CONSTRUCTION OF A GENETIC LINKAGE MAP OF PAPAYA AND MAPPING TRAITS OF HORTICULTURAL IMPORTANCE

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

DECEMBER 1994

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DEDICATED TO MY BELOVED MOTHER

ACKNOWLEDGMENTS

I take this opportunity to thank my wife, Mrs. Sandhya Sondur for her patience, support and encouragement during the course of this lengthy investigation.

I would like to express my thanks to Dr. Richard Manshardt, Chairperson of my committee for his support, encouragement throughout the course of investigation and for having gone through my manuscript critically. I am very thankful to Dr. John Stiles, for leading me to this project and providing me with valuable guidance during the course of investigation and, for providing me with excellent lab and working conditions to achieve the set objectives.

I wish to thank Dr. James Brewbaker, Dr. Francis Zee and Dr. John Hu, members of my committee, for their valuable suggestions, encouragement and constructive comments throughout the course of investigation.

I am highly thankful to East-West center for having provided my with me financial assistance during the early part of the investigation. I also wish to thank USDA for funding the project and for providing financial assistance during later part of the investigation.

Finally, I wish to thank all my friends in the departments of Horticulture and Plant Molecular Physiology, who made the difficult times look so easy and smooth.

ABSTRACT

A genetic linkage map of papaya based on 61 randomly amplified polymorphic DNA markers and one morphological marker has been constructed by using a computer program MAPMAKER/EXP. The map is based on a F_2 segregating population of a cross between a Hawaiian cultivar Sunrise and Line 356, a disease (papaya ringspot virus) tolerant selection from Florida. The map is comprised of 11 linkage groups covering a total distance of 1000 cM. The sex locus is mapped on linkage group 1, within a marker bracket of 14 cM. Assay for these flanking markers can be used to forecast the sex of the plant at the seedling stage.

Analysis of QTLs based on MAPMAKER/QTL reveals the presence of several loci affecting vigor and precocity. With regard to vigor defined as plant height and stem diameter, environmentally sensitive and stable QTLs have been observed. Analysis of growth rates for height and for diameter improved the efficiency of resolving for neighboring peaks in QTL analysis for vigor. The analysis of yield and yield related traits like carpellody, sterility and fruit weight, has indicated the occurrence of multiple QTLs. QTLs affecting carpellody indicate a possible interaction between qualitative and quantitative factors in influencing the phenotype. In the majority of the traits studied, genetic factors with an effect opposite to the overall effect have been detected in parent Line 356.

Genetic analysis of different components of papaya ringspot virus tolerance

(vigor, severity of symptoms and ELISA titer) has confirmed the complex nature of disease tolerance. QTLs affecting plant vigor (diameter and height) specific to the disease environment have been detected in Line 356. Stem diameter appears to be an important index of disease tolerance. QTLs in Line 356 with gene-dosage dependent effects in lowering PRV load as measured by ELISA titer show the presence of suppressive virus resistance, indicating a positive type mechanism of disease resistance. In light of these findings, disease tolerance in Line 356 is reclassified as resistance or 'tolerant to symptoms and resistant to virus'.

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CHAPTER 1

Introduction

Papaya is an important, all-season fruit of the tropical world valued for its excellent dessert qualities. The papaya industry is the fourth largest component of commercial agriculture in Hawaii with an annual production valued at $14.5 million (Anon, 1993). Over 92% of the total production comes from the island of Hawaii.

Improvement of papaya through breeding and selection is one important approach to increasing productivity. Considerable efforts have gone into improving yield and quality in the past, resulting in the release of Hawaiian gynodioecious cultivars like 'Sunrise' and 'Kapoho'. These cultivars have better yield and quality. However, they have a long pre-bearing stage and segregate for flower sex into female and hermaphrodite plants. Only hermaphrodite plants are of commercial value in Hawaii. Currently, three plants are planted per hill followed by thinning at flowering to keep a hermaphrodite plant. In recent years the spread of the papaya ringspot virus disease is threatening the papaya industry in Hawaii. This vector transmitted disease is very severe on Hawaiian cultivars, and is an impending threat to the industry. This disease is also a serious problem in most of the papaya growing regions of the world. The only source of tolerance to PRV disease in the species *Carica papaya* was identified in the 1970s (Conover, 1976) and later improved to usable levels in Line 356. Introduction of disease tolerance into commercial cultivars is often associated with occurrence of carpellody, sterility and other yield associated problems in the progeny.

Currently, there is no information on location and nature of genetic factors responsible for PRV disease tolerance, carpellody and sterility, all of which are quantitatively inherited. Traditional selection methods, based only on phenotype selection in field trials, are lengthy and expensive.

The discovery of DNA-based molecular markers designated RFLPs (restriction fragment length polymorphisms) and RAPD (randomly amplified polymorphic DNA) has provided powerful tools to locate, characterize and introgress traits of economic importance into commercial cultivars. These markers rarely affect phenotype and occur in large numbers. A saturated genetic linkage map can be developed in a reasonably short period of time by following segregation of markers in a suitable population (Botstein, 1980). The neighboring markers in a saturated genetic linkage map can act as reference points for inheritance of chromosomal segments contained between them. With an appropriate population and analysis, the inheritance of neighboring markers can be used to associate and characterize chromosomal regions responsible for a phenotype. A map with defined location of qualitative and quantitative traits provide speed, direction and precession in a breeding trial.

In the light of above information, and due to lack of a basic genetic linkage map in papaya, an investigation involving two field trials was undertaken with the following objectives:

1. Construction of a preliminary genetic linkage map of papaya using randomly amplified polymorphic DNA markers.

2. Map and characterize QTLs affecting vigor, precocity carpellody, sterility and other yield related traits.

3. Map and characterize QTLs affecting disease tolerance to understand the genetics of tolerance in Line 356 to papaya ringspot virus disease.

CHAPTER 2

Review of literature

2-1. Importance.

Papaya (*Carica papaya*) is a small soft-wooded tropical fruit tree grown for its palatable melon-like fruits. The tree is normally 3 to 8 m tall (Foster, 1943) with a single, hallow unbranched stem. It is cultivated throughout tropical regions as an orchard crop as well as in back yards. The fruit is consumed as a fresh dessert fruit, rich in vitamins A and C (Arriola et al., 1980). It ranks second only to mango, as a source of the precursor for vitamin A (Aykroyd, 1951). Fruits are used in jam, soft drinks and crystallized fruits. Young papaya leaves are also used as a vegetable in Java (Ochse, 1931). Dried latex from immature fruits yields a protease, papain, which is used as a meat tenderizer; in manufacture of cosmetics; in tanning industry; in degumming natural silk; and to give shrink resistance to wool (Purseglove, 1968).

2-2. Origin and taxonomy.

Papaya is the best known member of the dicotyledonous family Caricaceae. The Caricaceae is comprised of 4 genera and 31 species (Badillo, 1971). The genera *Carica, Jacaratia, Jarilla and Cylicomorpha* include 22, 6, 1 and 2 species, respectively. The first three genera are native to tropical America, while the last is native to equatorial Africa (Badillo, 1971).

2-3. Genetics of papaya.

2-3-1. Chromosome studies.

Papaya is a diploid having 9 pairs of chromosomes with 2N=2x=18 (Meurman, 1925; Suguria, 1927; Hofmeyr, 1938; Storey, 1941). The total DNA content of a diploid cell is 0.77pg with approximately 372 million base pairs per haploid genome (Armuganathan and Earle, 1991). There are no apparent detectible heteromorphism in any chromosome pair, either among somatic cells or in cells undergoing meiosis. However, Kumar et al., (1945) observed precocious separation of one pair of chromosome during early anaphase I of meiosis in male and hermaphrodite plants and not in female plants. Storey (1953) confirmed the occurrence of precocious separation of one pair of chromosome at a very high frequency, but not in every cell at anaphase.

2-3-2. Sex forms.

All the species in the family Caricaceae are dioecious, except for three species in the genus *Carica*. The species *C. monoica, C. pubescens* and *C. papaya* have sexually ambivalent forms which undergo 'sex reversals' in response to change in environmental conditions. *C. monoica* is strictly monoecious, but may lack pistillate flowers during certain parts of the year. Trees of *C. pubescence* exhibit three sexual forms; pistillate, staminate and andromonoecious. The pistillate and staminate flowers are unresponsive to seasonal variation, while the andromonoecious types are sexually

ambivalent, producing staminate, perfect and pistillate flowers in varying proportions during different parts of the year (Storey, 1976).

Several scientists have grouped sex forms in papaya based on floral composition consisting of normal and teratological forms of flowers on small, many flowered or short, few-flowered inflorescence. Higgins and Holt (1914) identified 13 sex types, while Hofmeyr (1938) classified the flowers into 9 major types. However, Storey (1938) simplified the classification of the flower types by grouping them into 5 types. This classification also explains the characteristic fruits that develop from them. These types are explained below.

a. Type I: This is a typical female, or female flower lacking even rudimentary stamens. The five petals are free and inconspicuously adnate with the base of the pistil. The pistil is pentacarpellory, smooth and regular. The fruits are generally round or obovoid with a circular or slightly lobed cross section.

b. Type II: This is a hermaphrodite flower referred to as "pentandria" (Higgins and Holt, 1914). The petals are like type I. There are 5 stamens arranged alternately with the petals. The pistil is more deeply lobed with the stamens arranged along the groves. The fruit is deeply lobed with a conspicuous glossy disk at the basal end.

c. Type III: This is a transition hermaphrodite between type II and IV, and is commonly referred to as "intermediate" type. It is characterized by considerable distortion. The petals may be connate from base to one half their length. The number of stamens varies from 2 to 10 and the degree of their adnation to the pistil or to the

corolla also varies. This intermediate type is characterized by a high degree of pistillody. The pistils are some times distorted with fusion of the carpel varying from 5 to 10. The fruits that develop from such flowers are often misshapen.

d. Type IV: This is the hermaphrodite flower that is referred to as "elongata" type. The petals are gamopetalous to three-fourths of their length. Ten stamens are arranged in two whorls at the throat of the corolla. The filaments of 10 stamens are adnate with the corolla and connate among themselves. This gives the appearance of the secondary thickening which encloses most of the pistil. The pistil is generally five-carpellate, elongate and shallowely lobed. The fruits vary from long-cylindrical to ellipsoid.

e. Type V: This is the typical male or staminate flower with petals and stamens fused as in elongata type. The corolla tube is slender and long and the rudiments of pistil extends up to one half of the tube. Due to the lack of a functional pistil, trees are devoid of fruits. However, these plants occasionally produce type III or type IV flowers which produce fruits.

The hermaphrodite flower of papaya is not stable and undergoes sex reversal in response to environmental conditions. The occurrence of "carpellod" or "cat-faced" fruits due to fusion of stamens with carpels has been reported by many workers (Higgins, 1914; Hofmeyr, 1938; Kumar, 1952). Storey (1941) suggested that cool winter weather conditions cause fusion of stamens to carpels resulting in carpellod fruits while the hot months of summer result in flowers without functional ovaries. Later, Awada (1958) correlated minimum temperature conditions at three locations to

increased production of carpellod fruits. Lange (1961) observed rapid reduction in male parts 4 to 6 weeks after the lowest drop in minimum temperature and/or greatest range between maximum and minimum temperatures. He further observed that flower bud differentiation occurred about one month before the flowers were big enough (5 mm in length) to be indexed.

