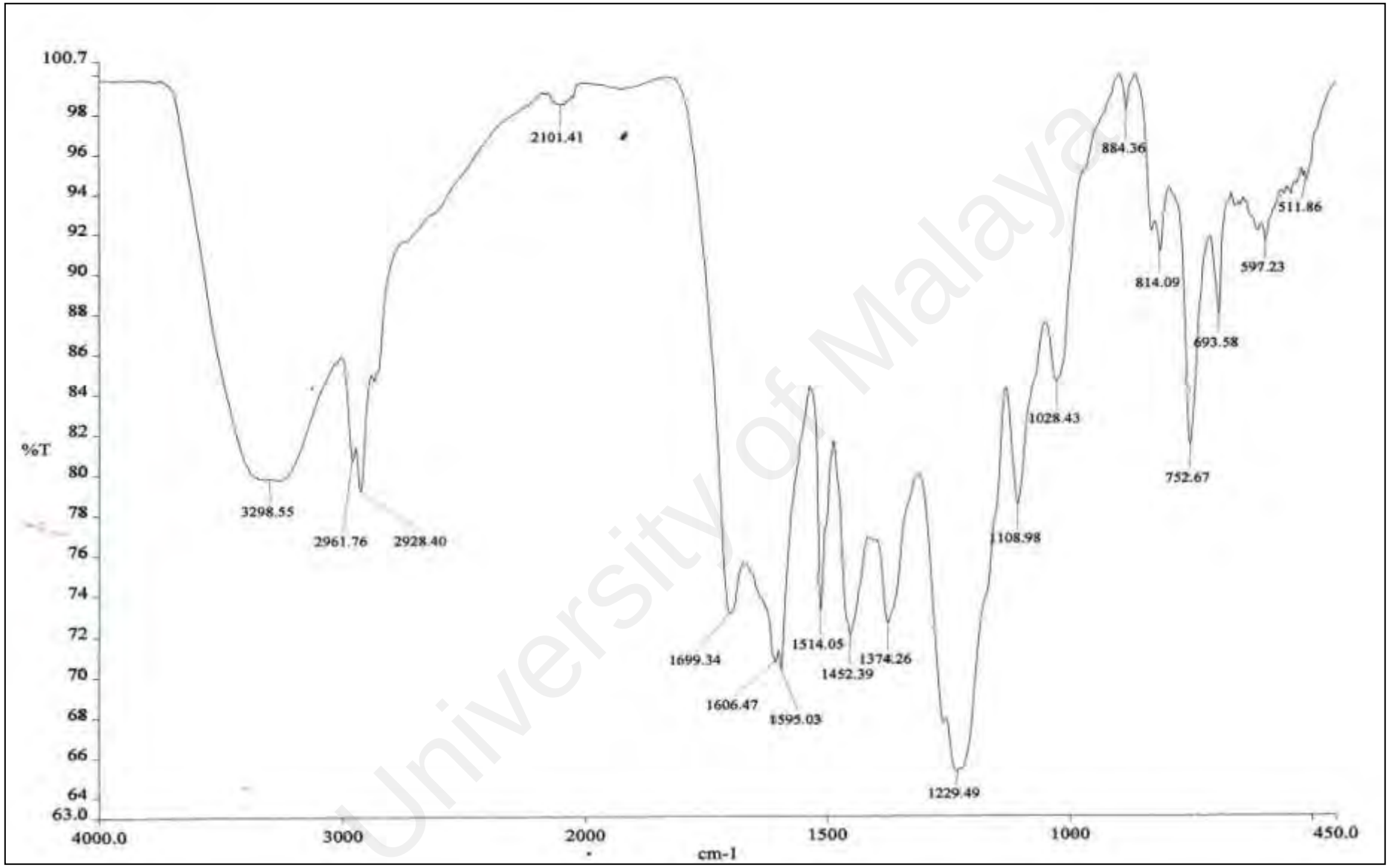
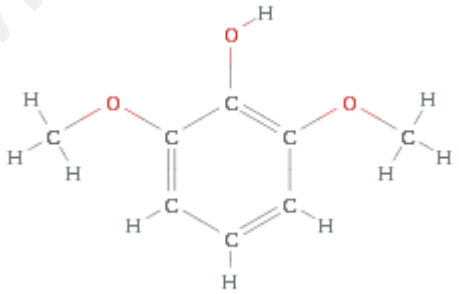
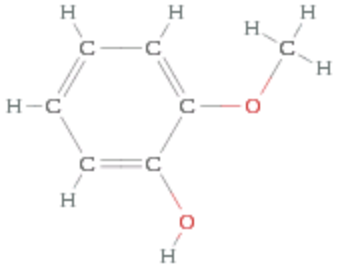


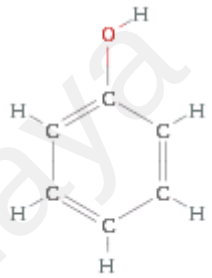
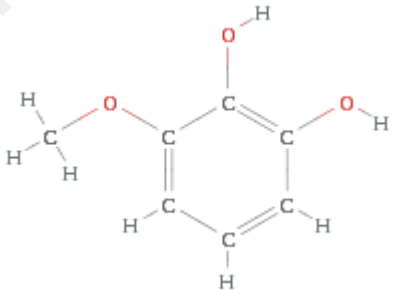
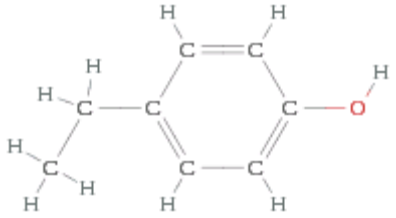
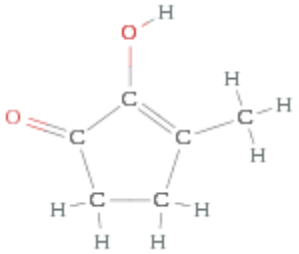
Figure 25. FT-IR graph for genotype E17 (F1)

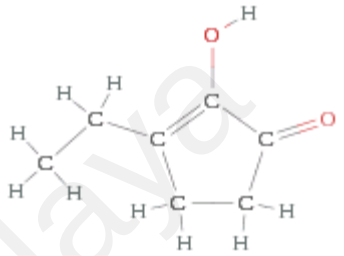
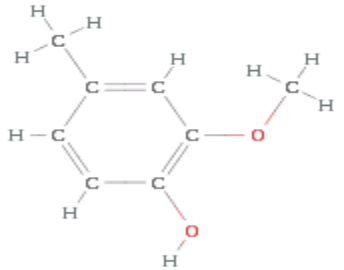
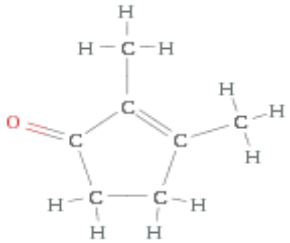
4.4.4 Chemical composition of bio-oil

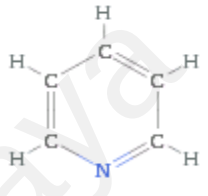
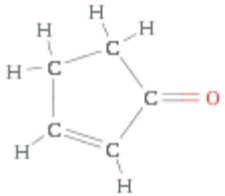
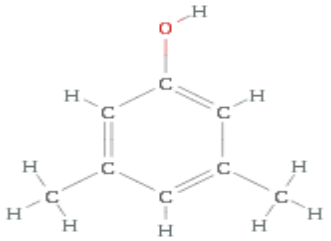
The chemical composition for each genotypes from GC-MS analysis were compute in Figure 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37. The peak for chemical compound was summarized in Table 9.

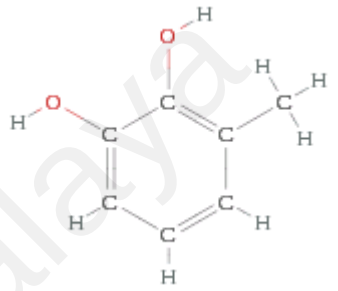
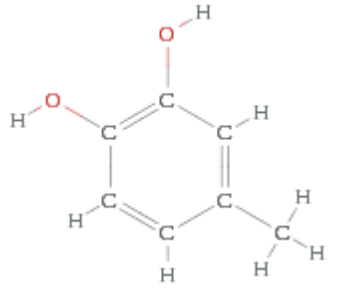
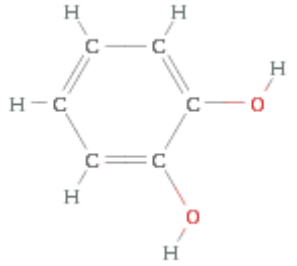
Table 10. Identification and quantification of chemical compound in bio-oil by GC-MS analysis.

No.	Chemical compound	Molecular formula	Molecular weight(g/mol)	Type of compound	Structure	Peak area (%)
1	Phenol,2,6-dimethoxy	$C_8H_{10}O_3$	154	Aro		10.41
2	Phenol,2-methoxy	$C_7H_80_2$	124	Aro		11.40

No.	Chemical compound	Molecular formula	Molecular weight(g/mol)	Type of compound	Structure	Peak area (%)
3	Phenol	C_6H_5OH	94	Aro		5.47
4	1,2-benzenediol,1,3-methoxy	$C_7H_8O_2$	140	Aro		3.55
5	Phenol,4-ethyl	$C_8H_{10}O$	122	Aro		4.19
6	1,2-cyclopentanedione, 3-methyl	$C_6H_8O_2$	112	Aro		7.94

No.	Chemical compound	Molecular formula	Molecular weight(g/mol)	Type of compound	Structure	Peak area (%)
7	2-cyclopenten-1-one, 3-ethyl-2-hydroxy	$C_7H_{10}O_2$	126	Aro		2.31
8	Phenol,2-methoxy-4-methyl	$C_8H_{10}O_2$	138	Aro		1.03
9	2-cyclopenten-1-one,2,3-dimethyl	$C_7H_{10}O$	110	Aro		0.78

No.	Chemical compound	Molecular formula	Molecular weight(g/mol)	Type of compound	Structure	Peak area (%)
10	Pyridine	C_5H_5N	79	Aro		1.18
11	2-cyclopenten-1-one	$C_6H_8O_2$	112	Aro		1.18
12	Phenol,3,5-dimethyl	$C_8H_{10}O$	122	Aro		0.43

No.	Chemical compound	Molecular formula	Molecular weight(g/mol)	Type of compound	Structure	Peak area (%)
13	1,2-benzeneiol,3-methyl	$C_7H_8O_2$	124	Aro		0.93
14	1,2-benzenediol,4-methyl	$C_7H_8O_2$	124	Aro		1.58
15	1,2-benzediol	$C_6H_6O_2$	110	Aro		2.81

