2. LITERATURE REVIEW

2.1. BACKGROUND OF PAPAYA

2.1.1. Origin and distribution

The cultivated species of *Carica papaya* L. has never been found wild in nature. Its origin is rather uncertain, but the consensus of opinion among botanists is that it originated in the lowlands of Central America between southern Mexico and Nicaragua (Storey, 1969a). It probably originated from hybridisation between two Mexican species of *Carica*.

The papaya was distributed along the tropical maritime trade routes by explorers and traders along Panama, Puerto Rico and Cuba around the middle of the sixteenth century. By 1611, it had spread to India and Malacca by the Portuguese and to the Philippines by Spaniards. By 1800, there was wide distribution in the Pacific islands. Today, papaya can be found in latitudes within 32° north and south of the equator, grown extensively as a plantation crop in India, Mexico, many African and south American nations, Hawaii and south east Asia.

2.1.2. Taxonomy

The cultivated papaya belongs to the family *Caricaceae* and genus *Carica*. Three other genera are present within this family, i.e. *Cyclimorpha*, *Jacaratia* and *Jarilla*. All members in the family are small trees or shrubs with latex vessels present in all parts of the plant. Of the four genera, *Carica* is the only one with species cultivated for its fruits. It has about 40 species in tropical and sub-tropical America. Other than *Carica papaya* L., the other edible species are *C. candamarcensis* Hook. f., *C. monoica* Desf., *C. pentagona* Heilborn, *C. erythrocarpa* Heilborn, *C. goudotiana* Solms-Laubach and *C. quercifolia* Benth. and Hook (Storey, 1969a). These are mostly eaten cooked because they lacked the palatability of papaya. However, parthenocarpic fruits of *C. pentagona*, known commercially as 'Babacos', are consumed fresh and is grown to a small extent in New Zealand (Little, 1982) and Ecuador (Oosten, 1986).

Some of the *Carica* species have resistance to diseases to which *Carica papaya* L. is susceptible e.g. *C. cauliflora*’s resistance to distortion ringspot virus and some attempts have been made to transfer resistance from wild to cultivated species. Successful interspecific
hybrids have been reported between other *Carica* species but *Carica papaya* L. was not cross-compatible with any of them (Mekako and Nakasone, 1975). However, hybrids of *Carica papaya* L. with *C. cauliflora* (Manshardt and Wenslaff, 1989a) and with *C. pubescens*, *C. quercifolia* and *C. stipulata* (Manshardt and Wenslaff, 1989b) were obtained using embryo rescue techniques to overcome postzygotic barriers to hybridisation.

2.1.3. Mating system

*Carica papaya* L. is a polygamous species. In nature, it is dioecious with male and female trees in the population, but possibly due to Man's interference and deliberate selection against non-productive male trees, gynodioecious populations with female and hermaphrodite trees also exist (Storey, 1969a).

For dioecious populations, several workers (Prest, 1955; Storey, 1969a) agreed that wind is the main agent of pollen dispersal. The long pendulous male inflorescence which readily shed pollen in the breeze, lend support to this belief. However, Allan (1963) reported that very little papaya pollen was airborne and suggested that honeybees were responsible, although papaya flowers were not the priority sites for visits. Free (1974) reported that papaya flowers were often visited by many Skipper butterflies *Perichares philetes philetes* during dusk in Jamaica and suggested their use as pollinators if the need arose. Dioecious papaya varieties are, therefore, enforced cross-pollinators because of separation of the androecium and gynoecium.

In gynodioecious populations, the role of wind as the pollinating agent is diminished. This is because the stamens are packed inside the corolla tube and seldom protrude prominently out of the flower. Many gynodioecious varieties such as Sunrise Solo, Kapoho Solo, and Eksotika are self-pollinated and are therefore, purelines. The hermaphrodite flowers are cleistogamous i.e. anthers dehisce and release the pollen to effect self-pollination prior to anthesis of the flower (Chan, 1980; Rodriguez-Pastor et al., 1990). Such varieties are enforced self-pollinators and seeds gathered from hermaphrodite fruits will usually breed true to type. Self-pollination in papaya does not appear to result in any loss of vigour (Hamilton, 1954).
2.1.4. General biology

2.1.4.1. Stem

The stem is herbaceous, usually single and erect and sometimes branched if the terminal shoot is injured. The stem is hollow, and marked by prominent half-moon shaped leaf scars on the surface.

2.1.4.2. Leaves

The leaves are clustered at the apex of the stem. They consist of large, palmate laminae, 40 - 60 cm in width, normally with 7 - 9 lobes and held by long, hollow, pale green or purple tinged petioles

2.1.4.3. Fruit

The papaya fruit is a fleshy berry, variable in weight from 200 g to well over 10 kg. Fruit shape is a sex linked character and ranges from spherical to ovoid in female flowers to long, cylindrical or pyriform (pear-shaped) in hermaphrodite flowers. The skin of the fruit is thin and usually green when immature, turning to yellow or orange when ripe. The flesh is succulent, usually yellow or reddish orange in colour. Yellow flesh is governed by a single dominant gene and red is homozygous recessive. The fruit has a central ovarian cavity which is lined with the placenta carrying numerous black seeds. The ovarian cavity is larger in female fruits than hermaphrodite. The shape of the cavity at the transverse cut ranges from star-shape with 5-7 furrows to smooth circular-walled.

2.1.4.4. Seed

The papaya seed consists of a small laterally flattened embryo with ovoid cotyledons surrounded by fleshy endosperm and a seed coat made up of a dark brown, hard, muricate endotesta and a translucent sarcotesta which contains a thin mucilaginous fluid. A well-pollinated fruit has about 800 - 1 000 seeds attached to the interior wall of the ovarian cavity.
2.1.5. Floral biology

2.1.5.1. *Flower types and sexes*

Storey (1941) classified papaya flowers into five basic types:

**Type I:** Pistillate or female flower devoid of stamens, with a distinct ovoid ovary terminating in a five-lobed stigma (*Fig. 2.1a*)

**Type II:** Hermaphrodite (pentandria) flower having five functional stamens and a globose five-furrowed ovary (*Fig. 2.1b*)

**Type III:** Hermaphrodite (carpelloid) flower having six to nine functional stamens and an irregularly-ridged ovary (*Fig. 2.1c*)

**Type IV:** Hermaphrodite (elongata) flower having ten functional stamens and an elongate, smooth ovary (*Fig. 2.1d*)

**Type IV+:** Hermaphrodite (barren) flower having ten functional stamens but the pistil aborts, becomes vestigial and lacks a stigma (*Fig. 2.1e*)

**Type V:** Staminate flower having ten functional stamens only. The ovary is completely absent (*Fig 2.1f*)

Although five basic floral types are listed, certain male and hermaphrodite trees undergo sex reversal and morphological changes in various degrees under the influence of climatic and environmental changes (Storey, 1958).

2.1.5.2. *Derivation of floral types*

The evolution and derivation of the pistillate (Type I) and staminate (Type V) flowers started basically from a common ancestor i.e. the elongata hermaphrodite flower (Type IV) (Storey, 1969b). From the elongata flower, two phylogenetic lines diverged intraspecifically, each terminating with the derivation of a unisexual form.
Figure 2.1. Flower types of *Carica papaya* L.
The staminate flower was derived along classical lines i.e. with the phylogenetic loss of the gynoeccium without appreciable disturbance to other floral organs. Transitional forms of hermaphrodite flowers leading to complete maleness showed reduction in ovary size, numbers of stigmatic rays, dorsal vascular bundles, carpels and placenta (Nakasone and Lamoureux, 1982).

Derivation of the pistillate flower, on the other hand, represents a departure from the classical theory. It arose not through the loss of stamens from the hermaphrodite (elongata) type but by the incorporation or fusion of the stamens to the ovary tissues. The sequence leading to the derivation of the pistillate flower begins in the upper whorl of five stamens of the elongata flower fusing with the ovary leading to the formation of the intermediate pentandria (Type II) flower. This is the start of the final steps to the formation of the pistillate flower. After further fusion of the remaining five stamens to the ovary is completed, the pistillate flower is the result. The process of fusion of the stamens to the ovary is called carpellody of stamens. Between the elongata flower and the pistillate flower, many intermediate, carpelloid forms therefore exist, depending on the number of stamens that are fused. Such flowers develop into misshappen or 'cat-faced' fruits which are not marketable.

With regard the female or pistillate flower, its morphological structure is strongly fixed genetically in the female tree. Therefore, unlike hermaphrodite trees where sex reversal is commonplace, the pistillate tree is virtually unknown in undergoing any change of sex (Storey, 1969b).

2.1.5.3. Genetics of sex expression

Sex of papaya is determined by monogenic inheritance involving three alleles (Hofmeyr, 1938). The alleles are M for male, M\textsuperscript{H} for hermaphrodite and m for female. All homozygous dominants i.e. MM, MM\textsuperscript{H} and M\textsuperscript{H}M\textsuperscript{H} are lethal to the zygotes. Therefore male genotypes (Mm) and hermaphrodite (M\textsuperscript{H}m) are enforced heterozygotes while the female genotype (mm) is a double recessive.