The occurrence of carpellod fruits is also associated with tree vigor. Hofmeyr (1939 b) observed that the favorable growing conditions promote a female-like tendency and unfavorable conditions a male-like tendency. Awada and Ikeda (1957) showed that higher levels of soil moisture and nitrogen content caused increased carpellody, while moisture stress and low nitrogen encouraged female sterility. They associated carpellody with higher plant vigor. Nakasone and Lamoureaux (1982) reported a reduction in fruit production during summer and autumn as a result of carpel abortion leading to female sterile flowers. The fruits that develop from these transitional forms tended to be cylindrical, longer and often showed curvature due to one or two poorly developed carpels.

Storey (1967) attributes the variation in flower type to two sets of genetic factors, one affecting female sterility and the other causing carpellody. Both sets of factors are intern influenced by a third factor that determines the time of expressivity. This instability indicates that though the primary sex of the tree is determined genotypically, the phenotypic expression of alleles responsible for the presence or

absence of androecium and those for the presence or absence of the gynoecium, are influenced by the environmental factors at the time of flower bud production.

2-3-3. Sex determination.

The early part of this century witnessed several attempts to understand the complex sex inheritance mechanism in papaya that produce male, female and hermaphrodite forms. Storey (1938) and Hofmeyr (1938) concluded from their genetic experiments that sex in papaya is determined by a single gene with multiple alleles following Mendelian inheritance. Staminate and andromonoecious plants are heterozygotes and pistillate flowers are recessive homozygotes, with a zygotic lethal factor eliminating the dominant homozygous types. The three hypotheses on the genetics of sex determination are presented below.

1. Hofmeyr's (1967) genic balance hypothesis.

This hypothesis is based on the genic balance of male and female determiners in the chromosomes and autosome. Female determiners predominate on the sex chromosomes and male determiners in the sum-total of autosome. The region M_1 and M_2 represent inert or inactivated regions of the sex chromosomes, with M_1 being slightly bigger than M_2. This accounts for the zygotic lethality of the dominant M_1M_1, M_1M_2 and M_2M_2 genotypes. The homologous region m is normal. The viable genotypes are mm (pistillate) M_1m (staminate) and M_2m (andromonoecious).

The greater concentration of female determiners on sex chromosomes explains greater phenotypic stability observed in female plants. Since M_1 is the longer inert region, it is expressed phenotypically as staminate (M_1m) due to great influence of autosomal factors. The M_2 being shorter, is less influenced by the autosomal factors resulting in the expression of andromonoecious types (M_2m). The heterozygosity of staminate and hermaphrodite flowers renders them susceptible to alteration in phenotypic expression by external influence.

2. Horovitz and Jimenez (1967) hypothesis.

The basic assumption of this hypothesis is that dioecism is a primitive state in the family Caricaceae and sex determination is the XX and XY type with heterogametic male and the genotype YY lethal. An ambivalent sex form occurred at some point of time which served as the progenitor for the present day dioecious forms in the family Caricaceae (*C. monoica, C. pubescens and C. papaya*). The ambisexual mechanism built up in *C. papaya*, giving rise to a modified homologue, Y_2 chromosome, without affecting the X chromosome, which explains the stability of the pistillate forms. The genotype XY_2 is expressed as the sexually ambivalent andromonoecious form. This hypothesis proposes that the andromonoecism and polygamy followed the evolution of the XY-XY system and are of recent origin.

3. Storey's (1976) hypothesis.

This hypothesis is based on the progressive evolution of dioecism from an unknown perfect flowered progenitor (elongata type). The staminate flower evolved in the classical way by elimination of a functional pistil. The pistillate form evolved progressively through carpellody and pentandria types accompanied by the change of the ovary from superior to partially inferior position. Hence, the present day pistillate forms are a morphological anomaly of the original elongata flower type (Storey, 1969). The derivation of unisexual forms was followed by dioecism.

Certain secondary characters of flowers are associated with sex types in papaya. Male flowers have a long peduncle and many-flowered cymose inflorescences while hermaphrodite forms have fewer flowers (< 15), that are borne on short peduncles. Female forms have very few flowers (< 5), again borne on short peduncles. These genes and the genes responsible for sex determination are linked and comprise differential segments occupying identical regions on sex chromosomes. However, the linkage is not absolute. They may also exhibit pleiotropic effects. The sex-determining genotypes are:

Staminate and andromonoecious: (sa) L C (SG)/(SA) + + (sg)

Pistillate: (SA) + + (sg)/(SA) + + (sg)

The symbol (SA) represents the sum of the factors involved in transmuting the ancestral androecium into the present day gynoecium; (sa) represents the normal androecium development; (SG) represents the factor or factors responsible for the

suppression of the gynoecium in the staminate flower. The symbol 'L' represents the recessive sex-linked zygotic lethal factor that enforces heterozygosity on staminate and andromonoecious plants. The symbol 'C' represents the factor that prevents crossing-over between the sex determining factors and the lethal factor, which explains the absence of pistillate forms carrying lethal factor L.

Recent studies in molecular biology of flower development in *Arabidopsis* and *Antirrhinum* have increased our understanding of the complexities of processes involved in flower development. Since the present day flowering plants arose from a common hermaphrodite ancestor (Cronquist, 1988), much of the floral development program is expected to be common in all species. A general overview of these developments is presented below.

Genetic studies have defined two main types of genes, meristem and organ identity genes, that are involved in flower development (Coen and Carpenter, 1993). Meristem identity genes affect the primordia as such, where as organ identity genes more specifically affect fate of primordia and hence, the type of organs develop from them. Based on genetic studies in *Arabidopsis* and *Antirrhinum,* Coen and Meyerowitz, 1991 propose the following model to explain the process of flower development.

The basic hermaphrodite flowers can be subdivided into four whorls. Whorl 1 contains sepals, whorl 2 contains petals and whorl 3 and 4 contain androecium and

gynoecium. Organ position and identity are controlled by combinatoral action of homeotic genes in three overlapping regions, A, B and C. If genes acting in regions A, B and C are required for three requlatory functions a, b and c, respectively, than the combination of functions in the four whorls of wild-type would be a, ab, bc and c. Expression of a alone is required for sepal development, while ab together determine petal development. Sex organogenesis takes place in whorls three and four by the action of homeotic genes in the regions B and C. The function of genes B and C is required in whorl 3 for stamen determination. Function of C alone is required in whorl four for carpel development. The difference between carpel and stamen determinations resides in the individual action of homeotic genes in regions B and C of the flowers.

2-3-4. Qualitative characters.

Several mutant phenotypes in *C. papaya* have been characterized with the main objective of identifying a phenotype linked to sex to enable identifying sex at an early stage. Hofmeyr (1949) reported a dwarf mutant form characterized by early excessive branching. Storey (1953) listed a number of mutant forms; albino plant, diminutive form (dp) (characterized by short slender trunk, small leaves with short slender petioles with small flowers and fruits), and rugose leaf (rg)(characterized by upward puckering of blade areas between veinlets and curling of margins). All these mutant forms are recessive to the normal form. Hofmeyr (1938) found red flesh of fruit (r), white flower color and green stem and petiole to be recessive to yellow fruit color, yellow flower color and purple stem and petiole. Grey seed coat color was reported to be dominant

over black seed coat color (Hofmeyr, 1939a). Based on inheritance studies, Hofmeyr (1939a) derived linkage between yellow flower color (Y), purple stem (P) and the sex of the plant (M_1). The linkage relationship of these three traits is:

M_1----25cM--Y---16cM----P.

Flavor and associated odors of fruits of some papaya strains are very strong and musky. This muskiness is attributed to a homozygous recessive allele of a single gene (Storey, 1969) that could be easily bred out of cultivated varieties.

2-3-5. Quantitative characters.

Fruit size, shape and quality: A wide range in fruit size and shape is observed in *C. papaya*. The fruit weight varies from 50g to 10kg and length from only few cms to over 50 cms. Fruit weight is determined genetically by multiple factors. The volume of the fruit is highly correlated with its weight (Storey, 1969). Wassee et al., (1984) reported that fruit shape, weight, cavity percentage, flesh color, flesh thickness and total soluble solids (TSS) are all quantitatively inherited traits. Fruit shape, weight, flesh thickness and TSS exhibited additive gene action with a narrow sense heritability of 0.03 to 0.66.

Plant stature and precocity: The stature of the papaya plant is largely decided by the length of the internode, which is influenced by multiple factors. The number of nodes produced by different strains in a given time appears to be constant (Nakasone and Storey 1955). Exceptions to the quantitative nature of the inheritance of the plant

height are the two mutant types described by Storey (1953) and Hofmeyr (1949), and the segregation ratio of 3 tall to 1 short plant observed in a cross between a tall and a dwarf variety (Gandhi, 1947). Nakasone and Storey (1955) observed that two of the economically important characters, earliness to flower and node to first flower, showed quantitative inheritance with an additive type of gene action. They noticed partial dominance of the early flowering parent over the late flowering parent in F_1 plants. Height at initial flowering was found highly correlated with number of nodes to first flower.

Yield of papain.

All parts of the tree contain papain in an anastomosing canal system of cells under turgor pressure. Papain is easy to collect from green fruits which have an extensive canal system in the mesocarp of the ovary wall. During maturation, the latex is converted to reducing sugars. Jones (1940) correlated the amount of fresh latex produced to the size of the fruit and the variation between several varieties and strains was about 0.7 to 1.0 per cent of fresh latex per unit fruit weight.

2-4. Genetic diversity and plant breeding.

Genetic diversity studies reported so far in species *C. papaya* are based on limited germplasm. The apparent higher genetic diversity observed in fruit size, shape and fruit quality aspects are perhaps due to human selection (Harlan, 1975), and may not be indices of true genetic diversity. Sharon et al., (1992), based on germplasm

study of papaya by DNA molecular markers (microsatellites and minisatellites), reported a limited amount of polymorphism in the species *C. papaya*. Likewise, Stiles et al., (1993) using randomly amplified polymorphic DNA markers (RAPD) on 7 cultivars, based on or related to Hawaii germplasm, and 3 unrelated cultivars, detected only a moderate degree of genetic diversity.

The objectives of papaya improvement through breeding are largely decided by the market preference for the type of fruit. In Hawaii, the gynodioecious 'solo' variety with small fruits are preferred, while in South Africa the bigger fruits from female plants are preferred. However, the breeding strategy remains the same. The breeding procedure most widely used is to make a cross between selected strains followed by either pedigree analysis or back-cross breeding. Since pedigree analysis is time consuming, intercrossing among the desirable genotypes offers a better and quicker alternative. The Hawaiian variety 'solo', the South African variety 'Hortus gold' and the newly released Malaysian variety 'Eksotica' are the most important varieties developed through these breeding approaches.

The Hawaiian variety solo may have originated as the F_1 or F_2 progeny from a cross between the male wild papaya 'Lechosita' of West Indies and a large commonly cultivated type (Storey, 1969). The present day solo is the result of successive generations of intercrossing among selected superior gynodioecious types. 'Sunrise' solo is an improved inbred strain of high quality solo papaya with reddish-orange flesh

and high TSS. This variety was developed by crossing two inbred strains 'Line 9' and 'Cairo solo' (Hamilton and Ito, 1968). Papayas do not show loss in vigor due to inbreeding. The variety 'Eksotica' was developed in Malaysia by a combination of pedigree and backcross breeding program involving the Hawaiian cultivar Sunrise and the local variety, 'Subang-6' with bigger fruits (Chan, 1987). The 'Eksoticas' combine the excellent fruit qualities of 'Sunrise' and the larger fruit size of 'subang-6'.