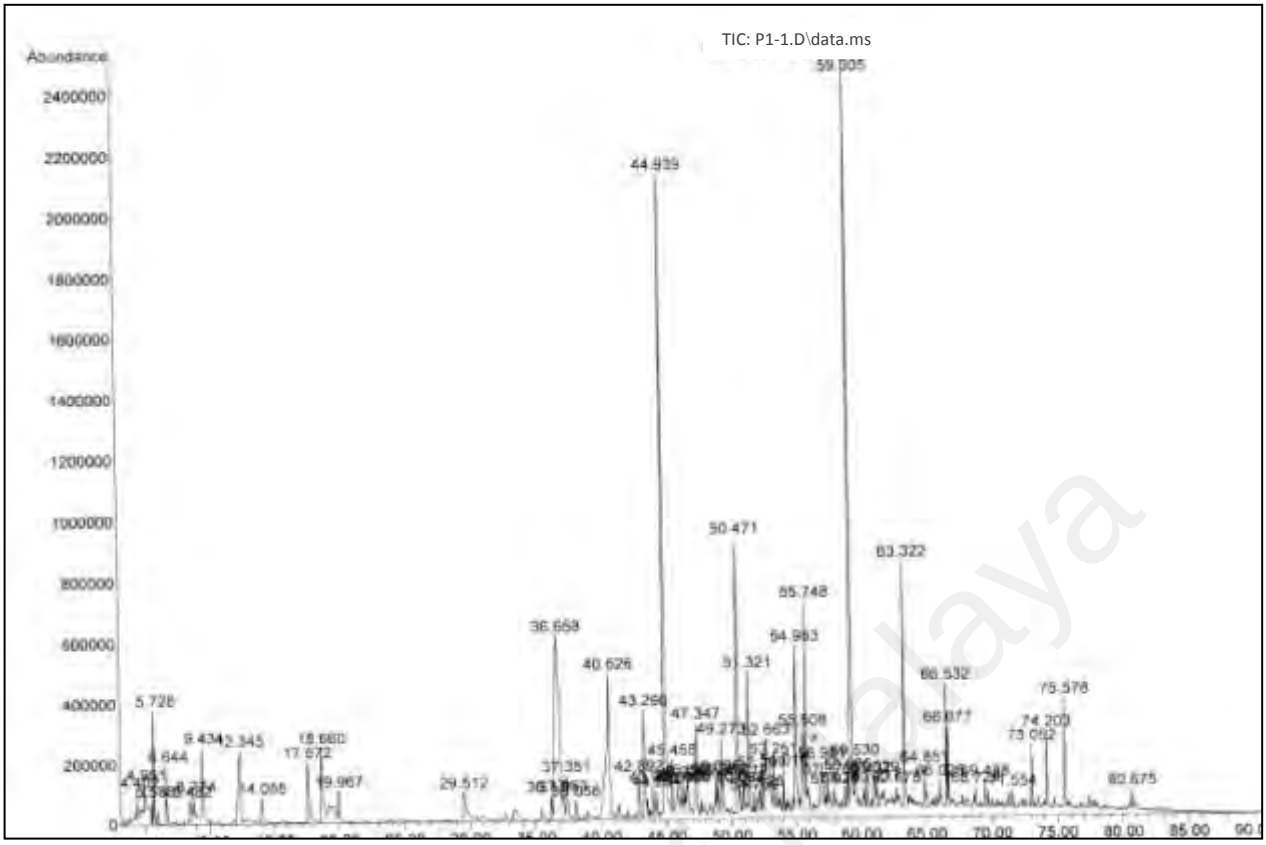


Figure 26. GC-MS ion chromatograph for genotype E1

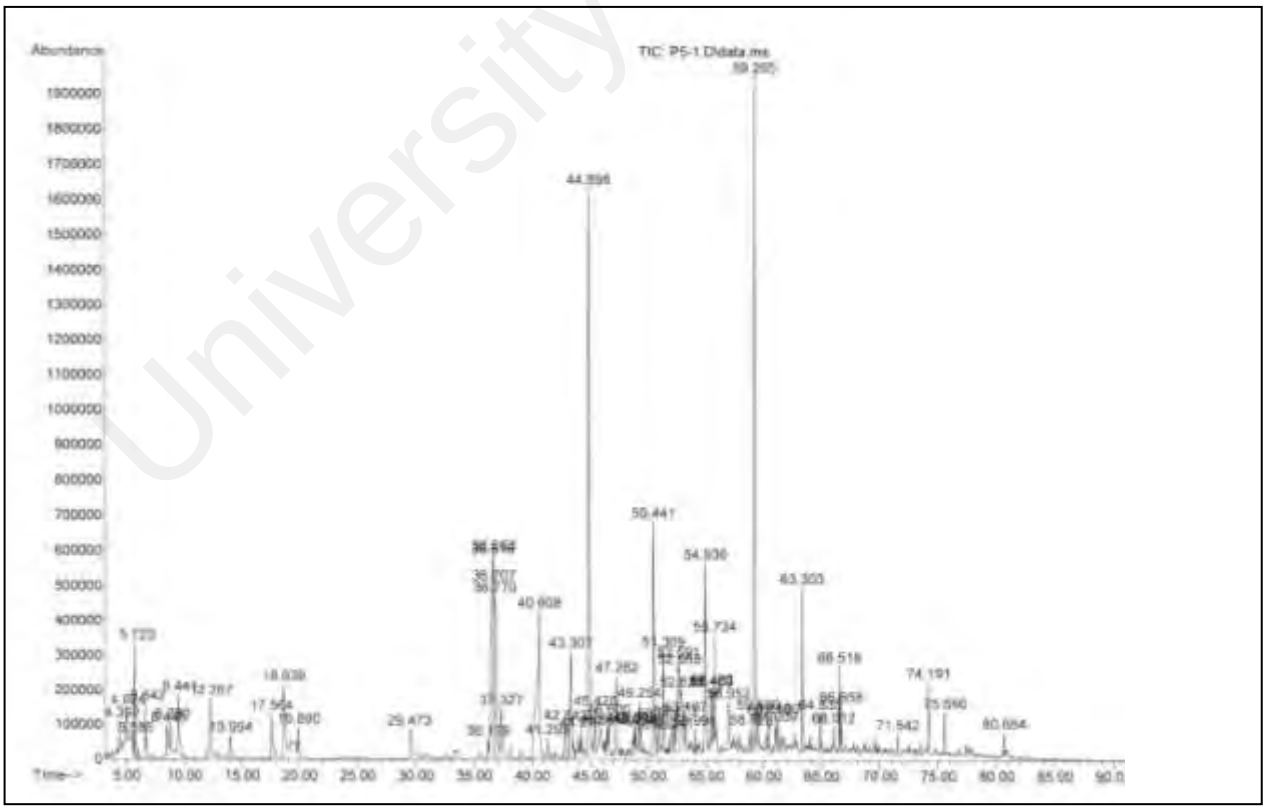


Figure 27. GC-MS ion chromatograph for genotype E5

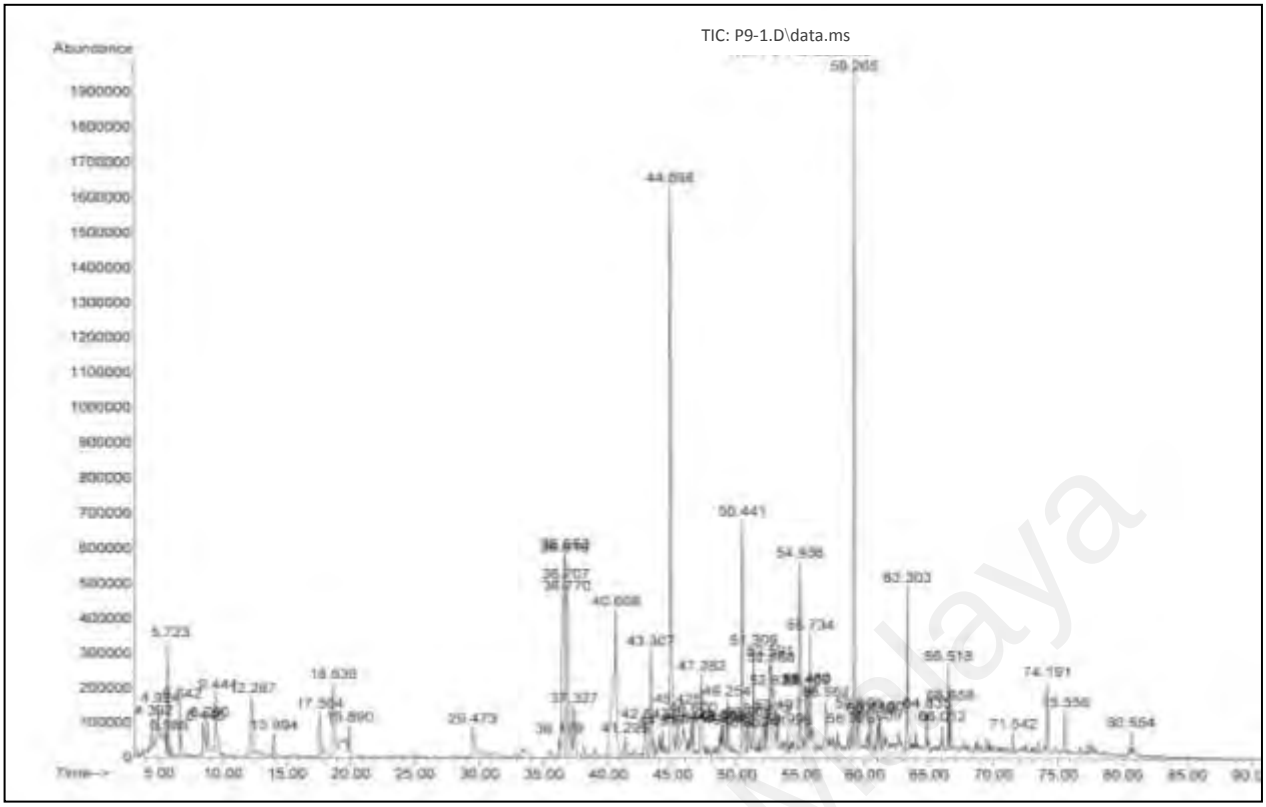


Figure 28. GC-MS ion chromatograph for genotype E9

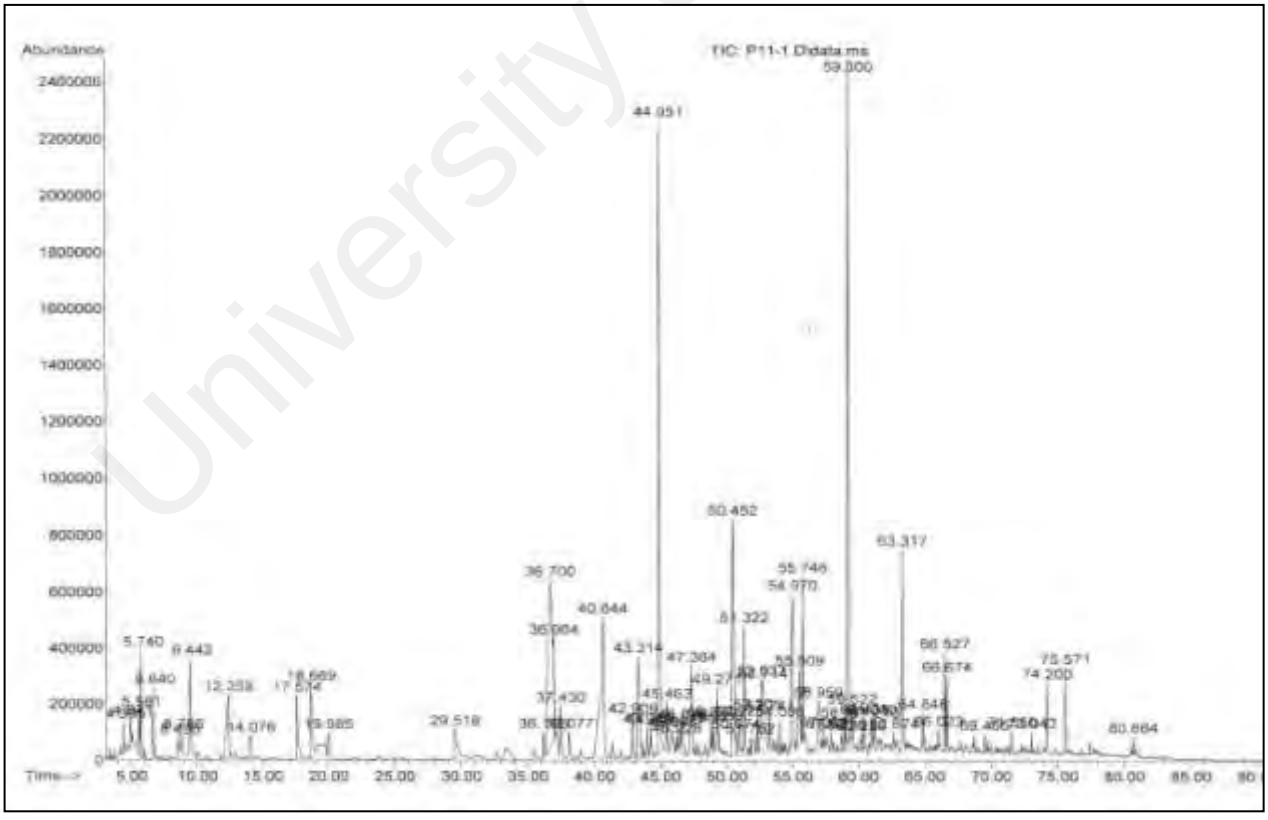