Table 2.1 shows the sex segregation obtained from eight possible combinations (after Storey, 1969a). For gynodioecious varieties such as Sunrise Solo and Eksotika, it is desirable to have a high proportion of hermaphrodite trees in the orchard because the pyriform hermaphrodite fruits fetch higher prices. In this case, selfing hermaphrodite flowers or hybridisation of hermaphrodite flowers with hermaphrodite pollen should be used in the
production of seeds. Seeds derived from these cross combinations will have twice the number of hermaphrodites compared with females.

Table 2.1. Pollination combinations and sex segregation in papaya

<table>
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<tr>
<th>Pollination</th>
<th>Segregation ratios</th>
<th>non-viable zygotes</th>
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<tbody>
<tr>
<td></td>
<td>Φ (mm)</td>
<td>Ô (M^Hm)</td>
</tr>
<tr>
<td>1. Φ x σ</td>
<td>(mm x Mm)</td>
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<tr>
<td>2. Φ x Ô</td>
<td>(mm x M^Hm)</td>
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<tr>
<td>3. σ selfed</td>
<td>(Mm ⊙)</td>
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<td>4. σ x σ</td>
<td>(Mm x Mm)</td>
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<td>5. Ô selfed</td>
<td>(M^Hm ⊙)</td>
<td>1</td>
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<tr>
<td>6. Ô x Ô</td>
<td>(M^Hm x M^Hm)</td>
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<tr>
<td>7. Ô x σ</td>
<td>(M^Hm x Mm)</td>
<td>1</td>
</tr>
<tr>
<td>8. σ x Ô</td>
<td>(Mm x M^Hm)</td>
<td>1</td>
</tr>
</tbody>
</table>

Φ = female  
Ô = hermaphrodite  
σ = male

For dioecious varieties, the preferred combination for seed production is to use pollen from male flowers for crossing to female flowers. A ratio of 1:1 male to female will be obtained. This high proportion of males in the seed far exceeds the amount that is required for pollination. Normally for dioecious varieties, a higher density is planted in the field with subsequent culling of male trees to about 10% when the sexes can be identified (Agnew, 1968).

It is possible to self or cross males (combinations 3 and 4) only if there is reversal of sex from staminate flower to a form that has a functional ovary. This reverted, bisexual 'males'
have been used for development of inbred lines in breeding of dioecious papayas (Aquilizan, 1987).

Crosses between female and hermaphrodites may be used for hybrid seed production because it obviates emasculation when female flowers are used as the maternal parent. The disadvantage of course, is the high proportion of females in the seeds. With regards to the other crosses i.e. male with hermaphrodite flowers, these produce variable, trioeocious populations and are not commonly used either for commercial seed production or in breeding.

2.1.5.4. *Relationship of sex with fruit shape*

Fruit shape in papaya is a sex-linked character. The female or Type I flower (*Plate 2.1*) has a globose ovary which develops into round or ovoid fruits (*Plate 2.2*). In contrast, the elongata or Type IV hermaphrodite flower has a slender, tapering ovary (*Plate 2.1*) and this subsequently develops into a fruit which is elongated and cylindrical or pyriform in shape, depending on the variety of papaya (*Plate 2.2*).

In some countries, notably Australia, the female fruits from dioecious varieties are preferred. One of the advantages of dioecious varieties is that no hermaphrodite trees are present, hence the shape of fruits from these orchards are round and consistently so. However, in the majority of papaya growing countries like Hawaii, Brazil, India and the south east Asian countries including Malaysia, the gynodioecious varieties are more popular. The obvious advantage of these varieties is that, without the presence of males, all the trees are productive. The disadvantage, however, is that there is variability in fruit shape arising from the production of elongated or pyriform fruits from hermaphrodite and round fruits from female trees. Further, even within hermaphrodite trees, variation in fruit shape and appearance will arise because of carpellocy of stamens. Under certain climatic and environment influences, the stamens are fused to the ovary wall resulting in unsightly scars and misshappen fruits.

In Malaysia, the hermaphrodite, pyriform fruit of the Eksotika variety fetches a premium price which is more than twice that of the round female fruit, although there was no difference in the eating quality and total soluble solids % of fruits between the two sexes (Chan, 1986). This is because only hermaphrodite fruits have a demand in the lucrative export markets and female fruits are treated as culls suited only for the domestic market. The inability of farmers to weed out the undesirable females from seed stocks, inspite of controlled pollination with hermaphrodite flowers, is indeed frustrating. One of the ways practised by
Plate 2.1. Female (left) and hermaphrodite (right) flowers

Plate 2.2. Female (left) and hermaphrodite (right) fruits
growers in Hawaii and Malaysia to increase hermaphrodite population is to carry out multiple planting of seedlings at each point. When the sex becomes known, thinning of females can be carried out. Usually two to three seedlings are planted at each point and after culling, the orchard would have 80% or more hermaphrodite trees (Chan et al., 1991). There is also good potential in using vegetative propagation and tissue culture to obtain 100% hermaphrodite stands (Nathan and Tan, 1989; Cheah et al., 1993) (see section 2.1.6.3).

2.1.5.5. Environmental influences on sex expression

The basic sex types in papaya are genetically determined. The pistillate or female is phenotypically very stable. However, certain male and hermaphrodite trees have been known to undergo sex reversal under the influence of various environmental changes (Storey, 1958).

(i) Temperature

Cool temperatures like those experienced during the winter months in Hawaii appear to promote more femaleness in hermaphrodite trees. The flowers revert from a Type IV hermaphrodite which has ten stamens to a Type III or carpelloid flower having a varying number of six to nine stamens and Type II (pentandria) having five stamens. This reduction in the number of stamens, as described previously, is brought about by fusion of the stamens to the ovary. At high elevations, e.g. cooler temperatures, the Solo variety have a greater number of Type II (pentandria) and III (carpelloid) fruits (Awada, 1958; Lange, 1961). The results also indicated that warm temperatures tend to promote production of Type IV+ (barren) hermaphrodite flowers resulting in sterility of the trees. When such conditions persist over a length of time, a production gap (sterility skip) along the trunk is clearly visible. Allan et al. (1987) in a study on environmental effects on clonal female and male papaya plants, also supported the view that cool temperatures favoured femaleness. They found that cool night temperatures of about 12°C and short daylengths appeared to be critical in causing sex reversal from the sterile staminate to fertile, elongata type hermaphrodite flowers.

(ii) Moisture

Hermaphrodite trees grown under high tension (low soil moisture) regime tend to produce more sterile Type IV+ flowers (Awada, 1961). On the other hand, consistently high moisture levels will promote the production of hermaphrodite flowers with reduced stamen
number i.e. Type II (pentandria) or Type III (carpelloid). In other words, high moisture regime promotes femaleness in papaya. Lange (1961) concluded that under moisture stress conditions, more sterile flowers are produced in hermaphrodites while vigorous growing trees produced a greater percentage of carpelloid flowers. It seems likely that sterile IV+ flowers and carpelloid (Type II or III) flowers are promoted by poor and good vigour of the plant respectively. Moisture levels probably affect the well-being and vigour of the trees and indirectly bring about the reversal in sex.

(iii) Nitrogen

Application of nitrogen increased the vigour of the tree and promoted greater tendency towards femaleness (carpelloid fruits) in hermaphrodite trees (Awada and Ikeda, 1957). Increased nitrogen application from 0.1 to 2 lb/tree at every six-week interval, brought about a 58% increase in fruit culls arising mainly from carpelloid fruits (Awada et al., 1979). Ghosh and Sen (1975) reported that they were able to control the expression of sex by manipulation of nitrogen levels applied to the plants. At levels greater than 30 g/plant, most of the flowers produced were female.

(iv) Growth regulators

Ethylene, which promotes femaleness in monoecious cucurbits, was also reported to have similar regulatory action in the sex expression of papaya (Ranvir and Sharma, 1976). Ethephon-treated papaya seedlings of a dioecious cultivar exhibited a significantly higher percentage of female trees at maturation. The proportion of females did not increase with an increase of concentration from 100 - 300 ppm, but increased when the number of applications were increased. The percentage of female trees was over 90% when applications were continuously given at 15-day or 30-day intervals until emergence of flowers.