2-5. Papaya ringspot virus.

Papaya ringspot virus (PRV) is a serious threat to papaya cultivation in Hawaii and elsewhere in the tropical world. The disease causes severe loss and renders papaya orchards economically unproductive. The first occurrence of this virus disease in Hawaii was reported on the island of Oahu by Parris (1938). This sap-transmittable virus disease was named "Wailua disease". Subsequently, another disease was reported from the island of Oahu by Linder (1945). This disease was named papaya ringspot virus disease.

Considerable confusion exists in the literature regarding naming the virus. The disease symptoms of PRV and papaya mosaic virus, two different viruses, appear similar on leaves. Gonsalves and Ishii (1980) used specific serological tests and electron microscopy to characterize papaya ringspot virus. The papaya ringspot virus is a potyvirus, while the papaya mosaic virus belongs to potexvirus group and is of minor importance in Hawaii (Purcifull and Hiebert, 1971). Distortion ringspot virus (DRV),

reported to be severe in Florida, is confirmed to be PRV (Gonsalves and Ishii, 1980), and is serologically identical to watermelon mosaic virus type I (PRV-W), which affects cucurbits (Yeh and Gonsalves, 1984).

2-5-1. Symptoms of PRV.

Symptoms of PRV disease appear on leaves, petioles, stems and fruits and show considerable variation. The first evidence of the disease appears on young leaves as puckering or bulging of leaf tissue between veins and veinlets resulting in upward curling of leaves (Jensen, 1949a). This is followed by chlorotic mottling and blistering of leaf surface. In severe cases the leaf is highly distorted. In later stages, linear chlorotic lesions appear on the petiole and stem (Conover, 1964). Older leaves may abscise prematurely, leaving a tuft of yellow terminal leaves (Holtzmann and Hines, 1965).

Symptoms can appear on fruits as small as 2.5 to 7.5 cm long (Jensen, 1949a) and as early as two weeks after fruit set (Holtzmann and Ishii, 1963). Fruits develop small green rings on their surface. Yellow rings with green centers on mature green fruits provide the most striking and reliable symptoms of the disease (Jensen, 1949a). The size of the ringspots range from 4 to 8 mm in diameter and the number of spots on the fruits may vary from a few to over 150. Fruit distortion is observed in severe cases, especially on the fruits infected during early stages of development.

The PRV causes a reduction in plant vigor. The size of leaf lamina and the length of the petiole are reduced and the plants appear stunted to various degrees, eventually leading to their death (Jensen, 1949a). Younger plants are more severely affected than older plants (Hollings and Brunt, 1981). Thomas et al., (1993) reported lower photosynthetic capacity, apparent quantum yield, photosynthetic CO_2-use efficiency and higher dark respiration in diseased plants. Diseased fruits are bitter to taste, have poor flavor and are low in sugar content (Ishii and Holtzmann, 1963; Khurana, 1970) but there is no effect on the latex content (Jensen, 1949; Khurana, 1970).

Symptoms appear on terminal leaves within two to four weeks after infection. Disease symptoms are milder during warmer weather conditions (Conover, 1962; Ishii et al., 1961). Variation in disease symptoms due to environmental conditions, mainly temperature, is also reflected in lower ELISA titers observed during warmer conditions (Gonsalves and Ishii, 1980).

2-5-2. Properties of PRV.

Papaya ringspot virus is classified as a potyvirus with flexueous, rod shaped particles about 780 X 12 nm. Virus particles are monopartite and have a single-stranded positive sense RNA enclosed in a protein coat. The molecular weight of the genome is about 330,000 daltons (Purcifull et al., 1984). The thermal inactivation point of the virus is 55° C and the dilution end-point is 10^{-3} to 10^{-4}.

Papaya ringspot virus is stylet-borne (Zettler et al., 1968; Conover, 1964), and is non-persistently transmitted by aphids (Watson, 1946). The chief vector of PRV is *Myzus persicae* Sulz (Namba and Kawanashi 1966; Cook and Milbrath, 1971). Other minor vectors shown to transmit the virus are *Aphis gossypii* Glover, *Aphis medicaginis* Koch, *Aphis rumicis* Linn (Jensen, 1949b), *A. craccivora* Koch, *Macrosiphum euphorbiae* Thomas and *Rhopalosiphum maidis* Fitch (Higa and Namba, 1971). *Myzus persicae* (peach aphid) feeds primarily in the veins and veinlets of the leaves. Normally the aphid selects undersurface of the leaves (Jensen, 1949b). The time required for virus acquisition by the vector varies from 5 sec to 5 min and successful transmission is brought about by feeding times as short as 10 sec (Cook and Milbrath, 1971; Namba and Kawanishi, 1966). The virus does not have a long latent period in the aphid vector, since the virus can remain virulent for only 30 to 60 min after it is acquired (Namba and Higa, 1975).

2-5-3. Host range and spread of PRV.

Most of the species in family Caricaceae are susceptible to PRV. The susceptible species are *C. papaya, C. cauliflora, C. goudotiana, C. monoica, C. parviflora* and *C. pubescence* (Cook and Milbrath, 1971), *C. quercifolia, and C. microcarpa* (Cook and Zettler, 1970). The species *J. stipulata, J. corembensis* (Horovitz and Jimenez, 1967), *J. spinosa* (Cook and Milbrath, 1971) and *J. mexicana* (Cook and Zettler, 1970) were reported to be immune to PRV disease.

Other than Caricaceae, some members of the family Chenopodiaceae and Cucurbitaceae are also hosts to PRV (Cook and Zettler, 1971; Hollings and Brunt, 1981). Virus particles are readily recovered from *Cucumis sativa* L., muskmelon, *Cucumis melo* L., watermelon, *Citrullus vulgaris* Thump., summer squash and pumpkin (Namba and Kawanishi, 1966), *Cucumis hardwickii, Cucumis anguria* var anguria, *Cucumis dipsaceus, Cucumis meeusii, Cucumis dinteri* and *cucurbita moschata* (Yeh et al., 1984). *Chenopodium amaranticolor* and *Chenopodium quinoa* are used as local lesion hosts (Cook and Milbrath, 1971).

Peach aphids do not usually colonize papaya orchards and are rarely seen feeding on papaya in the field. The vector-host relationship is characterized by transient visitation of the vector on papaya from hosts other than papaya (Ishii, 1972). The initial introduction of the inoculum into a papaya orchard is brought about by migrating alate aphids (Conover, 1964) and the further spread in the field depends on the vector population and activity. Ishii (1972), studying epidemics of the PRV disease spread, observed the spread to be logerthemic with a rate of 0.054 trees per day. The total number of plants infected increased from 0.7% to 88.9% in 84 days. Excess rain and/or wind was associated with a noticeable increase in new infections.

2-5-4. Prevention and control of PRV.

Rouging and sanitation.

Infected plants in abandoned orchards and backyards act as a source of inoculum. Hence, rouging of plants as symptoms appear reduces the chances of spread (Ishii, 1972). Continued loss of plants is only expected until all infected plants are eliminated from the area (Namba and Kawanashi, 1966; Wolfenbarger, 1966). Control of the vector as a measure to reduce the disease is ineffective in papaya due to the non-persistent nature of the vector (Bart et.al., 1960) and the presence of several alternate hosts. However, a conscious rouging program together with good insect control through sanitation, can minimize loss due to PRV. The disease was eradicated from Puna and Pahala areas of Hawaii and the island of Maui by strict rouging (Nakasone, 1979).

2-5-5. Cross protection.

Cross protection is a phenomenon in which plants systemically infected with one strain of a virus are protected from the effects of a second related strain of virus (Mckinney, 1929). The first commercial application of this phenomenon was demonstrated in tomato by Rast (1972). He showed that inoculation of plants with a mild mutant form of TMV (MII 16) provided protection to plants against the severe strains. Similar success reports have been made in controlling the tomato mosaic virus disease from Japan (Oshima, 1975) and tristeza virus (CTV) (Muller and Costa, 1977).

The success of cross protection depends on availability of a mild strain. Su and Lin (1979) reported the isolation of two mild strains of papaya ringspot virus from papaya by local lesion on *C. amaranticolor.* However, these strains were found neither stable nor mild in later field trials (Lin, 1980; Yeh and Gonsalves, 1984). An alternate approach to develop milder mutant forms by treating PRV with nitrous acid (a mutagen) led to isolation of two strains designated PRV 5-1 and PRV HA 6-1 (Gonsalves and Ishii, 1980). These strains caused infection without symptoms on plants or only with diffuse mottling with no reduction in growth (Yeh and Gonsalves, 1984). Papaya plants infected with these strains showed very strong positive reaction in enzyme linked immuno sorbant assay (ELISA) (Clark and Adams, 1977). The attenuated strain HA 5-1 offered complete or partial protection in field tests in Hawaii and Taiwan (Yeh et al., 1988). However, superinfection of the cross protected plants was observed by Wang et al., (1987) and Yeh and Gonsalves (1984). Under super infected conditions, symptom expression in cross protected plants was only delayed by one or two months. Other problems with cross protection are the strain specific nature and the appearance of symptoms on plants during cooler ($< 20^{\circ}$ C) months of the year (Yeh, 1990).

2-5-6. Disease resistance..

Several investigators have attempted interspecific hybridization with the main objective of introducing resistance to PRV. Interspecific hybridization between *C. papaya* and other species is difficult. Mekako and Nakasone (1975) were unable to

obtain progeny from a cross between *C. papaya* and *C. cauliflora* or *C. goudotiana.* Sawant (1958) reported failure in obtaining interspecific hybrids of *C. papaya* with *C. monoica* and *C. goudotiana* and *C. cauliflora.* The attempts by Horovitz and Jimenez (1967) to incorporate the gene for PRV resistance into *C. papaya* from *C. candicans* and *C. stipulata* were also unsuccessful. However, Khuspe et al., (1980), and Moore and Litz (1980) were able to obtain hybrids from a cross between *C. papaya* and *C. cauliflora* (a PRV resistant species). Khuspe (1980) carried the cross to F_2 generation and showed monogenic, dominant mechanism of resistance to papaya mosaic virus. Horovitz and Jimenez (1967) also observed a 3:1 segregation of resistance to susceptible plants in a cross between *C. monoica* (susceptible) X *C. pubescens* and *C. cauliflora* X *C. monoica.*

The search for resistance to PRV among members of *C. papaya* have also been unsuccessful (Conover, 1964; Cook and Zettler, 1970). Conover (1976) observed difference in papaya varieties and types from different sources, in their response to PRV virus. Two promising papaya stocks tolerant to PRV were identified. One of these was introduced from Colombia by Dr. S. E. Milo and the other was selected by Harold. E. Kendall (Conover and Litz, 1978). Plants selected as most tolerant from among the Colombian types were sib-mated for three generations. This resulted in an increase in tolerance from 4% to 55% (Conover and Litz, 1981). Resistance to papaya ringspot virus in papaya is controlled by multiple factors and is quantitatively inherited. Zee (1985) also reported that Line 356-3, a tolerant line selected from the Florida

accession was the most tolerant among the tested, and the tolerance was readily transferred to Line 356 X solo papaya hybrids in a quantitative manner. Currently, the Florida strain (356) and 'cariflora' (Conover et al., 1986) are the only source of usable resistance or tolerance to PRV in the species *Carica papaya*. The integration of cross protection with the tolerance may mitigate the damage caused by PRV (Yeh, 1990).