Figure 29. GC-MS ion chromatograph for genotype E11

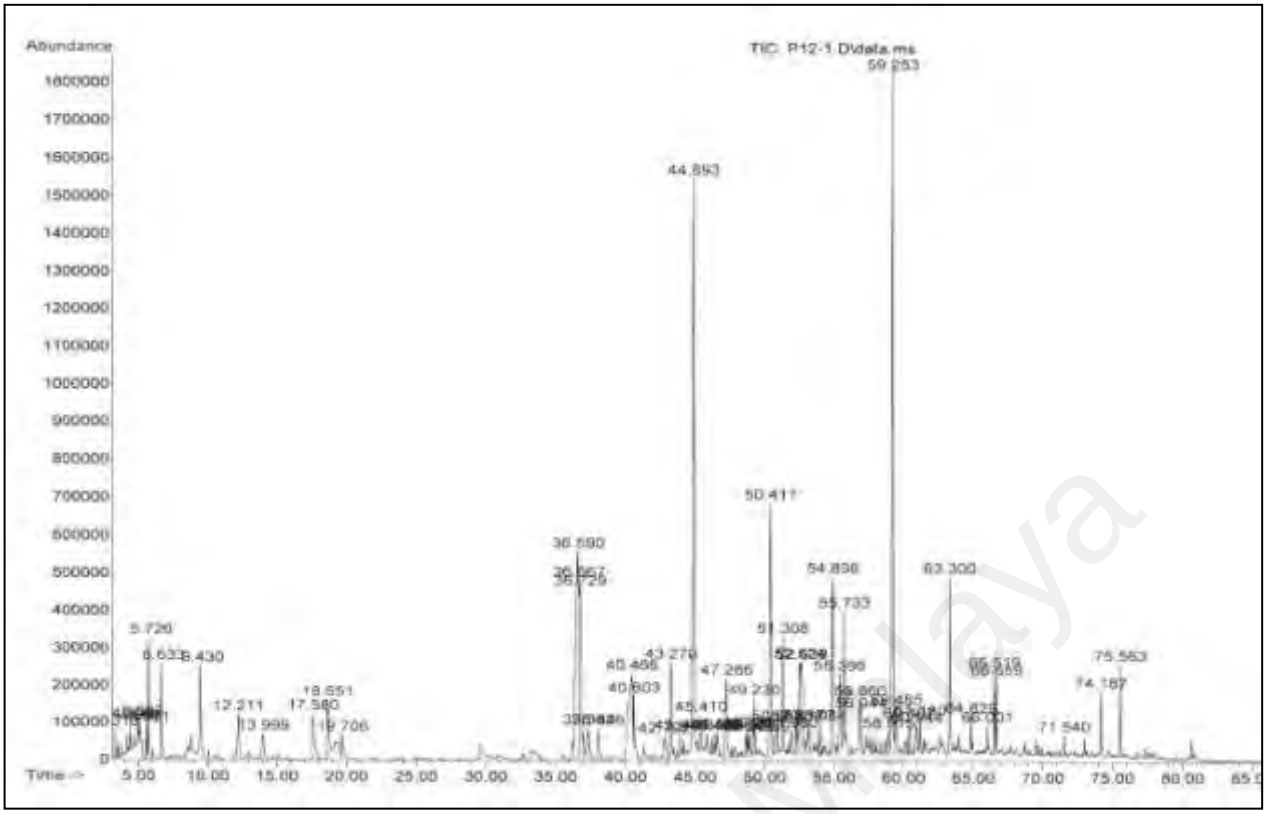


Figure 30. GC-MS ion chromatograph for genotype E12

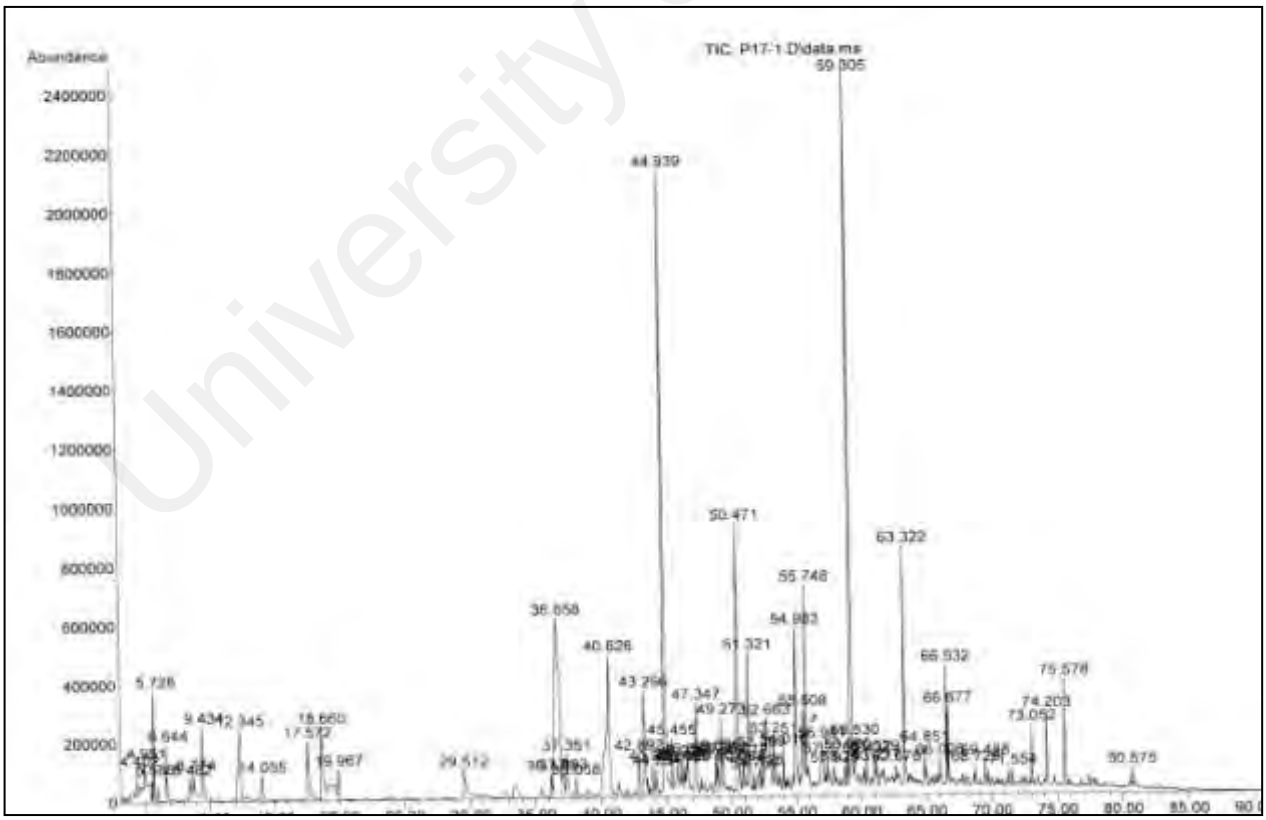


Figure 31. GC-MS ion chromatograph for genotype E17

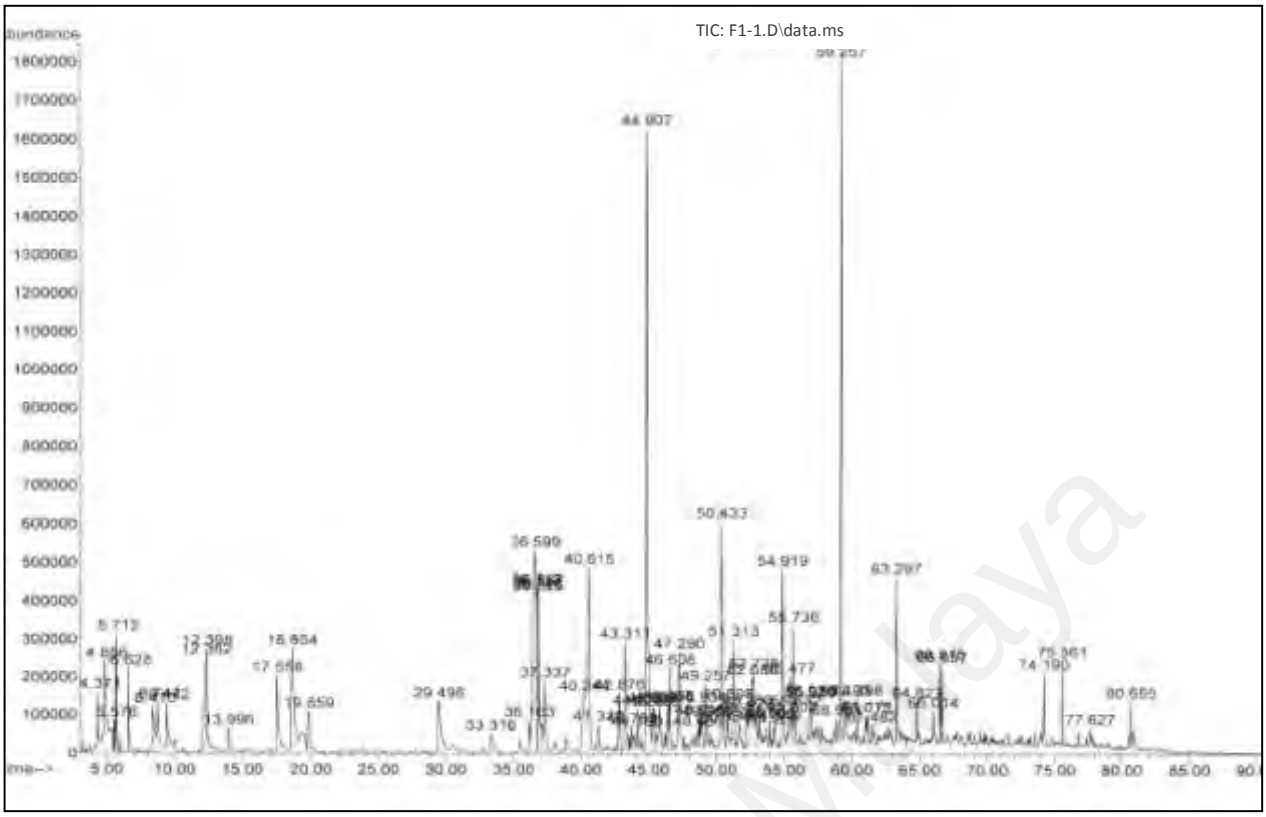