2.1.6. Propagation

2.1.6.1. Seed

Papaya is almost entirely propagated from seeds in commercial cultivation. The seeds are non-recalcitrant and can be dried to moisture levels of 9 - 12% for long term storage (Teng and Hor, 1976).
Seeds harvested fresh from fruits have very low and variable germination. This is because the sarcotesta (Arumugum and Shanmugavelu, 1975) and the seed itself (Yahiro and Hayashi, 1982), contain growth inhibitors which prevent wasteful germination while the seeds are still in the fruit. Certain treatments must be done to the seeds prior to storage or planting, so that they can store longer, as well as germinate with good viability and uniformity. Removal of the sarcotesta promoted germination considerably even in fresh, undried seeds but germination was further enhanced by seed drying and cool temperature storage at 15°C (Yahiro, 1979). The requirement of cool temperature to break dormancy in papaya is similar to vernalisation for temperate seeds although the temperature for vernalisation is lower (5°C - 10°C). Yahiro and Hayashi (1982) reported that storage of papaya for 30 - 50 days under 15°C greatly reduced the activity of growth inhibitors in the seed, resulting in improved rate and uniformity of germination.

In the tropics, the practical methods for drying of papaya seeds are either under the sun or air-drying in the shade. Chacko and Singh (1971), using the Washington variety, reported that there was no difference in germination using either methods. Chan and Tan (1990) however, found significant interaction between four genotypes and drying treatments (sun or shade and with or without sarcotesta) for seed germination. Sun or shade drying, with or without sarcotesta gave good germination for three of the varieties, but for Eksotika, sun drying without removal of the sarcotesta gave very poor results. Papaya seeds without sarcotesta, well-dried and stored at a temperature of 5°C can retain good germination of 60 - 70% even after five years of storage (Chan, 1991a).

The seeding rate for papaya is very low. This is because dry papaya seeds are relatively light, weighing about 14.5 g per 1 000 seeds. Further, the density of the crop at 2 000 trees/ha is relatively low compared with cereals and horticultural crops like vegetables. For establishing a hectare of papaya, about 3 000 seeds or only 50 g are required. This would be increased to 75 - 100 g if multiple plantings per point is practised. This amount is negligible compared with seeding rates of carrots (17 kg/ha) and barley (56 kg/ha).

Sound seeds usually germinate after two weeks in the polybags and ready for transplanting at the 8 - 12 leaf stage after another six weeks. The cost of production for each seedling is estimated to be 35 sen (Chan et al., 1991) and this works out to be about 3.5% of the total production costs for papaya (estimated to be RM 10/tree over 2 years).
2.1.6.2. Vegetative propagation

Vegetative propagation is the norm in perpetuation of perennial fruit trees and many horticultural crops particularly ornamentals. The main advantage in propagation by vegetative means is that it allows fixation of the maternal genotype and faithfully reproduces it from one generation to another. Several methods of vegetative propagation are available for papaya. Although these are still not widely used in commercial plantings, there are good prospects and potential for their adoption and utilisation in the near future.

Allan (1964) was the first to report the success of propagating papaya by cuttings. Large, leafy, lateral shoots which developed after winter, were initially used as cuttings for rooting under intermittent mist. In subtropical countries, the cool winter checks growth and temporarily overcomes apical dominance, resulting in the proliferation of lateral shoots. Availability of cuttings became less season dependent when they were induced from vigorous one to two-year old trees by topping off the shoot terminus to remove the apical dominance. The method for induction and proliferation of suitable sized lateral shoots for cuttings were improved further with the application of cytokinin and gibberellic acid mixtures (Allan, 1993). The ideal size of cuttings would be 50 - 150 mm long and 8 - 12 mm diameter with 4-5 leaves. These are harvested, trimmed to leave 3-4 small leaves and treated with fungicide and a basal dip in IBA to encourage rooting before they are planted in the intermittent mist beds with bottom temperature of 30° C. The cuttings will root in about three weeks.

Papaya can also be propagated by grafting. Airi et al. (1986) successfully cleft-grafted scion shoots from cultivars Co-1 and Honey Dew onto uniformly established seedlings. Patch and T-budding can also be used, but the success rate was poorer than cleft-grafting. In Malaysia, some papaya growers have used field grafting to replace female trees of the Eksotika cultivar in the orchard (Cheah et al., 1993). As soon as the sex of the trees can be determined, the female trees are side-cleft grafted with scion shoots (basal diameter 2-3 cm) harvested from hermaphrodite Eksotika trees. When the union is established in about 2-3 weeks, the female tree is cut back to about 60 cm from the ground. The precocity in bearing and yield of these in-field grafted hermaphrodites were not significantly different from seed propagated trees. This practice is economically justifiable because of the much better price paid for hermaphrodite Eksotika fruits (Cheah et al., 1993).
2.1.6.3. In vitro propagation

The early successes in in vitro propagation of papaya were reported by Mehdi and Hogan (1976) and Yie and Liaw (1977). However, they used seedling tissues as primary explants and as such, their findings have limited application because the sex of the plants, which is the major source of variation, was not established. Later, Litz and Conover (1978), reported successful regeneration of papaya plantlets by culturing apices of mature, field-grown papaya plants in modified Murashige and Skoog media. This success stimulated more in vitro research on papaya because of the prospects for mass propagation and greater uniformity of the crop.

Reports on field performance of in vitro propagated papayas have been encouraging (Pandey and Singh, 1988; Drew, 1988; Nathan and Tan, 1989). The most important is that in vitro plantlets propagate true to sex, i.e. when tissues from hermaphrodite trees are cultured, the subsequent plantlets will all be hermaphrodite. Therefore, the problem of sex segregation and variation of fruit shape which seed propagation faces, does not arise in this case. The other benefits of in vitro propagated trees are that they are more precocious (bear earlier and lower to the ground) and vigorous (Drew, 1988; Nathan and Tan, 1989) and higher yielding (Pandey and Singh, 1988).

In spite of all the clear advantages, the use of in vitro propagated papaya in commercial plantings appears to be an exception rather than the rule. The most likely reason may be related to economics. Demand for such planting materials may not be high enough to justify the economy of scale for the large capital investment. Under limited demand, some tissue culture laboratories in Malaysia have been known to sell in vitro propagated plantlets for RM 1.50 to RM 2.00 each (Girlie Wong, pers. comm., A.A.R., Kuala Lumpur). This is about five to six times the price of raising a plant from seed.

Besides the prospects of using in vitro as a method for rapid mass propagation of papaya, it has also been used in embryo rescue to obtain plants from otherwise incompatible interspecific crosses with C. papaya L. (Manshardt and Wenslaff, 1989b), rapid disease resistance screening (Sharma and Skidmore, 1988), anther culture for generating haploid papaya lines (Tsay and Su, 1985) and Agrobacterium-mediated gene transfer in papaya (Pang and Sanford, 1988) - particularly useful in transferring resistance of papaya ringspot virus disease.
2.1.7. Varieties

Storey (1969a) reported that the only *bona fide* varieties of papaya in existence were Solo and Bush of Hawaii and Hortus Gold of South Africa. Since then, however, numerous distinct, true-breeding varieties have been developed from many parts of the world, some from well-planned breeding programmes and others from judicious selection efforts by growers. These varieties can be self-pollinated, in which case they are purelines, or cross-pollinated. In general, gynodioecious varieties (having hermaphrodite and female trees) are self or cross-pollinated, while the dioecious varieties (having male and female trees) are enforced cross-pollinators.

2.1.7.1. Self-pollinated

The best known variety of papaya in the world today is the Solo. Within this variety are many lines such as Line 5, Line 8, Line 10, Kapoho, Waimanalo and Sunrise (Yee et al., 1974). Kapoho is the major cultivar in Hawaii while Sunrise which is the only pink-flesh line, is widely grown in many parts of the world. Sunrise Solo was used as a recurrent parent in the development of the Eksotika (Chan, 1987). This variety has similar features as Sunrise Solo except for the larger fruit size. It is a very popular variety for export and local markets in Malaysia. The Solo and the Eksotika are self-pollinated varieties (purelines) because the hermaphrodite flower is cleistogamous i.e. the anthers dehisce and release pollen for self-fertilisation before anthesis of the flower (Chan, 1980). Some cultivars such as Subang and Sitiawan were originally cross-pollinated, but after many generations of selfing and selection, have become purelines. Reliable seeds of these varieties can be easily produced by bagging unopened hermaphrodite flowers to prevent cross-pollination.

2.1.7.2. Cross-pollinated

Dioecious varieties such as Hortus Gold, Sunnybank, Cariflora and Washington which have male and female flowers on separate trees, are enforced cross-pollinators. There are also gynodioecious varieties such as Khack Dam, Maradol, Cibinong and Coorg Honey which are cross-pollinating. This is evident because selfing of hermaphrodite flowers of these varieties produces seeds with great difficulty. Seeds of these open-pollinated varieties can be produced by pollinating random hermaphrodite or female flowers with bulked pollen collected from the hermaphrodite or male flowers of the same variety. For maintenance of the gene pool of cross-
pollinated varieties, a large number of random individuals should be involved in the seed production so that genetic drift can be avoided.

Cross-pollinated varieties require pollinating agents such as wind for dioecious varieties and insect pollinators for gynodioecious varieties for good fruit production. For dioecious varieties, usually 10% of the population is kept as males to provide pollen for pollination.