2-6. Construction of linkage map and analysis of quantitative traits.

A primary genetic linkage map, well saturated with easily scored, polymorphic loci, evenly distributed throughout the genome is a prerequisite for a detailed genetic analysis and marker based breeding approaches in improvement of any crop plant. Since Mendel's discovery, a considerable amount of work has been done in monitoring, inducing, and mapping single gene markers in crop plants, such as tomato and maize.

Until recently, most of the single gene markers used in higher plant genetics were those affecting morphological characters, imparting a specific phenotype. Such phenotypic markers are limited in number. It has been possible to construct complete genetic linkage maps in a few intensively studied organisms like bacteria, yeast, or fruit flies, which have many visible mutations as genetic markers (Lander and Green, 1987).

With the exception of few qualitative characters like some specific disease resistance, morphological and color pattern, most of the agriculturally important characters like yield, quality, horizontal disease resistance etc. are quantitatively inherited. Such quantitatively inherited traits are thought to be decided by relatively large number of loci (<5), each of which make a small positive or negative contribution to the final phenotype. Classically, these traits are analyzed by biometrical techniques which do not provide information about the number of genetic factors involved in the expression of the trait, the location of these loci and the relative size of the contribution of individual loci to trait expression.

The discovery of molecular markers in recent years has greatly enhanced the scope for detailed genetic analysis and approaches to improvement of crop plants. The markers in a well developed linkage map act as points of reference for chromosomal segments and permit tracing their transmission in a segregating population. This opens up the possibility of assaying the entire genome, piece by piece, for genes controlling quantitative traits. Tanksley (1983) attributes the greater utility of molecular markers over morphological markers to the following inherent properties.

1. With molecular markers the genotypes can be determined at the whole plant, tissue or cellular levels, whereas the phenotypes of most morphological markers can be determined only at whole plant level, and frequently a mature plant is required.

2. A relatively large number of naturally occurring alleles can be found at molecular marker loci. Distinguishable alleles at morphological marker loci are less frequent and often must be induced through the application of exogenous mutagens.

3. Usually no deleterious effects are associated with alternate alleles of molecular markers while, morphological markers often have deleterious effects associated with alternate alleles.

4. Alleles of most molecular markers are co-dominant, allowing all possible genotypes to be distinguished in any segregating population. Alleles at morphological marker loci usually interact in a dominant-recessive manner, prohibiting their use in many crosses.

5. Molecular markers have fewer epistatic or pleiotropic effects, allowing a virtually limitless number of segregating markers to be monitored in a single population. Morphological markers often have strong epistatic effects that limit the number of segregating markers that can be equivocally scored in the same segregating generation.

2-6-1. Protein markers.

Protein markers are generally soluble enzymes separating by gel-electrophoresis and visualized using in situ activity stains. Isozymes are enzymes that share a common substrate but differ in their electrophoretic mobility (Markert and Moller, 1959). The crucial discovery of the presence of polymorphisms for isozymes with in a population (Lewontin and Hubby, 1966) revolutionized research in the fields of biochemical genetics, population genetics and evolution.

The most significant property of isozymes is the simple genetic basis of most polymorphisms. Isozymes directly reflect alterations in the DNA sequence through change in amino acid composition, that causes a change in their electrophoretic mobility. These electromorphs often represent variants or 'allozymes', encoded by alternate alleles at a single locus (Prakash et al., 1969).

The genetics of isozyme variants has been intensively studied in over 35 important crop species. Genetic analysis in crop plants like tomato, wheat and maize have progressed at a faster rate. More than 20 polymorphisms that show monogenic inheritance have been identified in each of these crops. Genetic linkage maps based on isozyme markers are available in tomato (Tanksley and Rick, 1980; Tanksley, 1983), maize (Goodman and Stuber, 1983; Wendel et al., 1986, 1988), wheat (Hart, 1983), and pine (Conkle, 1981). Association of many quantative trait loci (QTLs) with segregating isozyme loci have been shown by several investigators (Tanksley et al., 1982; Vellejos and Tanksley, 1983; Weller, 1983; Stuber et al., 1987) in different crops.

Although isozyme markers provide the basis for a relatively simple tool for genetic analysis and linkage studies, it is unlikely that a sufficient number of isozymes will be found to saturate the genome completely and uniformly (Tanksley, 1983). The enzymes extracted and subjected to electrophoresis are a tiny and probably non-representative sample of the total array of proteins present in them. Besides, for the

mapping purposes, marker loci are useful only if different alleles are segregating in the population of interest.

2-6-2. DNA based markers.

The most exiting feature of DNA based markers is the extent of detectible polymorphisms. It has been estimated that one in 100 nucleotides in human genome is polymorphic within a normal population (Jeffreys, 1979). The human genome is approximately $2x10^9$ base pairs (27 Morgans) long with a nucleotide polymorphisms of about $2x10^7$ in the population as a whole. A recent survey has shown that there are about 3500 recognized Mendelian traits in humans. Thus, only a small fraction of the total variation at the DNA sequence level reveals itself as a distinct trait.

Another advantage of DNA based markers is the flexibility it offers in sampling from any stage of development and from any tissue, including herbarium and mummified tissue (Rogers and Bendich, 1985). The longevity of DNA samples from organisms by far exceeds the life expectancy of the individual, enabling retrospective and post-mortem analysis.

The DNA content in higher plants is highly variable. Armuganathan and Earle (1991) estimated the DNA content in over 100 important crop species. DNA content varied from 0.30 picogram (pg) per 2C nuclei or 145 million base pairs (mbp) in *Arabidopsis* to over 50 pg or 24,255 mbp in leek. However, the DNA content of most

of the intensively mapped diploid species (tomato, rice, Arabidopsis etc.) is in the range of 0.30 to 1.0 pg.

Most higher plants have a considerable portion of DNA as repetitive non-coding DNA that is not transcribed. Species with larger genomes normally have more repeated DNA and a higher proportion of repeated DNA to single copy DNA (Tanksley and Pichersky 1988). Thus, only a small fraction of the total genetic variation at a DNA nucleotide sequence level reveals itself as a distinct trait, showing Mendelian inheritance. The degeneracy of the genetic code ensures that about one in three nucleotide changes will not affect the amino acid sequence of the protein produced. Thus, the great bulk of genetic variation at the nucleotide level may not have any detectable expression at a phenotypic level. It is this genetic variation that is exposed as DNA-based polymorphism.

The extent of polymorphisms detected by DNA-based markers covering the entire genome has brought within reach the development of well saturated linkage maps that have the potential of transforming quantitative trait loci into Mendelian and quasi Mendelian entities and fixing their physical location on specific segments of chromosomes (Tanksley, 1983; Saghai-Maroof et al., 1984). The total number of markers required to saturate a linkage map to the required density can be obtained by the following formula (Lange and Boehnke, 1982).

$$n = \text{Log}(1-P) / \text{Log}(1-2c/k)$$

where c = desired maximum distance (M) between a marker and a gene.

n = number of polymorphic markers required.

p = proportion of circular genome.

k = total length of genome (M).

This expression is based on a circular genome. In practice, the n would be about 20% to 30% higher due to the fact that the chromosome ends do not provide the same level of desired saturation.

For genetic analysis of QTLs, the marker should not be more than 20 cM from the loci (one marker every 40 cM) (Soller et al., 1976), whereas for introgression of such a QTL, a marker bracket of not more than 20 cM (marker-QTL distance not more than 10 cM) is desirable (Soller and Plotkin-Hazan, 1977). A genome with markers every 20 cM would enable tagging any gene of interest with a selection fidelity of 99% (Tanksley, 1983). Simulation studies by Beckmann and Soller (1986) have revealed that the proportion of coverage to any density, as function of the total number of randomly distributed markers per 1000 cM, is fairly independent of total genome size, and of the specific size distribution of chromosomes with in a chromosomal size range of 50-150 cM. The total number of markers required per 1000 cM in order to provide a given proportion of genome coverage is a function of the maximum spacing acceptable between markers.

The basic procedure in construction of a linkage map involves following inheritance of the markers in the appropriate pedigree. The approach of counting recombinants is not appropriate as the data is fundamentally incomplete (Lander and Green, 1987). The genetic distance estimated by two point analysis is a rough approximation of the actual distance as only a limited number of co-informative meioses are studied. Use of multipoint analysis can overcome this problem. Lander et al., (1987) developed a computer program 'MAPMAKER' for constructing primary genetic linkage maps of experimental and natural populations. It is based on simultaneous multipoint analysis of any number of loci. The linkage analysis is based on the maximum likelihood method. For each possible map, the probability that the map would have given rise to the observed data is computed. This probability is the likelihood of the map and the best map is the one with the highest likelihood. The ratio of the likelihoods between two maps provides a simple measure of how much better one fits the data than the other. Although no simple statistical test exists for this comparision of one order to another, odds of 100:1 or 1000:1 against are usually considered reliable for rejection of the order with smaller likelihood. This method can be applied even when the modes of inheritance and amounts of data vary among loci.

2-6-2-1. Restriction fragment length polymorphism (RFLP) markers.

Restriction fragment length polymorphisms are homologous fragments of DNA that vary in length after being cleaved with a restriction endonuclease (Grodzicker et al., 1974). Since its first use by Grodzicker et al., (1974), to map temperature sensitive

mutants of adenovirus, RFLP analysis has found many applications. Botstein et al., (1980) were perhaps the first to explore the application of the RFLP analysis for constructing a genetic map in humans which drew the attention of plant scientists. Despite its recent discovery, RFLP analysis has already been used in construction of linkage maps for many important crops such as tomato (Tanksley et al., 1988; Paterson et al., 1988), pepper (Tanksley et al., 1988), maize (Helentjaris et al., 1986), rice (McCouch et al., 1988) lettuce (Landry et al., 1985), potato (Gebhardt et al., 1989), lentil (Havey and Muehlbauer, 1989), Arabidopsis (Chang, 1988), *Brassica* sp. (Slocum et al., 1990; Landry and Hubert,1991; Song, 1991), barley (Huen, 1991) and sorghum (Whitkus, 1992).

The molecular basis of polymorphisms observed in the length of the restriction fragments, is most often due to single-base substitutions that create or abolish recognition sites for a restriction enzyme (Burr et al., 1983). Transpositions, deletions, insertions and other chromosome rearrangements also cause changes in restriction patterns. Such polymorphisms are stably inherited. Evola et al., (1986) followed 16 random genomic and cDNA RFLP markers in three inbred lines of maize for 6 to 11 generations without variation in restriction pattern. The rate of mutations, that cause differences in restriction patterns were found to be less than 10^{-4}/nucleotide/generation.

The presence of specific fragments (RFLP) in an individual can be tested by cutting the DNA with a restriction endonuclease, separating the fragments based on

size using agarose gel electrophoresis, transferring the DNA to a suitable membrane and hybridizing with the appropriately labeled DNA probe homologous to the fragment. DNA probes that include highly repetitive DNA sequence are not suitable as they hybridize with a large number of DNA fragments resulting in a continuous smear. Hence, unique DNA sequences are generally used as probes in determining RFLPs.