Figure 32. GC-MS ion chromatograph for F_1 derived from genotype E1 and sweet corn

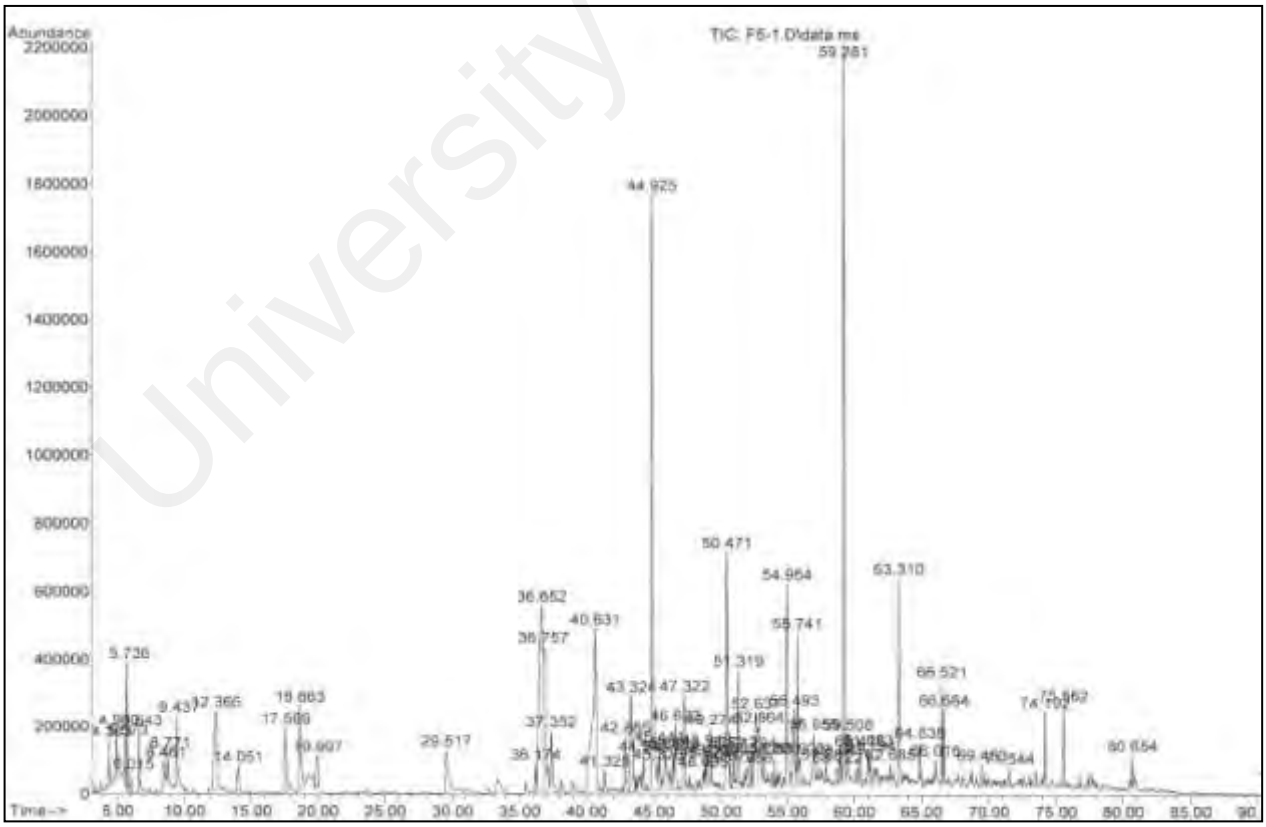


Figure 33. GC-MS ion chromatograph for F_1 derived from genotype E5 and sweet corn

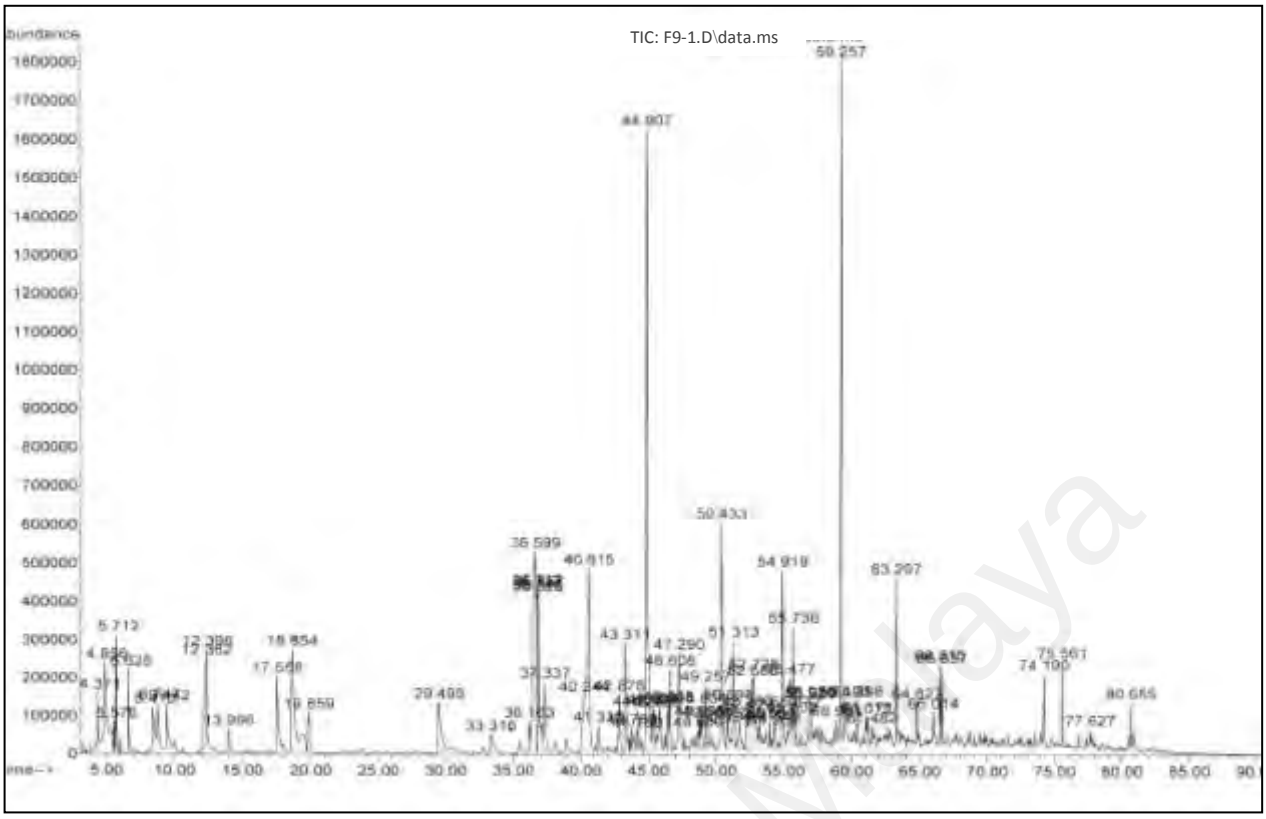


Figure 34. GC-MS ion chromatograph for F_1 derived from genotype E9 and sweet corn