2.1.7.3. Hybrids

F₁ hybrid varieties of papaya are rare, although there have been several reports on the heterosis and improved yields in cross combinations between different varieties of papaya (Subramanyam and Iyer, 1984; Aquilizan, 1987; Chan, 1992). Agnew (1968) described a vigorous F₁ hybrid derived from Bettina 100A and Petersen 170 that was important in Queensland. An important hybrid developed in Taiwan that has resistance to papaya ringspot virus disease is Tainung no. 5 (Lin et al., 1989). This was derived from a cross between Florida (FL-77-5) and the Costa Rica Red.

In Malaysia, a recent F₁ hybrid called Eksotika II was developed from hybridisation of Line 19 and Eksotika (formerly Line 20) (Chan, 1993a). The new hybrid has similar features as Eksotika, but the yield is 14 - 33% higher and the fruit cosmetics are improved.

Hybrid papaya varieties appear to show better adaptability, vigour and yield performance over traditional cultivars and they are expected to have a place in the seed catalogues in the near future.

2.1.7.4. Clonal varieties

Honey Gold is perhaps the only known papaya clone in the world today. It is a dioecious variety from South Africa which was selected and propagated from generation to generation by leafy cuttings for over 30 years (Allan, 1993). Clonal varieties have the advantage of greater uniformity, especially in fruit shape which is sex-linked. Clonally propagated Honey Gold gives fairly high yields of 25 - 30 tons/ha year under subtropical conditions and have been known to remain productive for 10 or more.

Carica pentagona or Babacos, which is grown to a small extent in Ecuador and New Zealand is also exclusively clonally propagated by cuttings because of its parthenocarpic fruits (Little, 1982).
2.2. HETEROESIS

2.2.1. Definition and computation

The word 'heterosis' was first coined by Shull in 1911 to mean the increase in size, yield and vigour (luxuriance in growth) of an organism arising from bringing together of unlike gametes to form a hybrid. The expressions of heterosis later included earliness in maturation, and survival fitness such as adaptive, selective and reproductive advantages (Mac Key, 1976).

The measurement of the relative superiority of the hybrids over a defined genotype or set of genotypes is the hybrid vigour or heterosis. There is considerable debate over what the 'defined genotype or set of genotypes' should be. There are generally three situations:

(i) comparison of the hybrid with its mid-parent (MP) value i.e.
\[
H = \left[ \frac{(F_1 - MP)}{MP} \right] \times 100 \quad \text{where } MP = \frac{(P_1 + P_2)}{2}
\]

(ii) comparison of the hybrid with its better parent (BP) value i.e.
\[
H = \left[ \frac{(F_1 - BP)}{BP} \right] \times 100
\]

(iii) comparison of the hybrid with the best contemporary cultivar (BC) i.e.
\[
H = \left[ \frac{(F_1 - BC)}{BC} \right] \times 100
\]

In the first case i.e. comparison with mid-parent values, the general consensus of opinion among breeders is that such estimates are not very meaningful in terms of genetic advancements because for hybrids to be truly superior, they should in fact be better than the better parent. In the case of open-pollinated crops, even comparison of hybrids with the better inbred parent also do not reflect satisfactory breeding progress. This is because many inbred lines of open-pollinated crops are so weakened by inbreeding depression that superiority over them is an empty victory and quite meaningless. In this situation, the worth of the hybrid should be compared with the best contemporary cultivar.

2.2.2. Genetic basis for heterosis

2.2.2.1. Non-allelic gene interaction

Non-allelic gene interaction that may contribute to heterosis include epistasis,
transgression and recombination. The heterosis in yield for many crops was due to favourable interactions of the primary components of yield (Grafius, 1959). In cereals, they are the number of spikes/unit area, number of seeds/spike and the weight of each seed. Williams (1959) explained that when two parents differed reciprocally for the interacting yield components i.e. if inbred A had low fruit number but large fruits and inbred B had high fruit numbers but small fruits, the F₁ levels compensate one another in such a way that their products are greater than the parents, thus expressing heterosis. This is supported by findings of some workers that widely divergent, phenotypically different inbred lines give maximum heterosis (Pearson, 1983).

2.2.2.2. Inter-allelic interaction: Dominant - overdominant theory

In the overdominant theory, heterozygosity per se is a prerequisite for heterosis. Alleles in heterozygote state may exercise a stimulatory, complementary or dosage-adjusting manner to result in higher superiority in performance of the hybrids (Mac Key, 1976). Berger (1976) reviewed heterosis at the gene product level and explained that overdominance heterosis of heterozygotes arises because of greater catalytic efficiency of complementary enzymes from heterozygotes. Griffing and Langridge (1963) experimenting with Arabidopsis thaliana, concluded that heterosis in heterozygotes resulted because of complementation between alleles with differentially temperature-sensitive products, thus making hybrids more adaptive or homeostatic over a wider range of environments. Since heterozygosity is a requirement in overdominance heterosis, such heterosis is said to be non-fixable in genotypes through sexual propagation.

In the dominant theory, which appears to have wider support, genotypes may be homozygotes or heterozygotes, but will still show the same heterotic expressions if the same complement of dominant genes are accumulated in the genome. The sheltering or inhibiting effects of dominant genes over the deleterious recessives prevent their expression of negative effects, thereby resulting in hybrid superiority. The dispersion of these genes of favourable expressions in the F₁ hybrids is the basis for heterosis (Jinks and Lawrence, 1983). Since heterozygosity per se is not a prerequisite for heterosis, such heterosis can be fixed in true breeding, homozygous dominant individuals. In practice, however, it is extremely difficult to get such individuals because of unfavourable gene linkages and further, gene effects may be quite small in phenotypic expression and identifying segregants which have all dominant genes
may be a near impossibility. There are, however, reports that certain homozygous inbred lines or pure lines have been isolated which performed as well as the best hybrids (Hayes and Foster, 1976).

2.2.2.3. Non-genomic heterosis

It is increasingly evident that heterosis is not a phenomenon regulated by the genome only. Mitochondria and chloroplast heterosis reflect to a great extent intergenomic interactions and are essential components of heterosis at the molecular level (Srivastava, 1983).

Mitochondria are cytoplasmic organelles that provide energy in the form of ATP. Research has shown that the oxidase activity of mixtures of mitochondria from different inbred lines of maize exceeded that of the lines considered separately. This heterotic effect is also called mitochondrial complementation (McDaniel and Sarkissian, 1966). Mitochondria are also important in maintaining intercellular homeostasis (Sarkissian and Srivastava, 1969) and thus provide an explanation on the stability of hybrids when exposed to environmental stress and disturbance.

2.2.3. Exploiting heterosis in crops

2.2.3.1. Crop range in heterosis breeding

Heterosis breeding had been very active since the turn of the century. Table 2.2, adapted from Mayo (1987), shows the range of crops in which hybrid varieties have been successfully developed. The first commercial hybrid released was for field corn in 1921. From that year until 1955, the range had extended very quickly to include many types of vegetables notably eggplant, tomato, onion, pepper and cabbage (Table 2.2). In the era from 1955 - 1974, heterosis breeding on cereals was evident and resulted in the development of hybrids for sorghum, millet, barley, wheat and rice. After 1975, hybrid oats, rye, soyabean and potato, to name a few, have made the appearance. Besides this list which covered mainly vegetables and cereals, hybrid ornamentals (Begonia) have been known to be commercially produced even before the advent of hybrid corn (Reimann-Philipp, 1983). Hybrid cultivars also existed for fodder grasses and pasture crops (Kobabe, 1983).

In retrospect, heterosis breeding was so significant for agricultural crops that there is hardly any crops which had not included development of hybrids in their breeding programme at one time or another. An exception, perhaps is for fruit crops which, in the majority of cases,
are vegetatively propagated. Even in such cases, hybridisation had been used in development of vigorous, high yielding clones. In pineapple for example, commercial cultivars were crossed and desirable selections were cloned from the segregating F₁ population (Chan, 1993c).