A. Development of probes.

A large fraction of the genome of most eukaryotic organisms is highly reiterated. Often these repeats are interspersed with unique DNA sequences in a manner that makes it difficult to isolate clones consisting entirely of single-copy DNA. The proportion of repeated DNA and the extent to which it is interspersed with single-copy DNA is generally a function of the overall DNA content of the species being studied (Flavell et al., 1980). For species with small genome like *Arabidopsis*, (haploid DNA 145 mbp, 501 cM) the majority of the nuclear DNA is single copy, interrupted by repeats (Pruitt and Meyerowitz, 1986). Crops like wheat (15966 mbp) and maize (2500 mbp) have unique sequences interspersed with repetitive sequences. The number of unique sequences and their interspersion, vary from species to species. Helentjaris et al., (1985) have observed abundant variability in maize whereas in contrast, domesticated tomato lines do not show appreciable variability (in tomato, only 3 out of 22 cDNA clones tried, detected polymorphism between two lines).

The presence of repetitive sequences in genomic DNA of higher eukaryotes necessitates selection of unique DNA sequences for use as probes in RFLP analysis. Currently there are two approaches to obtain unique sequences.

1. cDNA clones: Classical genetic studies and research at the molecular level both indicate that majority of the genes that are transcribed into mRNA are in single or low copy numbers (Tanksley and Pichersky, 1988). Complementary DNA (cDNA) clones derived from gene transcripts are therefore, a good source of low-copy DNA. Probes corresponding to mRNA's are made by reverse transcription followed by cloning of the resultant DNA. Bernantzsky and Tanksley (1986) have reported that, in tomato, out of 34 cDNA clones picked at random, 53% corresponded to single copy genes, 32% corresponded to two genetically independent loci, and 3-5% of the clones attach to multiple sites in the genome.

The length of cDNA clones is limited (less than 1kb), and consequently the development of autoradiographs may take more time because of low signal. Helentjaris et al., (1986) have reported that more than half of the cDNA probes used in maize resulted in very weak hybridization signals and were unfit for RFLP analysis. The cDNA probes map only transcribed regions, hence, they may be non-randomly distributed over the chromosome. It is possible that this could accentuate the distortion between the genetic map and physical map.

2. Genomic clones: The above mentioned problems are to some extent overcome by developing probes from genomic DNA. However, for most plant species the majority

of random genomic clones are likely to carry repeated sequences making them useless. Tanksley et al., (1988) suggest a two step process to obtain genomic clones of unique sequences.

a. Pre-cloning selection: Much of the DNA in eukaryotes is highly repeated. Highly repeated DNA is also highly methylated at cytosines. High copy DNA contains more methyl cytosine than low copy DNA. The proportion of low copy DNA in a digest could be enhanced by using a restriction endonuclease that acts on unmethylated portions of the genome (such as *Pst*I). It recognizes and cuts only sequences in which cytosine is not methylated. Tanksley et al., (1988) found that 92% of the *Pst*I-digested clones corresponded to sequences present only once in the genome, whereas, the majority of the *Eco*R1-digested clones of comparable size, had only 35% of low copy DNA.

b. Post-cloning selection: Large numbers of bacterial colonies can be grown directly on nylon hybridization filters, each of the colonies harboring a plasmid into which plant DNA has been cloned. The colonies are lysed on the filter, the denatured plasmid DNA is bound to the filter, and the filter is then probed with nick-translated, total nuclear DNA for 12-24 hours. The concentration of repetitive sequence in the genomic probe is relatively great and would result in a strong signal when used to probe a clone consisting of a complimentary, highly reiterated sequence. Conversely, a low copy number clone, probed with genomic DNA, would give a very weak signal. Figdore et

al., (1988) followed this stepwise procedure for obtaining low copy genomic DNA fragments and reported a high proportion (75-87%) of unique sequences.

2-6-2-3. Random ly amplified polymorphic DNA markers (RAPD).

This method of DNA polymorphism analysis was independently discovered by Williams et al., (1990) and Welsh and McClelland (1990). It is based on polymorphism of segments of the DNA that are amplified with single primers of arbitrary nucleotide sequence using polymerase chain reaction (PCR). These polymorphisms, detected as amplified DNA sequences from one parent but not from the other, are inherited in a Mendelian fashion. The amplified fragments serve as markers for generating linkage maps.

Possible sources of polymorphisms include deletion of a primer binding site, insertions or inversions that render priming sites too distant to support amplification or insertions that change the size of DNA fragment without effecting amplification (Williams et al., 1990). Williams and co-workers have also shown that RAPD markers in some cases can even detect single base changes in DNA. This was evident by the different amplification products they observed from primers differing in only one nucleotide.

The RAPD procedure is simple and involves amplification of polymorphic DNA fragments using short primers and the PCR machine. The resulting products are

run on an agarose gel and stained with ethidium bromide. The presence or absence of the fragment in a segregating population is scored directly from the gel. This method has several advantages over the RFLP method. It is easy and fast, requires a very small amount of DNA (25ng/reaction), does not involve the elaborate procedures of generating a recombinant library, isolation of low copy number fragments, restriction digestion, Southern transfer, hybridization, etc. Further, a universal set of primers can be used for genomic analysis in a wide variety of species without any prior knowledge about the DNA of the genome. Each RAPD marker is the equivalent of a sequence tagged site.

The extent of polymorphisms detected by the RAPD method is greater than that detected by RFLP method (Williams et al. 1992). In an intraspecific cross in tomato, the extent of polymorphism detected by RAPD was 63% while the RFLP and isozyme markers detected 16% and 0% of polymorphisms respectively (Foolad et al., 1993). The greater number of polymorphisms detected by RAPD is partly due to the ability of RAPD method to detect polymorphisms in repetitive DNA segments of the genome. Williams et al., (1990) reported 5 out of 11 polymorphisms were from repetitive segments in soybean, while, Reiter et al., (1992) observed 9 out of 18 polymorphisms were from repetitive DNA segments in *Arabidopsis thaliana*.

The Mendelian segregation of RAPD markers have been demonstrated in alfalafa, *Arabidopsis*, pine, diploid banana etc., (Echt et al., 1992; Reiter et al., 1992;

Carlson et al., 1991; Faure et al., 1993; Roy et al., 1992). The vast majority of the markers are dominant. However, a proportion (upto 43% in *Arabidopsis*) of the total polymorphisms do not show the expected Mendelian ratio. Huen and Helentjaris (1993) investigated the effect of genomic background on amplification of a fragment by subjecting a partial diallel in maize to RAPD analysis. Over 95% of the markers were unambiguously scored in the F_1 generation.

RAPD analysis is shown to be very efficient in identifying molecular markers linked to the targeted region of the genome. Near-isogeneic lines were used to identify RAPD markers specific to the small introgressed region on chromosome 6 containing gene (Mi) for nematode resistance in tomato (Klein-Lankhorst et al., 1991). Near-isogeneic lines were also used to identify RAPD markers tightly linked to genes or regions having genes for disease resistance (Martin et al., 1991; Paran et al., 1991; Haley et al., 1993). Chalmers et al. (1993) adopted RAPD assay on a pooled double-haploid population of barley to identify markers linked to a QTL for milling energy requirement.

A new approach, termed bulked segregant analysis, which obviates the use of near isogeneic lines, was developed by Michelmore et al. (1991). It involves identifying polymorphisms between two bulks or pools containing individuals from a segregating population. The two bulks are different with respect to a particular trait or genomic region and seemingly heterozygous with respect to all other regions.

Michelmore et al., (1991) were able to define a 25 cM marker windows, on either side of the locus responsible for downy mildew resistance in lettuce.

Williams et al., (1990) mapped 11 polymorphisms generated by various primers in soybean by using 66 F_2 individuals from a cross between *Glycine max* and *G. soja*. The map positions have been compared and confirmed using an existing RFLP map. They have also reported an average polymorphism of 1 RAPD marker per primer for maize, 0.5 per primer for soybean and 2.5 per primer for *Neurospora crassa*. Welsh et al., (1991) mapped four polymorphisms in mouse (*Mus musculus*) by analyzing a set of recombinant inbreds. The speed and efficiency of RAPD approach in constructing linkage maps have been well demonstrated. Chaparro et al., (1992) were able to create a 191 marker RAPD map of loblolly pine in only 6 person-months. While, Reiter and co-workers (1992) placed 250 new genetic markers on a recombinant-inbred population of *A. thaliana* in only 4 person-months. They also adopted a 'local mapping' technique to saturate a specific region by pooling recombinant inbred lines based on their genotype. Torres et al., (1993) used RAPD, isozyme and RFLP markers in an F_2 segregating population of *Vicia faba* to construct a preliminary linkage map consisting of 11 linkage groups.

A major disadvantage of RAPD markers is that most of them are dominant. Thus it is not possible to differentiate homozygotes from heterozygotes. Dominant markers linked in repulsion provide little information on genetic distance (Allard,

1956). Williams et al. (1990) suggest that the exact genotype could be assessed by using tightly linked RAPD markers, in pairs, each diagnostic for a different parental type. However, the confidence with which the heterozygote could be identified depends on how tightly the markers are linked. Another approach to overcome this problem is to select markers in coupling phase (markers residing on a single chromatid), as can be found in recombinant inbred or backcross populations, for generating linkage maps. In F_2 intercross populations, RAPD markers dominant in one parent provide the best linkage maps (Williams et al., 1992). Computer simulation studies have shown that dominant markers in coupling are as efficient for mapping as codominant markers (Tingey and del Tufo, 1993).

2-7. Mapping populations.

The basic procedure for developing a linkage map involves following the segregation of markers in a segregating population of a cross between two inbred lines. However, the choice of species and parental lines influence the frequency of detectible polymorphisms. Adequate inter-varietal polymorphisms have been reported in crops like maize (Helentjaris et al., 1985) and rice (McCouch, 1988), while in tomato, plymorphisms within the cultivated species is low (Helentjaris et al., 1985), necessitating the use of inter-specific crosses. As an alternative, in crop species with low polymorphisms, Beckmann and Seller (1985) recommend the use of a DNA insertion techniques to generate additional RFLPs *de novo*. A direct consequence of the insertion into host, is the interruption of the indigenous sequence, whether coding or

not-coding, and the generation of a RFLP at the site of insertion. Selfing of such individuals brings the inserts to the homozygous condition. This approach is limited by the time and efforts involved in generating a number of polymorphisms and the subsequent selfing to generate the homozygous lines. Besides, the insertion event may also cause a mutation, rendering it unfit for use as an RFLP.

The genetic analysis and mapping of QTLs depends on the magnitude of its QTLs phenotypic effect. The smaller the magnitude, the more progeny required to map it. An estimate of the magnitude of the QTLs effect can be made using Wrigtht's formula (Wright, 1968).

The number of QTLs (k) segregating in a backcross or F_2 intercross between two strains with phenotypic difference D, can be estimated by the following formulae:

$k = D^2 / 16 * \sigma^2G.$ (Backcross)

$k = D^2 / 8 * \sigma^2G$ (F_2 intercross)

provided 1: The QTLs have effects of equal magnitudes.

2: The QTLs are unlinked.

3: The alleles in the high strain all increase the phenotype.

The total genetic variance explained by 'k' QTLs would be (1/k) or $D^2/16$ for backcross and $D^2/8$ for F_2 intercross. The quantity 'k' is called the number of effective factors in the cross and each QTL effects the phenotype by (D/k) and explains (1/k) of the genetic variance. If the assumptions are not satisfied, as is often the case, the number of effective factors k may seriously underestimate the number of QTLs

(Lander and Botstein, 1989). Under these circumstances, the above formula could be used to indicate the presence of at least one to a few QTLs with large effects.