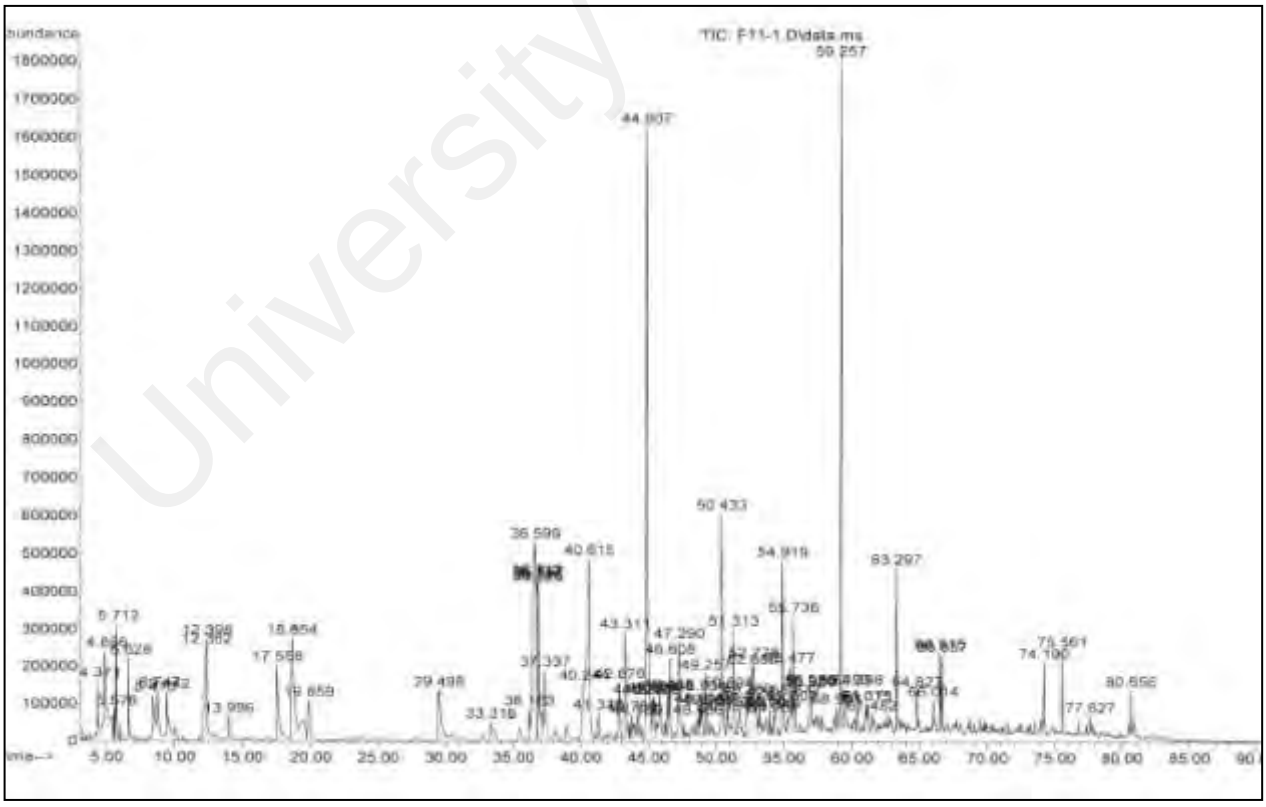


Figure 35. GC-MS ion chromatograph for F_1 derived from genotype E11 and sweet corn

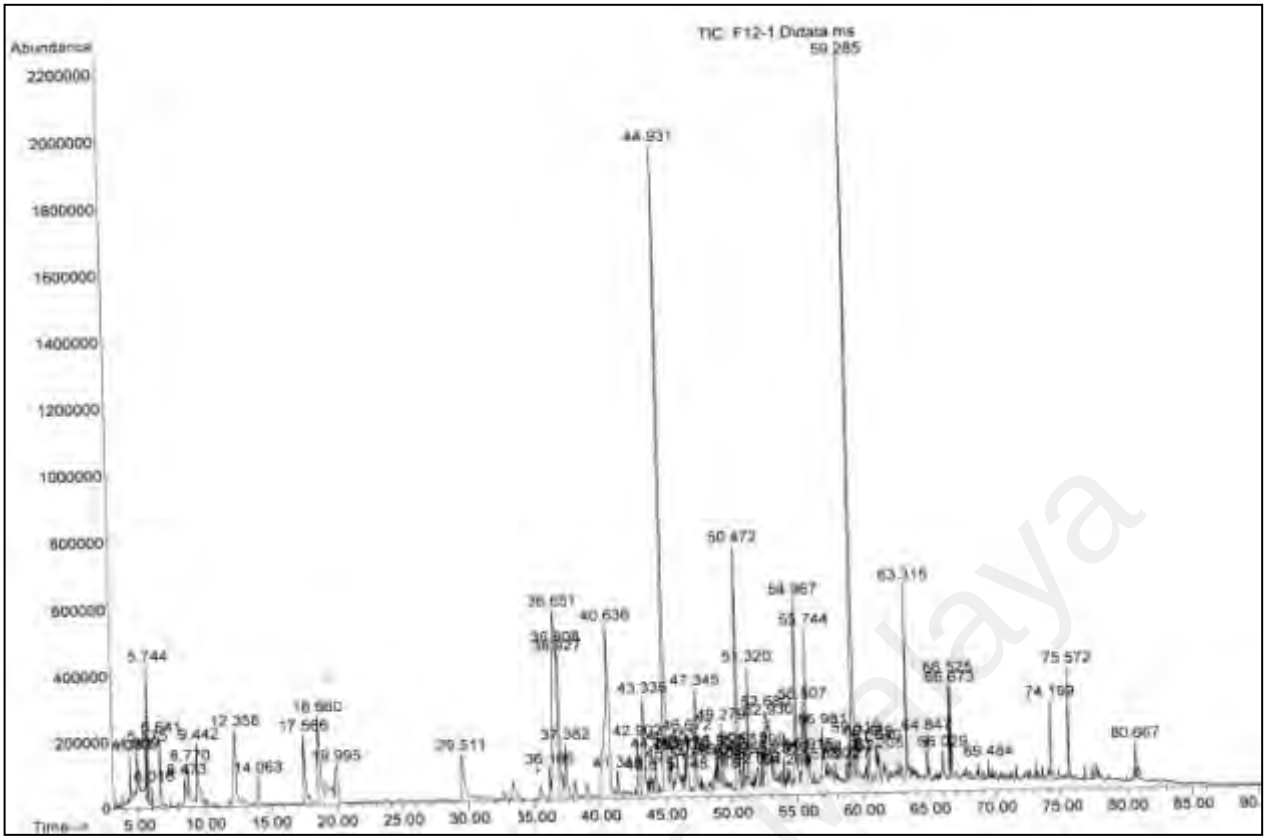


Figure 36. GC-MS ion chromatograph for F_1 derived from genotype E12 and sweet corn

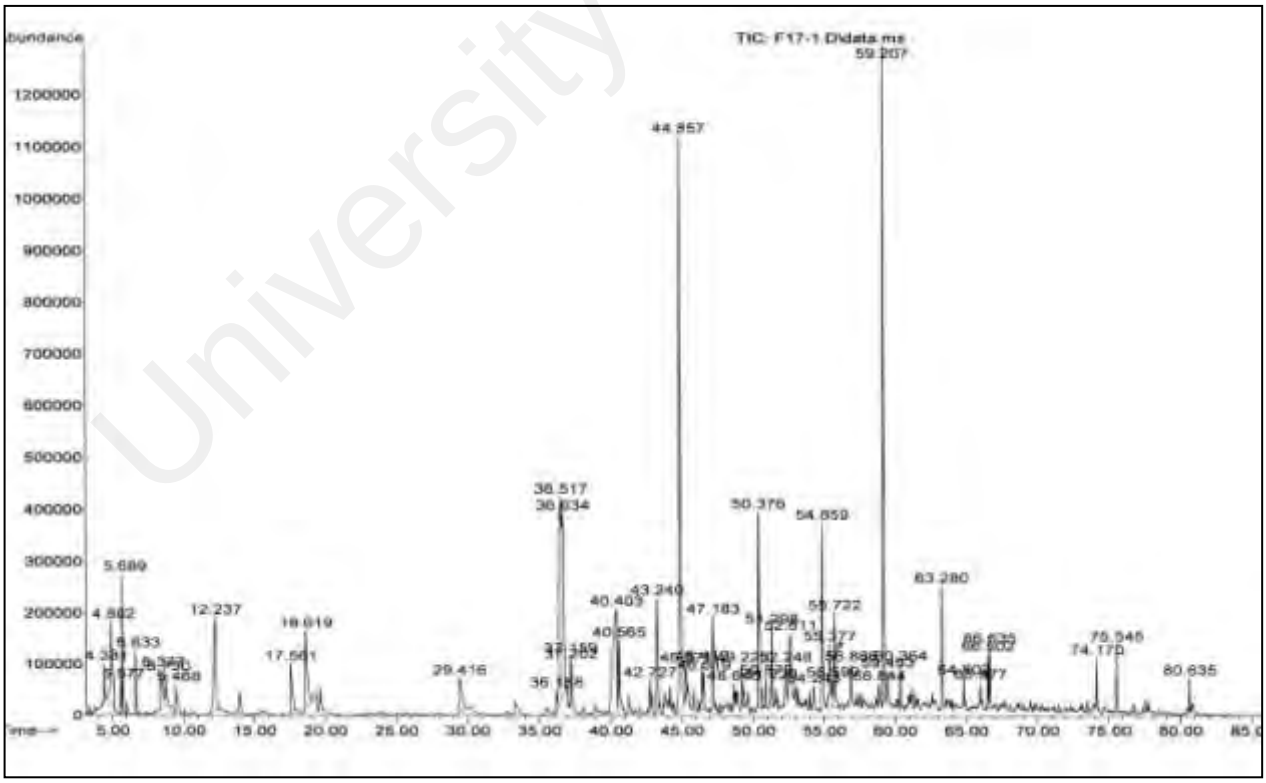


Figure 37. GC-MS ion chromatograph for F_1derived from genotype E17 and sweet corn

4.5 PRODUCT AND BY-PRODUCT FROM PYROLYSIS PROCESS ACCORDING TO GENOTYPES

The percentage of products produces by pyrolysis process for each genotype was listed in Table 11.

Table 11. Oil, Char and Gas weight percentage for parental genotypes and F_1 genotypes.

No	Genotype	Oil wt %	Char wt %	Gas wt %
1	E1 (F_1)	28.3	39.1	32.6
2	E5 (F_1)	42.0	29.7	29.3
3	E9 (F_1)	40.1	30.4	29.5
4	E11 (F_1)	38.5	31.2	31.3
5	E12 (F_1)	35.4	36.0	28.6
6	E17 (F_1)	33.6	36.5	29.9
7	E1	34.3	43.3	22.4
8	E5	26.5	42.4	31.5
9	E9	31.8	38.0	30.2
10	E11	45.8	28.6	25.6
11	E12	49.8	26.2	24.0
12	E17	44.0	27.6	28.4

The percentage of products produce by pyrolysis was varied through out genotypes (Table 11). Oil percentage was highest for genotype E12 with 49.8% and the lowest oil percentage was for genotype E5 with 26.5%. Char percentage was highest for genotype E1 with 43.3% and the lowest char percentage was for E12 with 26.2%. Gas percentage was highest for genotype E5 with 31.5 % and the lowest gas percentage was for E1 with 22.4%.