Table 2.2. Range of food crops and year of release of first commercial hybrids (after Mayo, 1987)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Year</th>
<th>1955 - 74</th>
<th>Year</th>
<th>Crop</th>
<th>1975 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field corn</td>
<td>1921</td>
<td>Sorghum</td>
<td>1955</td>
<td>Asparagus</td>
<td>1975</td>
</tr>
<tr>
<td>Sweet corn</td>
<td>1933</td>
<td>Sugar beet</td>
<td>1957</td>
<td>Celery</td>
<td>1975</td>
</tr>
<tr>
<td>Eggplant</td>
<td>1939</td>
<td>Broccoli</td>
<td>1961</td>
<td>Oats</td>
<td>1980</td>
</tr>
<tr>
<td>Summer squash</td>
<td>1941</td>
<td>Spinach</td>
<td>1961</td>
<td>Rye</td>
<td>1980</td>
</tr>
<tr>
<td>Tomato</td>
<td>1943</td>
<td>Beetroot</td>
<td>1961</td>
<td>Potato</td>
<td>1980</td>
</tr>
<tr>
<td>Slicing cucumber</td>
<td>1945</td>
<td>Brussels sprouts</td>
<td>1963</td>
<td>Soyabean</td>
<td>1980</td>
</tr>
<tr>
<td>Onion</td>
<td>1948</td>
<td>Carrot</td>
<td>1964</td>
<td>Haricot bean</td>
<td>1985</td>
</tr>
<tr>
<td>Watermelon</td>
<td>1949</td>
<td>Pearl millet</td>
<td>1965</td>
<td>Field bean</td>
<td>1985</td>
</tr>
<tr>
<td>Winter squash</td>
<td>1950</td>
<td>Coconut</td>
<td>1965</td>
<td>Peas</td>
<td>1985</td>
</tr>
<tr>
<td>Pepper</td>
<td>1954</td>
<td>Cauliflower</td>
<td>1966</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muskmelon</td>
<td>1954</td>
<td>Lucerne</td>
<td>1968</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickling cucumber</td>
<td>1954</td>
<td>Barley</td>
<td>1968</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td>1954</td>
<td>Wheat</td>
<td>1969</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice</td>
<td>1972</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sunflower</td>
<td>1972</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2.3.2. Expressions of heterosis

There are numerous reports on heterosis on yield for a wide range of agricultural crops. Many of these were compared with the performance of the better parent or with the established cultivars. The majority of the estimates indicated positive heterosis for yields e.g. 26% - 192% for onions (Jones and Davis, 1944), 35% - 40% for cucumber (Ells and McSay 1981), 10 - 15% for wheat (Wilson and Driscoll, 1983) and 2% - 35% for tomato (Valicek and Obeidat, 1987). While yield is undoubtedly the most used parameter in the measurement of heterosis in
many crops, it may not be applicable as a yardstick for some horticultural crops such as lettuce and pickle cucumber. These have to be picked at the immature stage and although gross yield would increase with later maturation, the produce quality would by then be unmarketable.

Plant vigour and earliness to maturation are other plant variables which often express heterosis. In the case of earliness, negative values of heterosis would of course be desirable. Earliness in maturation of hybrid tomatoes was reported to be the major reason for the superiority in overall yield compared with the inbred parents (Yordanov, 1983). Most of the difference in yield between hybrids and inbreds was accounted for by the high yields of the first season harvest in hybrids. In cabbages, cucumber and muskmelon, earliness was much sought after because of the premium prices given for early produce in the market.

For cucumber as well as crops where the fruits are harvested at a immature stage, heterosis in fruit number is the most important consideration. Heterosis in fruit number for cucumber was reported by Ells and McSay (1981) to be 35% - 40%. In the pursuit for higher fruit number, selection of the gynoecious gene that produces mostly female flowers in cucumber was necessary. Pearson (1983) warned that because of the abundance of female flowers and the heavy fruit load, there may be twice the culling rate of malformed fruits in hybrids which may result in poorer marketable yields than inbreds.

Heterosis for fruit weight was reported by El-Maksoud et al. (1984) for okra (124% over mean parent) and for papaya (Subramanyam and Iyer, 1984) who reported heterosis over the better parent for eight of the ten hybrids. However, in general, heterosis for fruit size or weight appeared to be an exception rather than the rule.

The above characters of the plant and yield components (vigour, earliness, fruit number and yield) showed potential for improvement because of the supporting evidence regarding expression of heterosis for these characters. There are, however, certain characters in which heterosis seldom exist. Quality characters such as total soluble solids content, capsicin, Vitamin A and C, and protein contents were often reported to have intermediate values in hybrids. In chilli breeding, Nair et al. (1986) found no significant heterosis for Vitamin A and C as well as capsicin contents while Valicek and Obeidat (1987) found that total soluble solids in hybrid tomatoes were intermediate or inferior to the parents. Bequette and Fischer (1980) found that wheat hybrids were generally intermediate to parents in dough properties (gluten protein content) and baking quality. These characters, governed predominantly by additive genes, would not be greatly improved by heterosis breeding.
2.2.3.3. Environmental influences on heterosis

Under less favourable environments, F₁ hybrids appear to show much better performance compared with their pure line parents because they are individually better buffered (Allard and Bradshaw, 1964). This was supported by findings that greater heterosis was shown when trials were carried out under less favourable environments. In a trial over three locations for processing tomato, Conti et al. (1990) reported that heterosis was highest at the least favourable environment because of the hybrids' adaptability and capability to set fruits under adverse conditions. Yordanov (1983) also reported that heterosis in tomato was more pronounced under unfavourable conditions such as low fertility, insufficient illumination (autumn-winter crop under glasshouses) and extremes of temperature because of their wider adaptability to set fruits under such conditions. Narula (1984) tested hybrid wheat under three fertiliser regimes and found that heterosis was 23%, 13% and 11% under low, moderate and high fertiliser regimes respectively. Hybrids therefore, appear to be able to withstand stress better than inbreds and have greater justifications for cultivation under less suitable or marginal environments.

One of the greatest difficulty in testing of hybrids in cereal crops lies in the production of sufficient seeds for the trial. Quite often, the trials were conducted in spaced planted plots at low seeding rates. Results from such trials compared with those obtained from commercial plots with high seeding rates indicated that heterosis under these two regimes were not the same. Dhindsa and Anand (1973) found that the heterosis for grain yield of barley varied with the density of seeding, with the highest expression from plants grown at the widest spacing. However, Yap and Harvey (1971) obtained opposing results, concluding that relatively higher heterosis was obtained for grain yield in densely seeded plots compared with spaced planting. The higher yield of hybrids at high density planting was because of its tillering ability which produced more heads/unit compared with the parents.

Environment factors also appear to influence the quality and production of F₁ seeds which in turn affect expression of heterosis in hybrids. Yordanov (1983), studied the variation of heterosis expression from tomato seeds produced at four different altitudes. He found that the best quality seeds which expressed the highest heterosis came from 1100 m altitude which was characterised by low night temperature and strong day illumination. Seeds produced in the plains (150 m) can be improved in vigour by pollination with pollen gathered from plants at 1100 m. The variation in heterosis may be a result of the differences in seed size at the various
altitudes. Sage (1973), segregated wheat seeds of three F₁ hybrids into large, medium and small categories and tested them with the parents. He obtained apparent yield heterosis of 9%, 3% and -3% respectively. Therefore, environmental influences on seed production and vigour will indirectly affect hybrid performance and may give rise to spurious heterosis estimates.

2.2.4. Limitations to exploitation of heterosis

Inspite of considerable reports on increased yields, early maturation, better adaptability and uniformity of harvests gained by F₁ hybrids, the success in implementation of commercial scale plantings of hybrids is hardly convincing for some of the crops. A case in point is the hybrid onion. Dowker and Gordon (1983) concluded that a wealth of scientific information is available regarding heterosis in onions after five decades of research in this area. However, there is very little clear evidence regarding its agronomic utility and they seriously doubted its economic feasibility. The breeders in North America have, since 1944, placed emphasis on breeding of hybrid onion while their European counterparts have relied on improving open-pollinated varieties. Today, both programmes appear to have achieved similar results. The same appears to be true for hybrid wheat. Wilson and Driscoll (1983) noted that 'it is now 20 years since research into the development of hybrid wheat began, and commercial hybrids have neither shown the yield advantages anticipated, nor have hybrids been used on any significant area anywhere in the world'.

2.2.4.1. Problems in hybrid seed production

Production of F₁ seeds requires that the seed-bearing parent be devoid of pollen to prevent production of self-pollinated seeds that would otherwise contaminate the purity of the hybrid stock. This can be achieved by hand emasculation and use of gametocides (chemicals that would render pollen non-viable), but by and large, the most successful would be to use male sterility either in the cytoplasmic or gametic form. Indeed, for many of the agricultural crops particularly the cereals, heterosis breeding gathered momentum only with the advent of male sterility lines. Without male sterility lines as seed-carriers, there will be no potential for commercial utilisation of the hybrids.

The classical example for cytoplasmic male sterility (CMS) and its inheritance was described for onion by Jones and Clarke (1943). Male sterility was governed by a combination of the cytoplasmic factor (S) together with a recessive nuclear gene in its homozygous form
Cytoplasmic male steriles have the constitution (S) msms and they are used as the seed parents for hybridisation with the pollen parent which has (N) MSMS constitution (normal pollen fertility). The CMS lines are reproduced by pollination with 'maintainer' lines with constitution of (N) msms. When CMS line [(S) msms] is crossed with pollen of the 'maintainer' line [(N) msms], the resultant progenies are all [(S) msms] because the normal cytoplasmic fertility factor (N) cannot be transmitted by the pollen. Male sterility genes and CMS lines are used in hybrid seed production for many cereals like barley, wheat, sorghum and rice and in vegetable crops like carrots, tomato and pepper.