A further improvement in the efficiency of QTL analysis is made possible by 'selective genotyping' (Lander and Botstein, 1989). This concept is based on the observation of changes in marker frequency in segregating population subjected to selection for a quantitative trait (Stuber et al., 1980). Individuals that provide the most linkage information are those, whose genotypes can be most clearly inferred by their phenotypes. Thus, more information is provided by progeny that deviate most from the mean phenotype, the extreme types. Lander and Botstein (1989) have shown that progeny with phenotypes more than 1 SD unit from the mean comprise about 33% of the total population and contribute 81% of total linkage information. Hence, they suggest growing a large segregating population and selecting only those individuals that are at least 1 SD deviation from the mean. This approach often results in a two to five fold reduction in the number of individuals genotyped for QTL analysis. However, selective genotyping probably cannot be applied to more than two independent traits simultaneously, without reducing the population to a very small number.

The F_2 intercross provides twice as much information as a backcross due to recombination in both the megagametophytes and microgametophytes. This allows detection of more distant linkages and resolution of tighter linkages (Tanksley, 1988), in addition to reducing the progeny size. A partially dominant QTL can be more easily

analyzed in a F_2 intercross than a backcross. The error in estimating genetic and environmental component is large, however, and requires larger population to resolve individual QTLs, especially when heritability of the trait under investigation is low.

Several strategies have been worked out to overcome these problems like the use of replicated progenies from F_3, F_4, vegetative clones, recombinant inbred lines (RILs), double haploids, S1 lines (selfed F_2), and backcross inbred lines (Soller and Beckmann, 1990; Cowen, 1988; Young et al., 1988). Although these strategies are elegant, they generally involve higher cost and more effort.

2-8. Mapping and analysis of quantitative trait loci.

Majority of economically important characters in higher organisms are quantitative. By definition, the difference between a quantitative and qualitative trait resides in the relative magnitude of allele substitution effects at a genetic locus (Comstock, 1978). In a quantitative trait, an allele substitution at a QTL shows a small effect compared to the total variation, and, the observed phenotype is the joint result of a roughly additive effect of a large number of genetic and environmental factors. Quantitative traits also show Mendelian inheritance (East and Hayes, 1911).

Classical biometrical techniques have been effectively used in partitioning the combined effect of loci affecting a quantitative trait into genetic (additive, dominant and epistatic) and residual (environmental and residual interactions) (Mather and Jinks,

1977). However, these methods do not provide information on the number of genetic factors involved in expression of the trait, their chromosomal location and the relative contribution of individual loci to trait expression.

A powerful approach for studying inheritance of quantitative trait is by using mapped genetic markers. The concept of using monogenic markers in the study and evaluation of QTLs was realized during the early part of this century. In fact, genetic maps of crop species were among the first to be constructed and predate the demonstration of DNA as the hereditary material. Sax (1923) was the first to demonstrate the linkage between seed color, a simply inherited trait, and some factors determining seed weight in beans. Subsequently, a number of scientists have contributed to the general concept and theory of using mapped genetic markers for identifying, locating and manipulating QTLs (Jaykar, 1970; McMillan and Robertson, 1974; Soller and Plotkin-hazen, 1977; Tanksley et al., 1982; Lander and Botstein, 1989). The advent of molecular markers has provided the required tools for extending this approach to map the entire genome at a marker density not possible before, and has led to refinement of techniques.

The determination of linkage between marker loci and QTL depend on the linkage disequilibrium between alleles at the marker and the QTL. Linkage disequilibrium can be created by crossing populations or individuals that differ in allele frequencies at marker locus and/or QTLs. This disequilibrium generates marker-

associated quantitative effects that can be detected by appropriate statistical analysis. Random genetic drift and epistasis can also cause disequilibrium, but hybridization has greater power to generate linkage disequilibrium.

The important conditions for marker-based analysis of QTLs are (Beckmann and Soller, 1986):

a. QTLs having effects large enough to be detected by linkage analysis must be present and not closely linked to deleterious alleles of same or some other trait.

b. Differentiating marker traits are located near QTLs.

c. Effects of QTLs are basically additive, relatively unaffected by genotype by environment interaction and relatively independent of their background.

There are three statistical approaches to map and study the QTLs, that have been used to varying degree of success. These approaches are discussed below.

2-8-1. Least-squares linear model estimation.

In a linear model the dependent variable is a linear function of the independent variable and is expressed as:

$$Y = b1(F) + b2(T) + e$$

where, Y is the dependent variable (a trait under consideration), F and T are the independent variables (a strain or a genotype), and b1 and b2 are the respective

regression coefficients, e is the residual of Y not explained by the effects included in the model.

The principle behind the least square estimation is to find the parameter estimates that minimizes the residual sum of squares, thereby explaining as much variation in the dependent variable as possible. The model can be extended to include interaction effects and nested effects (hierarchieal). Significance is computed by comparing various model sum of squares to the residual sum of squares. If the model explains some of the variation in the dependent variable, then the model sum of squares will be greater than residual sum of squares, and its deviation from unity is tested by F-statistics. Linear model estimates have the property of minimum residual variance and unbiasedness and are easy to compute. They are based on the assumptions of normal and independent distribution of residuals with equal variance, and normal distribution of dependent variable.

The traditional approach for detection of a QTL in the vicinity of the marker was based on the study of single genetic markers, one at a time. The phenotypic means of progeny in each marker class are compared and the effect of the allele substitution is estimated based on the phenotypic difference. The inferred phenotypic effect is tested for significance by a simple 't' test.

Tanksley et al., (1982) mapped 21 marker-QTL associations using a backcross between parents *L. esculentum* and *Solanum pennelli*. Certain QTL coding for stigma exertion and leaf ratio had opposite effects to those expected from parents indicating the presence of both positive and negative factors for this trait in parents. Similar marker-QTL associations were also reported between 25 yield and yield related traits in maize (Stuber et al., 1987; Edwards et al., 1987). A large number of F_2 progeny were used in this study due to the low heritability of yield related traits due to the strong environmental influence. The proportion of variation associated with individual marker loci varied from 0.3 to 16% of the phenotypic variation.

Weller et al., (1988) reported marker-QTL associations in a F_2 intercross between parents *L. pimpinellifolium* and *L. esculentum*. In 14 of the traits, they observed highly significant effect of opposite sign to the overall difference between the parental lines. Also a general linear model was used to establish linkage between eight QTLs and RFLP markers in a F_2 segregating population of a cross between *Glycene max* and *Glycene soja* (Keim et al., 1990).

Trait based mapping: An alternate approach to detect linkage between a marker and a QTL was suggested by Stuber (1980), who observed a change in the frequency of marker alleles in populations subjected to selection for higher grain yield in maize over several generations. This led to the concept that selection for a trait in a segregating

population between two inbred lines would result in an increase in marker frequency of linked markers in the high line.

Lebowitz et al., (1987), showed that trait based analysis could be an useful approach, especially under situations where only a selected portion of the population remain after exposure to stress (eg. disease or pest). Trait based analysis would also be particularly useful in detecting pleiotropic effects of marker loci on quantitative traits in a segregating population.

As a result of recombination, homozygous marker genotypes in progeny represent a mixture of genotypes at the QTL. The difference in the mean quantitative values between alternate homozygous-linked marker genotypes will be attenuated by recombination between the marker and QTL. This results in an under estimation of the QTL effect (Lander and Botstein, 1989). As the distance between the QTL and the marker increase, the variance within the marker genotype class increases. The apparent dominance at the QTL also increases within marker genotype variance (Asins and Carbonnel, 1988). Under these situations, the linear model does not provide a correct estimate of the QTL effect. However, if a QTL is bracketed by markers, unbiased estimates for the QTL effect can be obtained by a linear model analysis of the non-recombinant types. Recombination frequency can be estimated using the ratio between the effects measured for recombinant and non-recombinant types. Knapp et al., (1990)

demonstrated the possibility of estimating recombination frequency by using marker brackets.

The linear model also lacks the discriminatory power to distinguish between a linked QTL and a pleiotropic effect of the genetic marker. A major shortcoming of linear model, in the light of extensive molecular polymorphisms, is the inability to carry out multiple comparisons or to study the effect of several genetic markers on several quantitative traits.

2-8-2. Moments method estimation (MME).

The principle behind MME is that incomplete linkage between marker and a QTL results in the skewed distribution of the individual marker-genotype classes (Zhuenchenko, 1979a). Hence, the difference in skewness can be used to estimate linkage distance between the QTL and the marker. The degree of skewness is a function of recombination and it is possible to construct a series of equations in which the basic statistics of the marker-genotype distributions (means, variance, skewness) are expressed in terms of the means and variances of the QTL-genotype distributions and recombination. Mean, variance, skewness and kurtosis are the four central moments and the non-central moments are derived by substituting some other values for the mean.

Though calculations are fairly simple and the theory is sound, in practice, it often leads to many estimates outside the parameter space (negative values for recombination and variance) (Zhuenchenko, 1979b). The non-parametric nature of MME also reduces its statistical power.

2-8-3. Maximum likelihood method (ML).

This is the most satisfactory approach to study of linkage analysis. The concept behind the ML method is to find the parameter estimates that best match the sample data. The ML method is a parametric test and is based on the assumption of normal distribution of the data. For each possible map, the probability that the map would have given rise to the observed data can be computed. The probability is called the likelihood map.

Lander and Botstein (1989) proposed the application of Log of odds (LOD) approach and interval mapping for mapping and analysis of QTLs. They adopted the use of linear regression of phenotype on genotype as a special case of the ML method. The phenotype ⌀ and the genotype g1 in an individual are related by the equation;

$$\varnothing = a + bg_i + \varepsilon$$

Where, g_i is the number of B alleles (0,1), ε is the random normal variable with mean 0 and variance σ^2, and a b and σ^2 are unknown parameters. The linear regression solutions ($\hat{a}$, $\hat{b}$,$\hat{\sigma}^2$) are the maximum likelihood estimates (MLE) which maximize the

probability L(a ,b ,σ^2) that the observed data have occurred. The MLE are compared to constrained MLEs ($\hat{\mu}_A$, 0, $\hat{\sigma}^2_{B1}$) under the assumption that no QTL is linked.

LOD = log10 ($\hat{a}$, $\hat{b}$, $\hat{\sigma}^2$) / L($\hat{\mu}_A$, 0, $\hat{\sigma}^2_{B1}$).

The LOD or log of odds is a quantitative expression for the likelihood of linkage and is the ratio of the probability for the data set at the maximum likelihood values or r to the probability at r = 0.5 (marker-loci are unlinked). When the LOD score exceeds a predetermined threshold, a QTL is declared present.

A genome with markers spaced evenly throughout is amenable for 'interval mapping' suggested by Lander and Botstein (1989). This approach is seen as a major breakthrough in improving the efficiency of mapping. Interval mapping measures the effect of each genome segment, located between pairs of marker loci, rather than the effects associated with individual loci. This approach is based on the assumptions of no double-cross over between the markers. The threshold of the LOD score to declare the presence of a QTL varies from 2-3, and is higher in dense maps compared to sparse map.

To accomplish interval mapping, Lander and Botstein (1989) designed a computer program using the MLE technique with missing data and EM algorithm. This program, MAPMAKER/QTL (Paterson et al., 1988) can be used for analysis of backcross and F_2 intercross data.