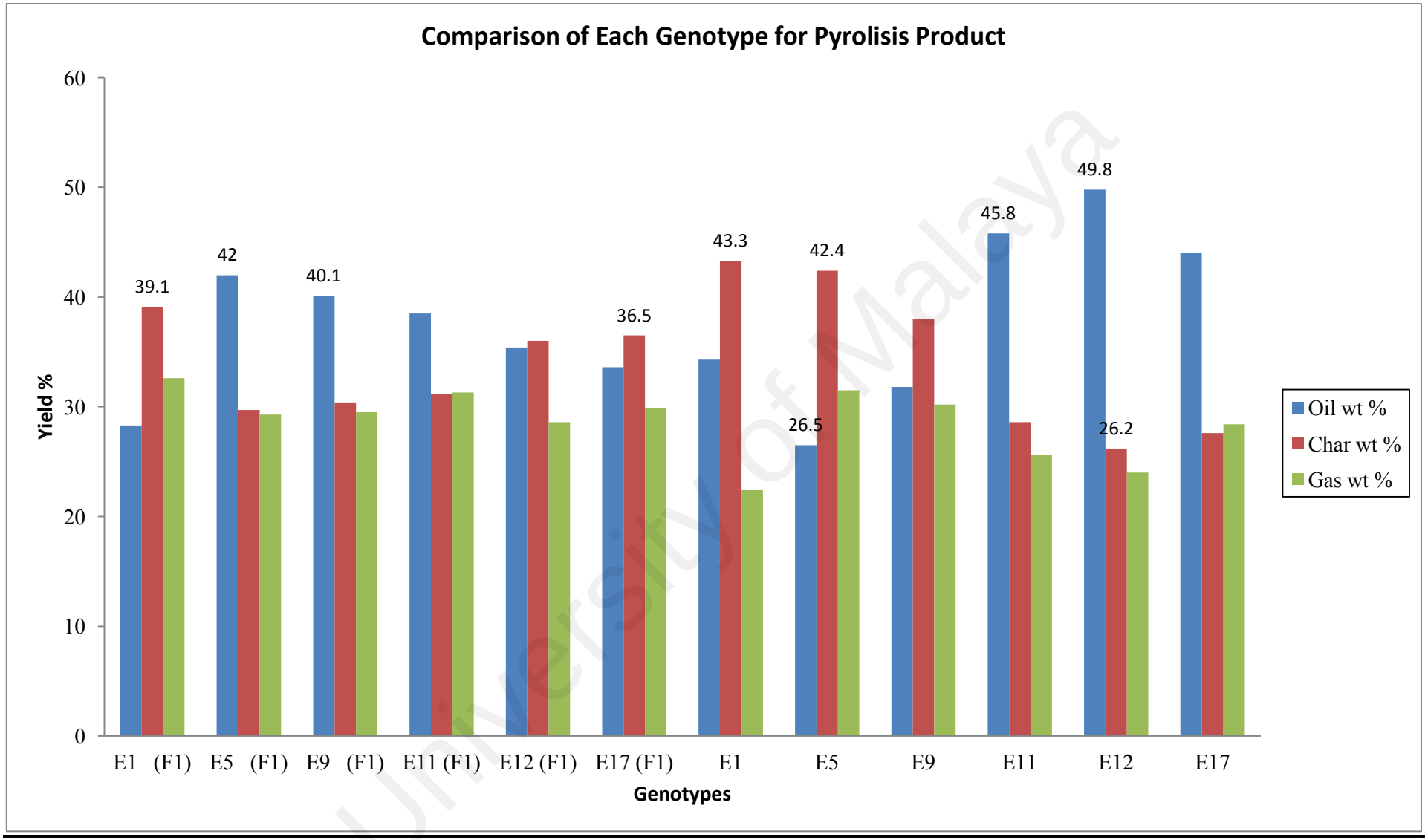


Figure 38. Products obtain from pyrolysis process for each genotypes ie. Oil, Char and Gas.

5.0 DISCUSSION

5. 1 YIELD CONTRIBUTING TRAITS

5.1.1 Plant height

In plant height, significant difference was observed among genotypes at P≤0.01 level while between replications in a genotype the difference was not significant (Table 3). This indicates that within genotypes, homogenous plant height can be observed. High and positive correlation were found between days to tasseling (0.826) and days to silking (0.843), days to maturation (0.767), grain yield (0.621) and stover weight (0.728) with plant height (Table 5). In earlier finding of Zsubori et al. (2002) there were association between plant height and flowering days (days to tasseling and days to silking) where the morphological and ontogenetically for both are strong. This leading to the initiation of flower will stop the internodes formation which means early flowering days usually ends the plant elongations process or plant growth. They also propose the earliness of flowering days is reciprocal with grain yield.

Other researchers who shares the same finding with Zsubori et al. (2002) was Iqbal et al. (2011) that observed higher plants have potentiality to produce better grain yield compared to the shorter plant. Plant height was used as an indicator of biomass yield which was in line with findings by earlier researcher (Lewis et al., 2010). Taller and larger plant may increase the dry matter as well as the grain yield. Significant and positive correlation of plant height and stover yield suggesting the contribution of higher amounts of dry matter accumulation in both phases of plant growth and development which lead to larger stover yield (Iqbal et al., 2011).

Moderate correlations were observed between number of cob and leaves with plant height. Higher plants have potentiality to have more number of leaves with broader leaf area. The indication of plant height have contribution towards the number

of cob which was align with Iqbal et al. (2011) where they reported the number of leaves and leaf area have positive relationship with yield. This is due to photosynthesis activity that attribute to accumulation of metabolites that are available for grain development during grain filling period (Tollenaar et al., 2004). The density of plant has a great influenced in cob number as the competition for light, water and nutrient may decrease grain yield per plant (Sangoi & Salvador, 1997). Negative and significant magnitude between stem diameters with plant height was observed. Contrary from general believed, stem diameter has no correlation with height of plant in this particular experiment.

In Duncan Multiple Range Test (DMRT, Table 4), genotype E17 appeared with highest mean (242.9a) and the lowest mean was from F1 derived from genotype E1 and sweet corn (118.7h). Genotype E11 and E12 were group together due to same range of mean (200.5cb and 193.0cb respectively).

Variance component for genotypic and phenotypic calculated for this trait was summarized in Table 3. Moderate broad sense heritability was profound in plant height. In previous studies, Lewis et al. (2010) and Guzman and Lamkey (2002), have similar results on plant height heritability. As were reported by Ojo et al. (2006), moderate heritability has potential to be exploited as large genetic determination for maize grain yield improvement. The genotypic coefficient variance (GCV) and phenotypic coefficient variance (PCV) ratios were large indicates the high influence of environment to this trait (Parveen and Ginwal, 2011). Moderate GCV shows the genetic components were moderately inherited to the next progeny while high PCV value indicates the trait were largely influence by environment. These would lead to improvement of traits for further selection in next generation.

5.1.2 Flowering days

In flowering days (days to tasseling and days to silking), significant difference was observed among genotypes at P≤0.01 level while between replications in a genotype the difference was not significant (Table 3). Tasseling days has positive correlation with days to silking and days to maturity. This positive correlation between days to tasseling and days to silking were useful as yield improvement tool as during pollination, the pollen shred from pollen sac were directly transferred to the silk that emerged simultaneously with the emergence of tassel. Effective synchronization of pollen shed with silking in field, are advantageous to obtained maximum kernel number (Anderson et al., 2004). Days to tasseling and days to silking have significant and positive association with grain yield which was also reported by Anderson. The synchronized time for tassel and silk will resulting in increasing of grain yield as the percentage for each silk to be pollinated by shredded pollen is higher. The synchronization of tasseling time and silking time will result in reduction of kernel abortion as endosperm development continued after fertilization.

Positive and significance correlation can be seen between flowering days and plant height. As were reported by Zsubori et al. (2002) elongation of plant stop at floral initiation which means earlier flowering days may result in shorter and stunted plant. Relationship between plant height and flowering days are crucial as it also resulting in dry biomass. Align with Zsubori et al. (2002) finding, the significant positive association between flowering days, height and stover indicates that longer flowering days will increase the plant height and eventually quantity of dry biomass.

In Least Significance difference (LSD) value for days to tasseling was 1.81 (Table 3) which shows the differences between mean for each entry were small and the days to flowering were not varied between genotypes. The highest mean for days to

tasseling were found in genotype E17 (72 days) and the lowest mean was found in genotype E1 (43 days) Using Duncan Multiple Range Test (DMRT), genotype E17 (72.0a) have the highest mean while F_1 derived from E11 and sweet corn have the lowest mean range (55.0f). Due to a small LSD value, many genotypes have same range of mean. Genotypes E12 and E11 (67.7b), E1 and E5 (59.7d - 58.7d) and genotype F_1 derived from E1, E9, E11, E5 and sweet corn (55.5fg- 53.3fg) were group together based on their mean range (Table 4).

In Least Significance Difference (LSD) analysis, the value for silking was small (1.91) indicates that the mean difference were also small. The highest mean were found in genotype E17 (72 days), the lowest mean was genotype derived from E9 and sweet corn for days to silking (53 days). Using Duncan Multiple Range Test (DMRT), genotype E17 (72.0a) have the highest mean range while F_1 derived from E9 and sweet corn have the lowest mean range (53.0f). Genotypes E12 and E11 have same mean range of 67.3b and 67.0b respectively. Genotypes E9 and E5 having the same mean range (62.3d and 61.0d respectively). While F_1 derived from E5, E11, E1 with sweet corn sharing the same group mean (55.0fg).

Based on genotypic variance and phenotypic variance summarized in Table 3, high broad sense heritability was observed. Reported by Mahmood at al. (2004), the broad sense heritability in number of days to tasseling and days to silking were high with small difference in GCV and PCV provided evidence that the parameters were under the control of additive genetic effect. Greater magnitude of broad sense heritability value couple with high genetic advance suggested that this trait is suitable condition for further selection (Mahmood et al., 2004). Genetic advance was 87.5 for days to tasseling traits while days to silking were 64.0. The small differences between GCV and PCV indicates that the presence of sufficient genetic variability for traits that facilitate selection.