Production of hybrid seeds using various male sterility systems in producing the maintainer and CMS lines as well as the inbred parents, will incur additional costs. In tomatoes, chili and eggplant, hand-emasculcation for production of hybrid seeds is still widely practised, and preferred over the use of existing male sterility genes. For these crops which produce fairly large seed number per pollination, it may still be economical to emasculate by hand although labour cost was estimated to be 40% of the total production costs (Yordanov, 1983). Hand emasculation is a safer method to ensure high seed purity because CMS lines are sometimes not stable and have been reported to revert to fertile males in chili (Shifiss and Guri, 1979) and field beans (Bond, 1989). In onions and carrots, the male sterile gene (ms) was reported to be sensitive to temperature and reverts to fertile state at 23°C (Dorsman, 1976).

When crops require very high seeding rates for example carrot (17 kg seed/ha) and barley (56 kg/ha), the seed cost will form a substantial part of the production costs. For carrot, the hybrid seed cost was estimated to be 7% of the production cost and this may be prohibitive for growers to use (Dorsman, 1976). One of the ways to reduce hybrid seed cost is to increase the seed yield. Several methods have been suggested to increase seed set in inbred parents. They are:

1. Using double cross or 3-way cross so that seeds are produced on the high yielding single cross hybrid. The first to use double cross hybrids was Jones (1918) for the production of hybrid maize.

2. Choosing the direction of cross so that the maternal parent is a more efficient seed carrier. In production of hybrids between Capsicum annuum var fasciculatum (pungent chilli) and C. annuum var grossum (bell pepper), it was found that the latter was a more efficient seed-carrier (Anand and Deshpande, 1985).
(3). Cold treatment of vegetative propagules. In onions, when the mother bulbs were stored at 14°C for 12 weeks and at 2°C for another 12 weeks, higher seed yields will be produced (Hesse et al., 1977).

(4). Effective pollination is very important for seed set for male sterile lines. In onions, a ratio of one row of pollen parent alternating with four rows of male sterile parents is adequate for pollination (Dowker and Gordon, 1983). However bees collecting both nectar and pollen preferred male fertile to male sterile plants (Williams and Free, 1974), but nevertheless, moved around sufficiently to effect pollination.

2.2.4.2. Inbreeding depression

Inbreeding depression, as opposed to hybrid vigour, is the decline in general well-being of the variety (loss of vigour, fertility etc.) when the variety is selfed. It influences not only the amount of seed produced by the inbred parent and hence the seed price, but also the reproducibility of the hybrid. It may restrict the number of inbreeding generations in the development of inbred lines, thus acting as a barrier to optimal uniformity of the hybrid. Cole crops can withstand inbreeding for five generations or more, but carrots showed severe depression after three generations of selfing. For some crops e.g. leek, hybrid programmes may not be suitable because of extremely severe loss of vigour after only two inbreeding generations (Dorsman, 1976). In such situations, the problem may be by-passed with the production of homozygotes through haploid breeding and anther culture. In papaya, however, inbreeding depression may not be a problem (Hamilton, 1954).

2.2.4.3. Undesirable characters accompanying yield heterosis

In the pursuit of high heterosis in yield, sometimes correlated undesirable responses may accompany the high yield, resulting in fact, in decline in economic yield. An example of high culm rates that accompanied heterosis for high fruit number in cucumber (Pearson, 1983), was discussed earlier. In barley, Khalifa (1973) obtained an average heterosis for grain yield of 26.7% over the mid-parent value. However, heterosis for vegetative vigour i.e. straw production, was higher at 37.9% because of increase in both straw length (17.2%) and number of culms (12.0%). This increase in length and quantity of straw in F₁ hybrids may be counter productive if it leads to higher yield losses due to lodging.
It was generally agreed that greater heterosis for yield may be obtained when more divergent inbreds were used for hybridisation. Williams (1959) stated that yield heterosis in tomato will arise if two parents differed reciprocally for interacting components i.e. fruit weight and fruit number. In the process of hybridising two genotypically very different inbreds, some of the characters related to quality such as total soluble solids and vitamin contents which are governed by additive genes, may be expressed as intermediate values in the hybrids. In so far as fruits and some horticultural crops are concerned, the decline in these fruit qualities in hybrids cannot be sufficiently compensated by the benefits of higher yields. Preference may still be given to the better quality, albeit lower yielding inbred parent.

2.2.5. Economic justifications for F₁ hybrids

The added costs incurred in production of F₁ hybrid seed and hence much higher seed price, is the primary reason for the reluctance of growers to use hybrids. However, there is still economic justifications for growing F₁ hybrids if their margin of returns over conventional varieties are higher than the added costs involved in their production.

In the case of carrots and barley, which have very high seeding rate of 17 kg/ha and 56 kg/ha respectively, the seed cost forms a substantial part of the production costs. Hayes and Foster (1976) estimated that 20%-25% increase in yield of barley hybrids over conventional self-pollinated varieties must be obtained before it is economically justified to use hybrids. In the case of onions, a 30% higher yield return would certainly offset the added seed cost, but a 10% higher return would probably be marginal and use of hybrid onions may need a review (Dowker and Gordon, 1983). For field beans, *Vicia faba*, Bond (1989) estimated that a mere 10% higher yield in hybrids would be enough to pay for the seed cost, although the seeding rate of 150 kg - 250 kg was very high because of the large seed size. For papaya, the seeding rate was extremely low, from 50 g to 100 g/ha (Chan, 1994) in which case, seed cost may incur only a fraction of the total production costs.

Another economic justification, not for the consideration of the growers, but for the seed producers, is the propriety and monopoly in seed production that seed agencies enjoy with hybrid varieties. The seeds are produced by cross pollination of inbred lines whose identities and propagation are maintained under strict security.
This model is concerned with the characterisation of each genotype in terms of a mean effect and a coefficient ($\beta_1$) describing its rate of change of expression with changing environment.

2.3.1.1. *Types of GE interaction*

The occurrence of GE can also be detected in graphical presentation. In the case of two genotypes tested over two environments, three situations may arise:

(i) the two regression lines are parallel in which case there is no interaction

(ii) the two lines are not parallel, but without intersection with each other. There are no changes in ranking of genotypes over the two environments. This is known as change-in-rate interaction or quantitative interaction (Peto, 1982).

(iii) the two lines are not parallel, and they intersect at some point. This is known as cross-over interaction and it results in a change in genotype ranking over the two environments. This is also known as qualitative interaction (Peto, 1982).

Cross-over interaction is the most difficult to deal with because the varieties appear to be specifically adapted to certain environments. Recommendation of varieties in this case cannot be generalised. Baker (1988), used a statistical test for GE to establish cross-over interaction or significance and tested the magnitude of the GE. He recommended that if there is no cross-over effects i.e. no significant change in ranking of genotypes over environments, then the GE component is deemed unimportant.

2.3.1.2. *Partitioning GE components*

The overall interaction can be elucidated from the combined ANOVA, and its significance can be tested against the pooled error in the random model below:

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>Expected mean squares</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>e-1</td>
<td>M1</td>
<td>$o^2 + r^2 + ge + go^2_{r(e)} + rgo^2_e$</td>
<td>M1+M5/M2+M4</td>
</tr>
<tr>
<td>Rep(enviro)</td>
<td>e(r-1)</td>
<td>M2</td>
<td>$o^2 + go^2_{r(e)}$</td>
<td>M2/M5</td>
</tr>
<tr>
<td>Genotype</td>
<td>g-1</td>
<td>M3</td>
<td>$o^2 + ro^2_{ge} + reo^2_{g}$</td>
<td>M3/M4</td>
</tr>
<tr>
<td>GxE</td>
<td>(g-1)(e-1)</td>
<td>M4</td>
<td>$o^2 + ro^2_{ge}$</td>
<td>M4/M5</td>
</tr>
<tr>
<td>Pooled error</td>
<td>e(g-1)(r-1)</td>
<td>M5</td>
<td>$o^2$</td>
<td></td>
</tr>
</tbody>
</table>
where $\sigma^2 = \text{error variance}$  
$\sigma^2_g = \text{genotype variance}$  
$\sigma^2_e = \text{environment variance}$  
$\sigma^2_{ge} = \text{genotype x environment variance}$  
$\sigma^2_{r(e)} = \text{replicate within environment variance}$

It is useful to partition the overall GE interaction into components for each of the genotypes in the test so that an evaluation of the behaviour and response of the genotypes over environments can be done. This partitioning of the interaction sum of squares was proposed by Wricke (1960). The component GE interaction ascribed to each genotype is known as the ecovalence ($W_i^2$). Ecovalence can be used as a measure of phenotypic stability (Weber and Wricke, 1990) and is computed as:

$$W_i^2 = \Sigma_j (\bar{y}_{ij} - \bar{Y}_i . - \bar{Y}_.j + \bar{Y}..)^2 = e_{ij}^2$$

Ecovalence depends strongly on the environments under tests. Different sets of environments for a similar set of genotype may yield different ecovalence estimates. Estimation of ecovalence of genotypes tested over seasons (years) instead of locations appear to be more reliable because season effects are less liable to be manipulated by the breeders (Lin and Binns, 1988a).