Weller (1986) used the MLE for mapping and analyzing QTL in F_2 segregating population. In an attempt to study marker-QTL linkage relationships in an interspecific cross between *L.esculentum* and *L. pimpinellifolium,* Waller (1988) employed MLE for estimating recombination frequency for those marker combinations that showed statistical evidence of linkage.

Paterson et al., (1988) demonstrated the efficacy of interval mapping (MAPMAKER/QTL) by dissecting QTLs into Mendelian factors in a interspecific backcross of tomato (*L. esculentum X L. chmielewskii*). They used a saturated linkage map (marker every 20 cM) and mapped 15 QTLs affecting fruit weight, fruit pH and soluble solids at a LOD score of 2.4. These QTLs accounted for between 44 and 58% of the phenotypic variation in these traits. Paterson et al., (1991) studied the genetic basis of quantitative variation in phenotype under different environmental conditions in a segregating backcross in tomato (*L. esculentum X L. cheesmanii)*. They mapped 29 putative QTLs effecting fruit mass, soluble solids and fruit pH under three different environments. Only four of the QTLs were active in all the three environments tested. The response of the remaining (25) QTLs indicate the considerable effect of environmental factors on QTL expression.

In a QTL analysis across environments in maize, Schon et al., (1994) observed relative consistency in QTL positions but differences in levels of significance and size

of estimated effects. Similar differences in magnitude of QTL effects across environments were also observed in barley by Hayes et al., (1993)

It is evident from these studies that the number of QTLS affecting a trait are far numerous than could be detected in a single environment. Stable QTLs active under different environments show a difference in magnitude of genetic effects. The number and relative importance of QTLs affecting a trait are under considerable environmental influence.

CHAPTER 3

A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers.

3-1. Introduction.

Papaya (*Carica papaya* L.) is a popular fruit crop in Hawaii and other tropical regions. The latex from the unripe fruits also yields the proteolytic enzyme papain, which has many industrial uses. *Carica papaya* is a polygamous species with both unisexual and bisexual tree types among cultivated papaya. Although hermaphrodite plants are preferred for commercial cultivation, sex expression and the fruit development is greatly influenced by environmental conditions (Awada, 1958; Awada and Ikeda, 1957). Early attempts to identify markers that co-inherit with sex led to the discovery of a loose linkage between sex, flower and petiole color (Hofmeyr, 1939). This is the only previous report involving genetic markers in papaya. The development of a detailed linkage map for papaya will greatly enhance our understanding of papaya genetics and improve the efficiency of crop improvement programs, especially those involving quantitative traits. Besides, the segregation of sex forms in cultivated papaya offers a good model to investigate the location and genetics of the factor involved in sex determination.

The discovery of restriction fragment length polymorphisms (RFLP) (Grodziker, 1974) provided a tool that offered a potentially unlimited number of DNA-based markers (Helentjaris et al., 1985) that could be used to map and

characterize an entire genome (Botstein, 1980). Within a span of 10 years, RFLP-based linkage maps have been constructed for several economically important crops including maize (Helentjaris et al., 1986), tomato (Bernatzky and Tanksley, 1986) lettuce (Landry et al., 1987), potato (Gebhardt et al., 1989), rice (McCouch et al., 1988) and soybean (Tingey et al., 1989). The potential utility of a saturated linkage map for understanding the complex nature of inheritance of quantitative traits has already been shown in tomato (Paterson et al., 1988).

Recently, a new class of DNA polymorphisms, based on the polymerase chain reaction (PCR) and called randomly amplified polymorphic DNA markers (RAPD), has been discovered (Williams et al., 1990; Welsh and McClelland, 1990). This approach is based on the PCR amplification of template DNA using 10-base-long random primers having a GC content of 50% or higher. The primer binds to the template DNA at random positions depending on the sequence of the primer used. If primers bind close to each other on opposite strands of the template DNA, a fragment will be amplified. Each primer can direct the amplification of several unrelated regions in the genome. The polymorphisms can be scored within hours by gel electrophoresis and ethidium bromide staining, compared to RFLP analysis which involves restriction digestion, southern blotting, labelling, hybridization and autoradiography. The added advantage of RAPD is its ability to detect greater polymorphism than RFLP analysis (williams et al., 1990; Foolad et al., 1993).

Due to the speed and ease with which it is performed, RAPD analysis has quickly found applications in population studies (Welsh et al., 1991; Hu and Quiros, 1991) biosystematics (Stiles et al., 1993), gene tagging (Klein-lankhorst et al., 1991; Martin et al., 1991) and especially in genetic mapping. The Mendelian segregation of RAPD markers has been demonstrated in crops like soybean (Williams et al., 1990), conifers (Carlson et al., 1991) and alfalfa (Echt et al., 1992). RAPD based linkage maps are available in pine (Chaparro et al., 1992) *Arabidopsis (*Reiter et al., 1992), faba bean (Torres et al., 1993). One limitation to RAPD mapping is that majority of RAPD markers are dominant and hence, they can not be used to distinguish dominant homozygotes from heterozygotes. Despite this disadvantage, RAPD methodology has an excellent potential for use in developing linkage maps quickly and easily. In this study we present the result of our efforts to construct the genetic linkage map for papaya based on RAPD markers as a first step towards understanding the complexities of the papaya genome.

3-2. Materials and Methods.

3-2-1. Mapping population.

The segregating F_2 population of a cross between the Hawaiian cultivar Sunrise, and Line 356-3, a selection made from Florida introduction, was used for the present study. An F_2 population was raised at the University of Hawaii Poamoho Experiment Station (153 plants) and Waimanalo Experiment Station (100 plants). The cultivar Sunrise, inbred for over 25 generations, was used as the male parent and the Line 356,

derived from the third sib-mated generation, was used as the female parent. Morphologically, the parents are distinct. 'Sunrise' is a gynodioecious, tall, late bearing commercial cultivar and Line 356 is a dioecious, early bearing, semi-dwarf selection.

3-2-2. DNA isolation.

One young leaf from the upper one-third of the canopy of each mature, field-grown plant was collected and stored on ice. Midribs and main veins were removed and the tissue was frozen in liquid nitrogen and stored at -20° C. The procedure for the DNA extraction was based on the method of Dellaporta et al., (1983). The chamber of a coffee grinder (Salton table top) was pre-cooled by grinding pieces of dry ice for one min. About 10 g of frozen leaf along with pieces of dry ice was homogenized to powder by grinding for 40s. The powdered tissue was poured directly into a beaker containing 150 ml of extraction buffer [100 mM Tris-HCL (pH 8.0), 50 mM EDTA (pH 8.0), 500 mM sodium chloride (NaCl), 1.25% sodium dodecyl sulfate (SDS), 200 ppm 2-mercaptoethanol] at 65° C. After incubation for 10 min at 65° C, the beaker was cooled on ice and 50 ml of ice-cold 5 M potassium acetate (KOAc) was added. The contents were incubated on ice for 30 min and the debris was removed by centrifugation at 6870xG for 20 min. The DNA was precipitated by adding 0.8 volume of isopropanol and incubation on ice for 30 min. The precipitate was collected by centrifugation at 6870xG for 15 min. The pellet was dried and redissolved in 10 ml of TE [10 mM Tris-HCL (pH 8.0), 0.5 mM EDTA (pH 8.0)]. NaCl was added to bring

the concentration to 0.7 M. And to this mix, an equal quantity of 1% cetyltrimethylammonium bromide (CTAB) was added after incubation for 30 min at room temperature, the pellet was collected by centrifugation at 12100xG, washed with 70% ethanol containing 0.1 M sodium acetate, and dissolved in 2.75 ml of TE containing 0.3 M of sodium acetate. An equal quantity of 4 M NH_4OAc was added, after 30 min at O° C. The supernatant was collected by centrifugation at 12100xG for 15 min. The DNA was precipitated by adding 0.8 volume of isopropanol, incubating on ice for 30 min, and centrifugation at 12100xG for 15 min after drying under vacuum for 20 min. The pellet was dissolved in 0.5ml TE and made 0.3 M in NaOAc by the addition of 1/10 volume of 3 M NaOAc. Contaminating RNA was removed by digesting with 10 μg of RNAse A for 30 min at 37.5° C. This was followed by extraction with equal volumes of phenol, phenol-chloroform (1:1), and chloroform. The DNA was precipitated by adding 0.8 volume of isopropanol and pelleted by centrifugation as before. The pellet was dissolved in 250 μl of TE and the DNA content was estimated by fluorometry using the Hoefer DNA fluorometer (San Francisco, USA) and DNA standards and procedure supplied by manufacturer.

3-2-3. Markers and RAPD analysis.

Sex of the plant (female or hermaphrodite) and the color of the fruit flesh (pink or yellow) were the two morphological markers scored for the population.

RAPD analysis.

A total of 596 (10 base-long) primers were used for PCR amplification. Five hundred primers (kits A to Y) were obtained from Operon™ Technologies (Alameda, California) and 96 primers were synthesized at the University of Hawaii Biotechnology-Molecular Biology Instrumentation Facility. Each potential polymorphism was checked and confirmed at least three times. Ninety-six primers that detected polymorphisms between the parents were scored in the F_2 population.

3-2-4. DNA amplification.

The PCR procedure described by Williams et al., (1990) was followed with minor modifications. Amplification reactions were carried out in 25 μl reaction mix containing 0.2 μM random primer, 150 μM of each deoxytrinucleotide triphosphates, 2 mM $MgCl_2$, 10 mM TRIS-HCl (pH 8.3), 50 mM KCl 0.001% gelatin with 15 to 25 ng of template DNA and 0.75 to 1.25 U (units) of Taq DNA polymerase. Reaction conditions were 45 cycles consisting of 1 min at 95° C, 1 min at 35° C and 2 min at 72° C. The PCR reaction was concluded by a 5 min extension at 72° C. Products were analyzed by electrophoresis at 50 V for 6-8 hours in 1.5% agarose gels, stained with ethidium bromide and photographed under UV light by using Polaroid 667 film (Fig. 1). The negatives were used for scoring bands. Gels were scored for presence or absence of the corresponding band and the absence of a band was confirmed by repetition.

3-2-5. Data analysis.

Goodness-of-fit to the expected segregation of 3:1 (dominant), 2:1 (sex) or 1:2:1 (codominant) for the F_2 population was tested by chi-square analysis. The linkage map was constructed using MAPMAKER/EXP software (Lander et al., 1987). Polymorphic markers were grouped at LOD 4.0 and recombination frequency (r) of 0.35. Within a group, a LOD threshold of 2.0 was used to order the markers by using MAPMAKER 'order' command. The markers in a group with a distance <35 cM were ordered and the remaining markers in that group (with $r = 0.35 - 0.45$) were placed with a minimum threshold of LOD 1.5. Since the RAPD markers are dominant markers with lower information per individual (especially in repulsion phase), two separate maps (not shown) with markers in coupling phase were constructed to confirm the linear order of the markers. The map distances were reported in centiMorgan (cM) using Haldane correction.

3-3. Results.

3-3-1. Polymorphisms.