5.1.3 Grain filing period

In grain filling period, significant difference was observed among genotypes at P≤0.01 level while between replications in a genotype the difference was not significant (Table 3).Grain filling period has positive association with kernel weight. Echarte et al. (2006) reported that final kernel weight is a result of the kernel growth rate during the linear phase or effective grain filling period (KGR) and of the duration of this period (Echarte et al., 2006). This result was supported by Badu-Apraku et al. (1983) and Muchow (1990) that suggesting the decreasing in grain yield were almost entirely determined by short grain filling period. In both papers, the association of grain yield with grain filling period were greatly influence by the mobilization and assimilation of photosynthesis product. During photosynthesis process, carbohydrates stored in vegetative part of plant were assimilating then being remobilized to kernels. In stem particularly, maximum amount of stored carbohydrates normally present in stalk at 2-4 weeks after pollination. However, a portion of stored assimilates (sink) will mobilized into the grain during grain filling period (Rajcan & Tollenaar, 1999). The capacity of kernels to used up assimilates store in stem were proportional with the duration of grain filling period (Echante et al., 2006). Longer the duration of grain filling period, the greater the tendency of remobilization of carbohydrate to kernels. Never the less, the kernel weight was also affected by lower night temperatures due to decreased proportion remobilization.

Wang et al. (1999) found out that the ear-filling rate was the most conducive in increasing the final yield. The kernel-filling rate was partly control by additive gene action with the involvement of dominant gene actions. Other than that, increased in chlorophyll concentration and early silking can serve as selection index for longer grain filling period which was crucial in kernel development.

The positive association between grain filling periods with kernel weight contribute to significant but low correlation between grains filling periods with stover weight. As were suggested by Badu-Apraku et al. (1983), the remobilization of sink materials from stem and leaves to kernel happen during grain filling period. This will lead to depletion of sink material in stover which decreases the total weight.

In Least Significance Difference (LSD) analysis, the value for grain filling period was small (1.54) indicates that the mean difference were also small. The highest mean were found in genotype E9 (29 days), the lowest mean was genotype derived from E9 and sweet corn for days to silking (21 days). Using Duncan Multiple Range Test (DMRT, Table 4), the highest mean was found in genotype E9 (29.0a) and the lowest mean was F_1 derived from genotype E9 and sweet corn (20.7g). Several genotypes have same mean range such as F_1 derived from genotype E1 and sweet corn and genotype E1 (26.7bc and 26.8bc), E11 and E12 (25.3cde), F_1 derived from genotype E5 and E12 (25.0ef and 24.33ef respectively).

Variance component summarized in Table 3, high broad sense heritability was observed. Similar results were also obtained by Perenzin et al. (1980) with similar observation on heritability for grain filling period. Screening for high yielding genotype is by feasibility of selecting for a longer grain filling length supported by high heritability and high genetic advance. The moderate difference between GCV and PCV value indicates the trait were slightly influenced by environment but were viable enough to be used to facilitate selection in future.

5.1.4 Days to maturity

Days to maturity was defined as the time at which kernels dried down to a selected kernel moisture percentage (Wang et al., 1999). Days to maturity or were also known as black layer maturity days occurs when the corn kernel continue to accumulate seed weight until it reached the physiological maturity (28-35 % moisture content). The black layer can be identified as it is located within the base of the kernel which normally forms 60 days after silking or 20 days after denting (University Mississippi States [MSU], 2010)

Significance difference at $P \leq 0.01$ was observed in days to maturity among the genotypes. These were expected as days to silking and grain filling period were also varied. These two traits have direct influence on days to maturity. Positive correlations were found in the days to tasseling (0.92), days to silking (0.83) and grain filling period (0.45). In earlier research, Ojo et al. (2006) found that days to maturity have significance and positive correlation with flowering days. This is due to physiological maturity were evaluated from the fertilization that occurred after pollinations of silk. Longer silking days have impact on the maturation of cob. Wang et al. (1999) found the same positive association between sowing to midsilk and effective grain filling period with black layer maturity date. However, they also reported that the delayed physiological maturity was resulted from the duration of grain filling period rather than the delay in flowering days as the paper was focusing on grain filling trait.

A positive correlation was found between days to maturity with plant height and stover weight. The same correlation was observed by Ojo et al. (2006) which plant height has direct influential to the maturity stage of corn. Align with this result, higher plant provided larger amount of stover which was reported by Iqbal et al. (2011).

A positive correlation was found in maturation days and grain yield. Different physiological maturity stages in corn have varied impact on its end yield. This was supported by several researchers (Ojo et al., 2006 & Wang et al., 1999) which also found the positive and direct link between these two traits. Minimum stage of maturity that a plant must reach to gain the highest yield was when the kernel developed at least into dent stage where at this point 65-75% of potential grains were made. When the kernel reach fully dented stage, 90-95% of maximum yield were obtained with moisture content of 35-40% in grain and when the kernel reach physiological maturity 100% maximum yield were gained with moisture content of 25-35% (Rankin, 2011).

In Least Significance Difference (LSD) analysis, the value for silking was small (4.19) indicates that the mean difference were small. The highest mean were found in genotype E17 (97 days), the lowest mean was genotype derived from E9 and sweet corn for days to silking (74 days). Using Duncan Multiple Range Test (DMRT, Table 4), highest mean was obtained from genotype E5 while the lowest mean was from F_1 derived from genotype E9 and sweet corn. However, many genotypes were group together such as genotypes E5 and E9 (88.0b) and F_1 derived from genotypes E17, E1, E11, E12, E5 and E9 with sweet corn (82.7c, 81.0c, 81.0c, 80.3c, 80.0c, and 73.7c respectively).

Variance component shows that the broad sense heritability for black layer maturity days was high which give indication of the environment have slightly effect on the trait that enabled the trait to be passed on to the next generations. Wannows et al. (2010) give the conformity to the result as their finding also appeared to be similar. Small difference between GCV and PCV suggesting the trait were controlled by additive gene effect. High heritability coupled with greater genetic advance gave the opportunity for further selection in the future.

5.1.5 Grain yield

In grain yield, significant difference was observed among genotypes at $P<0.01$ level while between replications in a genotype the difference was not significant (Table 3). A positive association were between grain yields with plant height, flowering days, maturity days, number of leaves and leaves length. In previous study conducted by Iqbal et al. (2011) has revealed the positive relationship of plant height and maturation days with grain yield. Plant with greater height would be the best selection criteria to improved shelling percentage thus increased grain yield. Height of plant with greater leaves number and longer leaves length contributes to the increasing total photosynthesis process (Richards et al., 2000). It was further elaborate as the rate of leaf photosynthesis will affect the total crop photosynthesis. This will enhance the production of grain yield as the accumulation of sink material product from photosynthesis will be channel to the kernel during grain filling period (Badu-Apraku et al., 1982). Higher rate of photosynthesis will increases the concentration of sucrose and other non-cell wall carbohydrates to be mobilized from stover into kernel (Lewis at el., 2010). However grain yield were not solely depends on the plant height, genetic means also play a role in high grain productivity.

Maturity day association with grain yield as were reported by Iqbal et al. (2011), where the delay in maturity would simultaneously increase grain weight and hence directly improve grain yield. It could be attributed that delay in maturity might have direct assimilation of dry matter towards grain development and thus resulted in heavier grain. They also found out the delay in maturity were used as one of the tool during partitioning of dry matter accumulation towards ear development which resulted more kernel rows on ear. The similar result were obtained in this research where the longer the maturity days resulting in higher grain yield which can be seen in the genotypes.

Badu-Apraku et al. (1982) also suggested the difference in night temperature will affect maturity which is primarily associated with kernel progression.

Positive correlation was found between grain yield and days to tasseling and days to silking. Emergence of male and female flowering simultaneously offers advantages as the exposure of silk to shedding pollen were higher. Anderson et al. (2004) has described in their research where the rate of pollination were directly effect by the emergence of tassel and silk. The concern was the receptivity of silk has to be synchronizing with the pollen shed during pollination. Such synchronization of activation both male and female flower were called nicking (Halsey et al., 2005). The changes in grain weight usually by the low kernel number that were cause by the number of floret positions that could be pollinated early has loss the silk receptivity to be pollinated again later (Anderson et al., 2004). These will reduced the kernel set for late pollinations. The kernel weight was greater for early pollination as the silk were form from mid base ear position that normally produces larger kernel (Anderson et al., 2004).

Other than receptivity of silk, the shedding of pollen grain that were released within 5 to 8 days need to be fertilize directly as it will dies throughout the rough condition or higher temperature. The abundance of pollen grain will reduced with the extended period of fertilization due to not similar tasseling and silking emergence (Halsey et al., 2005). Thus direct association of flowering day synchronization with grain weight were undeniable as it was proven by many researchers.

In Least Significance Difference (LSD) analysis conducted for grain yield was (39.2) indicates that the mean difference were also large. Using Duncan Multiple Range Test (DMRT, Table 4) the highest mean were found in genotype derived from E12 with sweet corn (365.7a), the lowest mean was genotype derived from E1 and sweet corn for

grain yield (188.4f). F1 derived from genotype E17, E5 with sweet corn (300.8bc and 230.6e) were in the same mean range. Genotypes E1 and E11 (288.8cd and 273.8cd respectively) were in one mean range, while F1 derived from E11 and E5 and sweet corn (230.8e) in the same range of mean.

Moderate broad sense heritability was profound in this trait. This is accord with finding of (Yousuf & Saleem, 2002) who also reported similar heritability for grain yield. However, it was not align with Rafiq et al. (2010); Yusuf (2010) and Salami et al. (2007). High broad sense heritability was found by Rafiq et al. (2010) and Yusuf (2010) as the genotypic relationship observed among traits affecting grain yield revealed that the association exclude the environmental effect. This lead to the high heritability of grain yield based on the trait contributing to grain yield. Salami et al. (2007) reported a different heritability in grain yield where lower heritability was found. GCV and PCV differences were large due to environment influential in this trait which to maintain the trait in future going to be a difficult task.

5.1.6 Thousand grain weight

Thousand grain weights is a vital yield contributing factor, which plays a decisive role in showing the potential of a variety (Rehman et al, 2008). Thousand grain weights were calculated by separating exactly 1000 kernel for weighing. The process was done randomly without any selection was applied to the kernel size or weight. In thousand grain weight, significant difference was observed among genotypes at $P \leq 0.01$ level while between replications in a genotype the difference was not significant (Table 3). A positive association were observed between plant heights, days to silking, grain filling period and days to maturity with thousand grain weight. The similar observations were reported by Rafiq et al. (2010) and Iqbal et al. (2011) where the positive correlation were seen in plant height with 100 grain yield. Larger and higher plant contributes to greater number of leaf. Its strongly suggest that such association are important in simultaneous improvement of grain yield. As were stated before in grain filling period and maturation days, grain yield and grain size were greatly influence by photosynthesis process. During photosynthesis, sink material were accumulate from vegetative parts (leaves and stem) to the grain (Iqbal et al., 2011). This occurs during grain filling period and end when the kernel reaches the dent stage (Rankin, 2011).