Small values of ecovalence would generally mean a small component of GE ascribed to the genotype. This means that the genotype is more stable and show less unpredictability in performance over a set of environments. It can also be described as 'stable', a term that goes hand in hand with GE studies.

2.3.2. Stability

Stability is an attendant terminology that appears in all GE studies because the degree of stability of a genotype is concomitant with the magnitude of its GE interaction. There are many interpretations of stability, but one that is simple and acceptable generally is that a stable genotype is one which shows very small GE interaction in the characters concerned, i.e. the genotype is consistent in performance regardless of the changes in a set of environments.

Allard and Bradshaw (1964) however, emphasised that stability does not imply constancy of phenotypes in varying environments. It only implies stability of economically important characters such as yield and quality. Thus stability of these characters may depend on holding some aspects of morphology and physiology in steady state while allowing others to
vary. Recommended stable varieties thus may show stability in economically important characters but may have large GE interaction in other traits.

2.3.2.1. *The basis for stability*

Bradshaw (1965) used the concept of 'phenotypic plasticity' to explain the differential responses of genotypes to different environments which can include spatial (over locations) or temporal (over seasons or years). A genotype that showed lower plasticity (least changes) appeared to be better adapted to environmental changes. Marshall and Jain (1968) added that phenotypic plasticity of a species was related to their genetic makeup and greater degree of heterozygosity exhibited less plasticity to environmental changes and therefore would appear more stable.

Allard and Bradshaw (1964) introduced the term 'buffering' to describe the ability of a variety which can adjust its phenotypic or genotypic state in response to transient fluctuations in environment. A 'well-buffered' variety is one whose adjustments can yield high and stable economic returns for a wide range of locations and over a number of years. The term 'buffering' is equivalent to 'homeostasis' used by Lewontin (1957).

Allard and Bradshaw (1964) further elaborated that stability or 'buffering' may be achieved in two ways: individual buffering or populational buffering.

(i) *Individual buffering*

This occurs when the individuals themselves may be well-buffered so that each member of the population is well adapted to a range of environments. This type of buffering applies to genetically homogeneous populations such as pure lines or F₁ hybrids. In general, the effectiveness of individual buffering appear to depend on the degree of heterozygosity of the individuals. F₁ hybrids appear to be better buffered than their pure line parents. This is especially more evident under less favourable environments, when heterozygotes show much better performance compared with homozygotes. This was demonstrated by Clausen and Hiesey (1958) with the work on the adaptation of *Potentilla glandulosa* at three different altitudes. The parental races cannot survive at all three diverse environments but the hybrids were found to be as vigorous as each parent in its optimal environment.
(ii) Populational buffering

This occurs when the population or variety is made up of a number of genotypes each adapted to a somewhat different range of environments. An obvious example of populational buffering is disease resistant multilines or multiclonal populations consisting of genotypes each having resistance to a different race or strain of the pathogen. In wheat, mixtures of several genotypes appear to have better stabilising effect and perhaps synergism that promote higher yields compared with combined yields of each component genotype. Although populational buffering may be effective in reducing GE interaction and therefore promote greater stability in performance, it may not be applicable for some horticultural crops such as fruits and vegetables where product standards are very specific and cannot be compromised.

2.3.2.2. Statistics for evaluating stability

Lin et al. (1986) reported that nine stability statistics were commonly used. They are:

1. The variance of a genotype across environments \( S_i^2 \) can be a measure of stability.

2. Coefficient of variability (CV). Francis and Kannenberg (1978) used the conventional CV of each genotype as a stability measure.

3. Plaisted and Peterson's (1959) mean variance component for pairwise GE interaction \( (\theta_{ij}) \). The mean of the estimated variance components of the GE interaction for all pairs of genotypes that include genotype \( i \) is the stability measure for genotype \( i \).

4. Plaisted's (1960) variance component for GE interaction \( (\theta_{ii}) \). One genotype \( i \) is deleted from the entire set of data and the GE interaction variance from this subset is the stability index for genotype \( i \).

5. Wricke's (1960) ecovalence \( (W_i^2) \). The GE interaction effects for genotype \( i \), squared and summed across all environments, is the stability measure for genotype \( i \).

6. Shukla's (1972) stability variance \( (s_i^2) \). Based on the residuals in a two-way classification, the variance of a genotype across environments is the stability measure.

7. Finlay and Wilkinson's (1963) regression coefficient \( (b_i) \). The observed values are regressed on environmental indices defined as the difference between the marginal mean of the environments and the overall mean. The regression coefficient for each genotype is then taken as its stability parameter.

8. Perkins and Jinks' (1968) deviation parameter \( (\beta_i) \). Similar to (7) except that the observed values are adjusted for location effects before the regression.
9. Eberhart and Russell's (1966) deviation parameter \((\delta_i^2)\). The residual mean square (MS) of deviation from the regression defined in (7) or (8) is the measure of stability for each genotype.

2.3.2.3. Concepts of stability

Lin et al. (1986) further classified these nine stability statistics into four basic groups depending on whether they are deviation from the average genotypic effect (DG) \((x_{ij} - x_i)\) or on the GE interaction \((x_{ij} - x_i - x_j + x..)\). The first two groups, A and B make use of sum of squares in estimation, while groups C and D used the regression coefficient and regression deviation respectively in the division of the group. From these four groups, three concepts of stability may be derived:

Type 1: A genotype is considered to be stable if its among-environment variance is small.

Type 2: A genotype is considered to be stable if its response to environments is parallel to the mean response of all genotypes in the trial.

Type 3: A genotype is considered to be stable if the residual MS from the regression model on the environmental index is small.

The four groups of stability estimates and their three types of stability are summarised as follows:

<table>
<thead>
<tr>
<th>Group A:</th>
<th>DG</th>
<th>SS</th>
<th>Type 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B:</td>
<td>GE</td>
<td>SS</td>
<td>Type 2</td>
</tr>
<tr>
<td>Group C:</td>
<td>DG/GE</td>
<td>Regression coefficient</td>
<td>Type 1 or Type 2</td>
</tr>
<tr>
<td>Group D:</td>
<td>DG/GE</td>
<td>Regression coefficient</td>
<td>Type 3</td>
</tr>
</tbody>
</table>

DG = based on deviation from average genotypic effect  
QE = based on deviation on the interaction term

The types of stability for Groups A, B and D are clearly defined as Type 1, Type 2 and Type 3 respectively, but for Group D, it may be interpreted as Type 1 or Type 2 depending on the definition of a stable genotype. If, as in Finlay and Wilkinson (1963), where the regression coefficient \(b_i = 1\) or as in Perkins and Jinks (1968) where \(\beta_i = 0\), then Type 2 is implied but when they are defined as \(b_i = 0\) or \(\beta_i = -1\), then Type 1 is implied.
Implications in using the 3 types of stability

Type 1:

Genotypes with Type 1 stability show very small GE interaction and when their means are regressed against the environment mean, they show an almost horizontal line with the x axis, indicating minimal changes in their mean when moved from one environment to another. The genotype is said to be homeostatic (Lewontin, 1957), well-buffered (Allard and Bradshaw, 1964) or static (Kang, 1990) in stability. This is the biological concept of stability (Becker, 1981) and has little use for the plant breeder. This is so because genotypes with Type 1 stability are also very poor performers and do not respond to favourable changes to the environment. This was demonstrated by Finlay and Wilkinson (1963) who plotted the regression coefficients against their mean yields. The best yielders were centred around those whose regression coefficients $b_i = 1$ and in cases where $b_i = 0$, poor yielders were invariably found.

The advantage of Type 1 stability is that it has a broad inferential base because its stability definition does not depend on the other genotypes in the test and it is therefore unambiguous (Lin et al., 1986).

Type 2:

Type 2 stability is referred to as the agronomic concept as opposed to the biological concept in Type 1 (Becker, 1981). It is also analogous to Kang's (1990) 'dynamic' concept of stability. It is related to the agronomic concept of stability because Type 2 genotypes respond readily to favourable changes in environment (e.g. improved agronomic management practices). Their regression coefficients $b_i = 1$ (Finlay and Wilkinson, 1963) or $\beta_i = 0$ (Perkins and Jinks, 1968). However, the interpretation of Type 2 stability is genotype dependent and should be only be confined to the test set of genotypes. This is so because the mean of all the genotypes is used as the standard response in each environment. A genotype stable by this definition is only so with respect to the attendant genotypes in the test. It may well be unstable in the company of another different set of genotypes. This point was well illustrated by Knight (1970).

Type 2 stability is useful for comparing a specific set of genotypes and has limited inferential value when other genotypes are considered. There are also arguments against using Type 2 stability for selection of genotypes. Since computation of Type 2 stability estimates is based on average stability i.e. $h_i = 1$, it precludes selection of those genotypes ($h_i > 1$) which
perform better than stable Type 2 genotypes at the extreme end of favourable environments. It also precludes selection of genotypes ($b_i < 1$) which do better under unfavourable environments. Eisemann et al. (1990) suggested that GE under such situations should be exploited rather than avoided. They proposed that in assessment of biological performance, there is a strong case for better definition of the nature and extent of environmental challenges or constraints that influence differential genotypic adaptation. Characterisation of the important variables in the environment that influence genotypic performance will lead to development of 'structured stress' environments. Genotypic probes which have established behavioral patterns over specific environments can also be developed. These will become the paradigm with which others can be compared with in varietal trials.

This approach will help tremendously in the study of the causes and occurrence of GE interaction. The information on environment and its influence on genotypic behaviour will translate to effective exploitation of GE resulting in identification of best genotypes for specific environments. This may be far superior to the conventional, conservative strategy of selection of genotypes with Type 2 (average) stability.

Type 3:

A genotype is stable under Type 3 classification if its deviation from regression of genotypic mean with Environment Index is very small. Thus Eberhart and Russel's (1986) estimate of $\delta_i^2$ or Tai's (1971) estimate of $\lambda_i$ are statistics which determine the genotypes' Type 3 stability.

Deviation from regression is advocated by Breese (1969) to be a more reliable estimate for stability because it computes the 'unpredictable' part of the genotypes' variability as opposed to the regression coefficient which computes for the 'predictable' part. Chan (1985), found two papaya varieties in his trial to be unstable and each was unstable for a different reason. The variety Sitiawan had a high regression coefficient and although it had large changes in yield over environments, such changes were nevertheless predictable. On the other hand, Subang was unstable due to the large deviations from its regression and was subsequently less predictable in performance.

Lin et al. (1986), however, argued that linear regressions and deviations from regression cannot be used as a predictive model but only as a descriptive model. This is because their computations were derived from a restricted data set, with the mean of all
genotypes at a certain location used as environment index of that location. For a useful predictive model, an independent variable for the environment index must be used. Further, the relationship between genotypes and this independent variable must be established before it has any predictive properties. In many cases, this is not done and therefore the deviation MS does not have deterministic property and its value does not extend beyond estimating the goodness of fit for the regression. Because of this, Type 3 stability estimate is perhaps the weakest indicator in the evaluation of genotypic stability (Lin et al., 1986).

2.3.3. Selection for yield and stability

There is general consensus of opinion that genotypic performance (mean yield for example), and stability are antagonistic in relationship. Finlay and Wilkinson (1963) demonstrated that in a population of 277 barley varieties, the high yielders were found to have average stability (b1 = 1) and that genotypes that have above average stability (b1 < 1) were invariably poor performers. Kang and Pham (1991) further illustrated this point when they found that stable genotypes were missed out in selections when greater weights were given to yield in the selection process (when rank sum index was greater than 1).

More specifically, the negative correlation of yield with stability were reported in several horticultural crops including cassava (Tan, 1984), papaya (Chan, 1985) and tomato (Poysa et al., 1986).

Integration of stability in performance with yield or other economic traits is desirable and necessary for selection of high yielding, stable genotypes. But can this be done in view of the inherent antagonism of their relationship? Poysa et al. (1986) argued that high yielding tomato genotypes classified as unstable by the regression analysis was misleading because these have high mean yield and did not have lower yields than the test mean in any of the five environments. Chan (1984) suggested that high genotypic means and instability should be reconciled and high genotypic means should take priority, especially in situations when stability arose because of the 'predictable' variances of the genotype (i.e. in situations when b1 > 1). In this case, high yields will be obtained at favourable environments although under less favourable environments, such genotypes' yields may be lower than others. This may not be too much of concern for fruit breeders because in normal circumstances, choice areas are selected for growing fruits with high quality (Chan, 1991b).

A few methods for simultaneous selection of yield and stability are as follows:
2.3.3.1. Mean and CV distribution

Francis and Kannenberg's (1978) concept of stability was based on CV and mean distribution of the genotypes. The use of CV per se was analogous to the Type I stability of Lin et al. (1986). The mean and CV of each genotype can be plotted in a scatter diagram and the average CV and average mean lines of the population can be drawn to demarcate four quadrants. Genotypes in Quadrant 1 which are high yield and low CV would be considered the most desirable. Genotypes with high CV and average yield would be considered as having below average stability, while those with low CV and average yield would be above average stability. The worst performers are those in Quadrant 2 which have above average CV and below average yields. These are clearly unsuitable and undesirable. Chan and Ooi (1975) have used this method for selection of papaya varieties with good adaptation and uniformity.

2.3.3.2. Non-parametric yield rank

Hühn (1979) proposed two non-parametric statistics which can be used for selection of combined yield and stability. These statistics are $S_i^3$ and $S_i^6$ and are computed based on yield ranks in each environment as follows:

$$S_i^3 = \Sigma_j (r_{ij} - \overline{r_i})^2 / \overline{r_i}$$

$$S_i^6 = \Sigma_j |r_{ij} - \overline{r_i}|^2 / \overline{r_i}$$

$r_{ij}$ = rank of $i^{th}$ genotype in $j^{th}$ environment

$\overline{r_i}$ = mean of ranks over all environment for the $i^{th}$ genotype

The advantage of this method lies in its simplicity in computation and the flexibility in interpretation. The parameters $S_i^3$ and $S_i^6$ confound and simultaneously evaluate stability and yield. The numerator measures the stability expressed as the variability of the ranks ($r_{ij}$), while the denominator reflects the yield level expressed as the mean of ranks ($\overline{r_i}$). Leon (1986) and Kang and Pham (1991) found that $S_i^3$ was more correlated to stability than yield and conversely, $S_i^6$ was more correlated to yield than stability. Therefore, in the analysis and interpretation, the choice between $S_i^3$ or $S_i^6$ will give the opportunity to weigh priority for selection either for stability or for yield performance respectively.
2.3.3.3. **Superiority measure (P_i)**

Lin and Binns (1988) developed a superiority measure (P_i) of a cultivar in a cultivar x location barley trial. The superiority measure (P_i) is defined as the distance mean square between the i\(^{th}\) test cultivar's response and the maximum response averaged over all locations and is expressed as follows:

\[
P_i = \frac{\sum_{j=1}^{n} (X_{ij} - M_j)^2}{2n}
\]

where
- \(P_i\) = superiority measure of the i\(^{th}\) cultivar
- \(X_{ij}\) = yield of the i\(^{th}\) cultivar in the j\(^{th}\) location
- \(M_j\) = maximum response (check or otherwise) among cultivars in the j\(^{th}\) location
- \(n\) = number of locations

Since \(P_i\) represents the distance MS between the cultivar and the best response over all the locations, a small \(P_i\) value would indicate a superior cultivar. A genotype which ranks highest in all environments will have \(P_i = 0\).

\(P_i\) value is a measure of the cultivar’s superiority in the sense of general adaptability because its computation is done over all locations. It will not be efficient for picking out cultivars which have high \(P_i\) but with specific adaptability i.e. high yielding genotypes in specific locations only.

2.3.3.4. **Rank-sum and rank-product**

A simple method for selecting high yield and stability is based on ranking of mean yield and stability. In the rank-sum method, Kang (1988) assigned ranks for mean yield (highest yield = rank 1) and stability variance (lowest \(\sigma_i^2\) = rank 1) and both were summed to obtain the rank-sum value.

In the rank-product proposed by Schuster and Zschoche (1981), similar rankings were carried out, but ecoalence (\(W_i^2\)) was used as the statistic for stability. Both ranks were multiplied to obtain the rank-product. Low values of rank-sum and rank-product indicate desirable genotypes which have a balance of superior performance and good stability.

Kang and Pham (1991), extended the method of rank-sum to include a procedure for weighting in favour of selection of yield. The rank-sum indices were varied from 1 to 5
depending on the extent of weights given to yield selection. Index 1 is the normal index as
described earlier, with equal weighting given for both yield and stability. In Index 2, the yield
rank is multiplied by a factor of 2 and then summed as usual with the stability variance rank. In
Index 3, the yield rank is multiplied by 3 and so on until Index 5, where the yield rank is
multiplied by 5 before adding on to the stability variance rank.

With this procedure however, they found that when yield was given higher weighting in
the selection of genotypes i.e. when rank-sum Index > 1, the methodology quickly loses its
efficiency in the selection for stable genotypes. The result of the selection which used rank-sum
Index 2-5, did not differ very much from selection based on mean yield only (Kang and Pham,
1991). They concluded that the most effective methods for simultaneous selection of
performance and stability were Hühn's (1979) non-parametric ranking estimates $S_i^3$ and $S_i^6$,