Other than trait influencing 1000 grain weight, Abuzar et al. (2011) and Rehman et al. (2008) described the association of plant densities with 1000 grain weight. Different plant densities give out different size and weight of kernel. The increase of grain weight was due to availability of more resources which is the nutrient, water and sunlight. Low grain weight in high plant population density was probably due to less photosynthates for grain development on account of high inter-specific competition which resulted in low rate of photosynthesis and high rate of respiration as a result of enhanced mutual shading (Zamir et al., 2011). Rahman et al. (2007) has mentioned on

the increasion in grain weight could be attribute to favourable soil condition and proper cultural practises.

In Least Significance Difference (LSD) analysis conducted for 1000 grain yield was (15.64) indicates that the mean difference were also large. Using Duncan Multiple Range Test (DMRT, Table 4) conducted for 1000 grain weight shows the highest mean was found in genotype E9 (342.9a), the lowest mean was found in F_1 derived from genotype E9 with sweet corn (231.3f). Genotypes E5, E17, E1 and E11 were group together. F_1 derived from genotype E1 and E11 (263.3d and 231.3f respectively) were group together.

High broad sense heritability was profound in this trait. This is align with finding of (Yusuf, 2010; Mahmood et al., 2004; Yousuf & Saleem, 2002; Ojo et al., 2006) who also reported similar heritability for 1000 grain weight. The genotypic relationship observed among traits affecting 1000 grain weight revealed that the association exclude the environmental effect. GCV and PCV differences were small due to less environment influent in this trait (Ojo et al., 2006). Improvement in 1000 kernel weight has immediate effect on end grain yield. Higher grain yield were due to size and weight of individual grain despite the environmental effect and traits influencing factors.

5.2 PRODUCTIVITY OF BIO-OIL

5.2.1 Physical characterization of bio-oil

The physical properties of bio-oil were resumed in Table 7 where the density of bio-oil at 24°C was 1032.9 Kg/m^3 which is denser than heavy fuel oil (Abnisa et al., 2011). The viscosity of bio-oil is 1.192 cP at 40°C. These two physical properties have important impact on the performance and quality of atomization of fluid. Fluid atomization has three important fuel properties which are surface tension, viscosity, and density where the interaction between these properties lead to atomization (Lefebvre, 2011). The low viscosity was influenced by the high value of water content in bio-oil which is 46 % (Abnisa et al., 2011). The total base number (TBN) and total acid number (TAN) for bio-oil was 18.55 mg/KOH and 0.35 mg/KOH respectively. As were reported by Sutton and Stow (2010) the high TBN value indicates the nonexistence of strong acid which the addition of these acids at combustion will reduce the value of TBN of oil. Such property was favourable for modern lubricants. As the value of TBN increases the value of TAN reduced, the relationship between TBN and TAN is complicated as the total number of dielectric constant of the oil media, the strength of the acid and base components (Sutton & Stow, 2010)

5.2.2 Chemical characterization of bio-oil

5.2.2.1 Fourier Transform infra-red

Fourier Transform infra-red (FTIR) conducted on bio oil shows the different functional groups consist in bio-oil. The experiments were carried out on KBr pellet. FTIR spectra indicate the present functional groups of a compound at different stretching vibrations. According to Wiley (2009), vibrational modes are given descriptive mode such as stretching, bending, scissoring, rocking and twisting. There are two types of molecular vibrations, which is stretching and bending (Feist, 2002). The vibrational modes referred the covalent bonds of molecules that can be stretched and bent as a spring, where it is not rigid. At normal temperature, the molecule bonds vibrate at consistent vibrational energy. However, with the applied of infrared radiation, transition between vibrational energy states will be elevated which will be shown in the film as the streaching or bending spectrum. Analogy to the spring stiffness, molecule with different covalent bond has different bond strength and thus having different stretching frequencies. Double bond (C=N) is twice stronger than single bond (C-N), while the triple bond is (C≡N) similarly stronger than double bond (C=N) (Wiley, 2008). This led to the different infrared stretching frequencies which ranging from 1100cm^{-1} for single bond, to 1660 cm^{-1} for double bond, to 2220 cm^{-1} for triple bond (Wiley, 2008).

Bio-oil formation from corn stover using thermochemical conversion was evaluated using FTIR analysis. Alcohol, phenol, alkanes, arenes, carboxylic acid, amines, aldehydes, and ketones were identified (Table 5). The O-H stretching vibrations between 3200 cm^{-1} to 3500 cm^{-1} of bio oil indicate the presence of alcohols and phenols (Abnisa et al., 2011). The O-H stretch have broad and intense region. Stretching

vibration frequencies for each genotype were different yet within the alcohols and phenol region. Frequencies for each genotype were tabulated in Table 7 and Table 8.

The C-H stretching vibrations between 2800 cm^{-1} to 3000 cm^{-1} indicated the present of alkanes. Simple alkane were characterized by absorptions due to C-H stretching and bending where the C-C stretching and bending band are too weak or the frequency was to low to be detected. Frequencies for each genotype were tabulated Table 7 and Table 8.

The C-H stretching in aromatic usually was observed at region 3100 cm^{-1}- 3000 cm^{-1} aromatic hydrocarbon show absorption at different region due to carbon carbon stretching vibrations in aromatic ring. Besides the C-H stretch above 3000 cm^{-1}, there were two other regions that distinguish between aromatics and organic compound without aromatic ring. These regions were 2000 cm^{-1}-1665 cm^{-1} and 900 cm^{-1}- 675 cm^{-1}. The carbonyl stretch C=O of alpha, beta-unsaturated aldehydes at 1710-1685 cm^{-1}. Frequencies for each genotype were tabulated Table 7 and Table 8.

5.2.2.1 Gas Chromatography Mass Spectrometry

Gas Chromatography Mass Spectrometry (GC-MS) is a confirmation tool for a sample. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically (Douglas, 2011). Bio oil was dominated by aromatic compound. Light oil was considered as a mixture of several micro families. As were reported by Yang at al. (2010) the macro-families may include polycychc aromatic hydrocarbon (PAHs), phenols, esters, furan derivatives aliphatic hydrocarbons. The analysis conducted on bio oil from different entries show almost similar abundant product. The highest peaks were observed at retention time of 59.300 (±0.05) s for 2, 6-dimethoxy phenol with area of more than 2 %. Second highest peak at retention time of 44.951 (±0.05) s (Table 10) was methoxy phenol with area of more than 2 %. Peak at 50.452 (±0.05) s indicates the abundancy of methoxy phenol with area of more than 2 %. Other compound were also observed with GCMS analysis such as 4-ethyl-2-methoxy phenol, 1,2,4-trimethoxybenzene, 3-methyl- 1,2-benzendiol, 2-hydroxy-3,4-dimethyl-2-cyclopentene-1-one, 3-methyl-1,2-cyclopentanedione, and pyridine.

5.3 RELATIONSHIP OF BIO-OIL PRODUCTIVITY AND GENOTYPES

Bio-oil production for each genotype was differs in the quantity as well as the by-product. Among parental genotypes, E5 produce the least bio-oil amount (26.5%) while for F_1 genotypes hybrid derived from E5 and sweet corn produce the least bio-oil amount (28.3%). The highest amount of bio-oil was obtained from parent genotypes, E12 with 49.8% while bio-oil obtains from hybrid derived from E5 and sweet corn with 42.0%.

Bio-oil productivity was based on several factors other than physical condition in pyrolysis. Bio-mass plays an important role in oil production where the composition that makeup biomass influenced the end product of pyrolysis. As were reported by Lewis et al., (2010), amount of ethanol produced from a fixed amount of corn stover depends on the concentration of cellulose in the stover and the proportion of the cellulose that is released as free glucose which can then be fermented to ethanol. Lignin is known to limit the accessibility of enzymes to cell wall polysaccharides, thereby reducing degradability of cellulosic biomass by ruminant livestock and in ethanol production. The ideal biomass composition for cellulosic oil was high concentration of cell wall glucose and low concentration of lignin. However the simultaneous improvement of yield and quality cellulosic biomass was antagonist as during photosynthesis process, carbohydrates stored in vegetative part of plant were assimilating then being remobilized to kernels (Rajcan & Tollenaar, 1999). Hence, the composition of maize stalk will be reduced. Higher yield weight will have impact on stover composition as well as the bio-oil productivity.

6.0 CONCLUSION

In earlier research conducted on twenty-two maize genotypes, only six genotypes managed to produce hybrid with yellow sweet corn. F_1s derived from this crosses were planted and undergo agronomic evaluation process and the stover were submit to pyrolysis process for bio-oil production. Traits that were evaluated were plant height, days taken to flowering, grain filling period, days taken to maturity, stem diameter, number of leaves, leaves length, number of cob per genotype, thousand grain weights, and stover weight. Among these traits, several traits which include plant height, days taken to flowering, grain filling period, days taken to maturity, thousand grain weights contribute and gave direct association to the final yield. Genetic analysis conducted provides that high heritability for traits days taken to flowering, grain filling period, days taken to maturity, stem diameter, thousand grain weights, while moderate heritability were found in plant height, number of cob, leaves length and grain yield. High genetic advance were observed in plant height, days taken to flowering, grain yield, thousand grain weight and stover weight. These traits with high heritability and greater genetic advance were vital component for improvement of maize breeding where it plays a major role in increasing the end yield of maize.

Product of pyrolysis can be divided into three major component, bio-oil, bio-char and gases. Viscosity of bio-oil at 40°C was 1.192 cP, density at 24°C was 1032.90 kg/m^3, Karl Fisher water content was 46.00 wt%, TBN was 18.55 mg/KOH and TAN was -0.38 mg/KOH. The chemical properties of bio-oil were analysed using Fourier Transform Infra-red (FT-IR) and Gas Chromatography Mass Spectrometry (GC-MS). From these analysis it can be deduced that bio-oil have high content of phenol and alcohols with the present of alkanes, acid carboxylic, aldehydes and ketones. GC-MS analysis shows the most abundant chemical compound in bio-oil was 2, 6-dimethoxy phenol with area of more than 2 % at retention time of 59.300 (±0.05) s. Second highest peak at retention time of 44.951 (±0.05) s

was methoxy phenol with area of more than 2 % (Figure 26 - 37). Peak at 50.452 (±0.05) s indicates the abundancy of methoxy phenol with area of more than 2 % (Figure 26 - 37). The high proportion of phenol and alcohol enables bio-oil to be further used as alternative fuels with stages of distillation.

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