The parents, 'Sunrise' and Line 356, were screened with 596 random primers. Sixty (10%) of these primers detected a total of 96 polymorphisms of which 61 polymorphisms satisfied the mapping criteria (Table. 1). An average of 8 strong bands, ranging from 200 bp to 2500 bp, were amplified per primer, although about 15% of the primers did not give any amplification product. One of the 96 polymorphic markers (OPM6) was inherited in codominant fashion. Sixty percent of the polymorphic

markers gave a single polymorphism per primer, 37% gave two polymorphisms per primer, and 3% gave three polymorphisms per primer. Three polymorphic markers did not segregate in the F_2 population. Two of these markers were dominant in Line 356 (OPA4, UHE5) and one was dominant in 'Sunrise' (OPL8).

3-3-2. Segregation analysis.

Chi-square analysis was performed to check for goodness of fit to the expected Mendelian segregation (Table 2). Inheritance of sex and 48 RAPDs (80%) showed the expected Mendelian segregation in the F_2 population. Eight of the 13 polymorphisms that did not follow expected segregation exhibited very strong deviations from expected ratios (significant at $P<0.01$).

3-3-3. Genetic linkage map.

A total of 72 polymorphisms were grouped into linkage groups using a minimum LOD score of 4.0. Ten of these that could not be ordered unambiguously and that did not meet the set criteria were discarded. Hence, the genetic linkage map was constructed with 61 RAPD markers and 1 morphological marker, which were ordered into 11 linkage groups (Fig. 2), comprising a total of 999.3 cM of the papaya genome. About 57% (29) of the intervals were 0-20 cM wide, 20% (10) were 21-30 cM wide, 20% (10) 31-40 cM wide and 3% (2) were 41-45 cM wide.

Linkage group 1, with 9 loci, included the sex locus. The markers flanking the sex locus (OPT12 and OPT1C) were inherited in expected dominant fashion (3:1), while, the sex locus itself, segregated in the expected ratio of two hermaphrodite plants to one female. Analysis of the recombinants in this region revealed an overabundance of female plants (18) with cross overs as opposed to hermaphrodites (3).

Linkage group 7 is the largest group (217.1 cM), with 12 loci accounting for over 1/5th of the total distance covered by the map. Seven of the 11 linkage groups have 5 or more loci.

3-4. Discussion.

3-4-1. Polymorphisms and segregation.

We have screened a total of 596 random primers to detect 96 polymorphisms between the parents 'Sunrise' and Line 356. The observed frequency of 0.16 polymorphisms/primer appears to be low compared to polymorphisms observed in inter-varietal crosses of faba bean (Torres et al., 1993), alalfa (Echo et al., 1992), and *Arabidopsis thaliana* (Reiter et al., 1992). Though the parents for the present study were selected to maximize segregating loci, the magnitude of the morphological differences between the parents does not seem to correlate well with differences at the molecular level. This observation is in conformity with the findings of Stiles et al., (1993), who reported a narrow genetic diversity among 10 domesticated papaya cultivars, including the parents used for the present study.

Mendelian segregation of 80% of the RAPD loci observed in the $_F$ segregating population is higher than that reported in *Betula alleghaniensis* (Roy et al., 1992), alfalfa (Echo et al., 1992) and conifers (Carlson et al., 1991). The larger size of the $_F$ population and the use of an intraspecific cross may have contributed to the reduced level of segregation distortion observed in the present investigation.

The lack of segregation for two polymorphisms having dominant markers in Line 356 can be attributed to maternal inheritance of plastids. The similar unexpected uniparental inheritance in the F_2 population of the marker (OPL8), which was dominant in the male parent 'Sunrise' is not clear. We have previously shown that RAPD markers in the progeny from third generation sib-mated Line 356 segregated for other RAPD markers (Stiles et al., 1993). Perhaps, the plant of Line 356 used as female parent in the generation of the F_2 in the year 1986 might have been heterozygous or homozygous dominant for this marker.

3-4-2. Sex determination.

We have mapped the sex locus to a region of linkage group 1 flanked by markers separated by 14 cM, and these markers are in coupling phase. Since the sex of a papaya plant can be determined only at the time of flowering, the current practice for growing hermaphrodite plants is to plant three plants/hill, followed by selection of desired type at flowering. With RAPD analysis for the flanking markers, we can now forecast the sex of the plant at the two leaf-stage with an accuracy of 98%.

Interestingly, the segregation of the flanking markers and the sex locus fit the expected ratios, which are different. Sex in papaya is determined by a single gene with multiple alleles (Storey, 1938; Hofmeyr, 1938). The alleles for male (M_m) and hermaphrodite (M_h) are dominant over female (m). The homozygous dominant types (M_mM_m, M_mM_h, M_hM_h) are non-viable. The lethality of these types is attributed to a closely linked recessive zygotic lethal factor 'L' and a cross-over suppression factor 'C' (Storey, 1976). Hofmeyr's (1967) genic balance theory of sex determination in papaya proposes that the male and hermaphrodite regions are inert, which accounts for the zygotic lethality in homozygous condition. The observed segregation of the sex locus and flanking loci, and the overabundance of female plants (18) among recombinants in this region compared to hermaphrodite plants (3), provide strong evidence in support of Storey's hypothesis. In summary, we have used RAPD analysis to delimit the sex locus and putative associated factors, to a 14 cM marker bracket in the linkage group 1.

3-4-3. Linkage map.

We have developed a primary genetic linkage map of papaya using 61 RAPD (60 dominant and 1 codominant) markers and 1 morphological codominant marker. The map is comprised of 11 linkage groups with a total mapped distance of 999.3 cM. We have covered over 70% of the papaya genome if as expected, the genome size is about 1000-1400 cM. This is a major improvement over the previous map involving three markers, covering only 41 cM of papaya genome (Hofmeyr, 1939). At present,

we can not assign any linkage group to a chromosome as the chromosomes are not characterized morphologically.

We feel that our papaya linkage map, having 51 intervals, with a mean distance between markers of 19.6 cM, and over 75% of the intervals shorter than 30 cM, is adequate for genetic analysis of quantitative traits. We have mapped several economically important quantitative traits such as plant vigor, node at first flowering, carpellody, fruit weight, and papaya ringspot virus disease resistance. The results of our study confirms the utility of RAPD markers in constructing genetic linkage maps.

Table 3.1 Sequence of primers from the 5' prime end.

Name	sequence	Name	Sequence
UHC5	'GAGTTCCGCA'	UHC11	'AGCAAAGGCC'
UHD4	'TTGGGACAGT'	UHD12	'TGGCGTTGCT'
UHE12	'ACCATCCCCA'	UHF3	'ATCTGTGTGG'
UHF5	'CACAGGTTCT'	UHF9	'GCATCTCAGT'
UHG12	'CCCTAGCTGT'	UHH11	'TTAGGGCCTC'
OPB11	'GTAGACCCGT'	OPD2	'GGACCCAACC'
OPD18	'GAGAGCCAAC'	OPD20	'ACCCGGTCAC'
OPE2	'GGTGCGGGAA'	OPE7	'AGATGCAGGCC'
OPE16	'GGTGACTGTG'	OPF12	'ACGGTACCAG'
OPG10	'AGGGCCGTCT'	OPH3	'AGACGTCCAC'
OPH13	'GACGCCACAC'	OPH18	'GAATCGGCCA'
OPI9	'TGGAGAGCAG'	OPI14	'TGACGGCGGT'
OPJ19	'GGACACCACT'	OPK3	'CCAGCTTAGG'
OPL12	'GGGCGGTACT'	OPL15	'AAGAGAGGGG'
OPM6	'CTGGGCAACT'	OPM13	'GGTGGTCAAG'
OPO10	'TCAGAGCGCC'	OPO15	'TGGCGTCCTT'
OPP15	'GGAAGCCAAC'	OPP5	'CCCCGGTAAC'
OPQ12	'AGTAGGGCAC'	OPR15	'GGACAACGAG'
OPR20	'ACGGCAAGGA'	OPS12	'CTGGGTGAGT'

Table 3.1 (cont.)

OPT1	'GGGCCACTCA'	OPT4	'CACAGAGGGA'
OPT12	'GGGTGTGTAG'	OPU13	'GGCTGGTTCC'
OPV14	'ACCCCCTGAA'	OPV16	'ACACCCCACA'
OPW2	'ACCCCGCCAA'	OPX17	'GAGACGGACC'

Table 3.2. Segregation of RAPD markers in F_2 population.

Primer	Present	Absent	Expected ratio	Chi-square
UHC5A	183	62	3:1	0.004
UHC5B	189	54	3:1	0.85
UHC11	187	60	3:1	0.028
UHD4	176	70	3:1	1.39
UHD12	163	35	3:1	5.27*
UHE12	196	46	3:1	4.36*
UHF3	183	63	3:1	0.015
UHF5	183	62	3:1	0.001
UHF9	176	66	3:1	0.55
UHG12	195	57	3:1	0.640
UHH11	176	74	3:1	2.58
OPB11	140	37	3:1	1.07
OPD2A	156	97	3:1	23.29**
OPD2B	187	60	3:1	0.028
OPD2C	176	72	3:1	1.93
OPD18A	160	80	3:1	8.44**
OPD20A	161	29	3:1	9.09**
OPD20B	128	57	3:1	3.02
OPE2	146	43	3:1	0.38

Table 3.2 (cont.).

OPE3	141		59	3:1	1.95
OPE7C	157		30	3:1	7.53**
OPE7D	125		40	3:1	0.034
OPE16A	134		109	3:1	49.94**
OPE16B	183		60	3:1	0.001
OPF12	140		46	3:1	0.001
OPG10A	198		50	3:1	2.84
OPH3	198		51	3:1	2.36
OPH13	192		56	3:1	0.64
OPH18B	162		86	3:1	11.86**
OPI9A	187		58	3:1	0.16
OPI9B	200		45	3:1	5.93*
OPI14	187		62	3:1	0.005
OPJ19	196		50	3:1	2.61
OPK3	139		55	3:1	0.90
OPL12A	150		43	3:1	0.61
OPL12B	177		10	3:1	37.46**
OPL15A	116		78	3:1	23.12**
OPL15C	143		42	3:1	0.40
OPM6 (Codominant)	43	95	58	1:2:1	2.73

Table 3.2 (cont.)

OPM13	152	45	3:1	0.37
OPP5C	163	33	3:1	5.27
OPO10	147	51	3:1	0.026
OPO15	149	51	3:1	0.006
OPP10	155	34	3:1	4.59*
OPP15A	157	38	3:1	2.87
OPP15B	146	40	3:1	1.02
OPQ12A	149	51	3:1	0.013
OPQ12B	156	42	3:1	1.30
OPR15A	150	50	3:1	0.005
OPR20A	143	44	3:1	0.14
OPS12A	127	58	3:1	3.61
OPT1B	155	42	3:1	1.23
OPT1C	145	52	3:1	0.12
OPT4	154	40	3:1	1.75
OPT12	135	47	3:1	0.027
OPU13A	153	43	3:1	0.82
OPV14A	139	51	3:1	0.10
OPV14B	152	39	3:1	1.89
OPV16	136	33	3:1	2.41

Table 3.2 (cont.)

OPW2	158	34	3:1	5.15*
OPX17	142	50	3:1	0.05
Sex	173	80	2:1	0.21

* indicates Chi-square of 3.84 (significant at $p < 0.05$).

** indicates Chi-square of 6.70 (significant at $p < 0.01$).

Foot note: For 25 markers, a total of 253 plants were scored and for the remaining markers 200 plants were scored. Those polymorphisms which could not be scored unambiguously or cold not be repeated were reported as missing, hence, the difference in total number of plants scored.

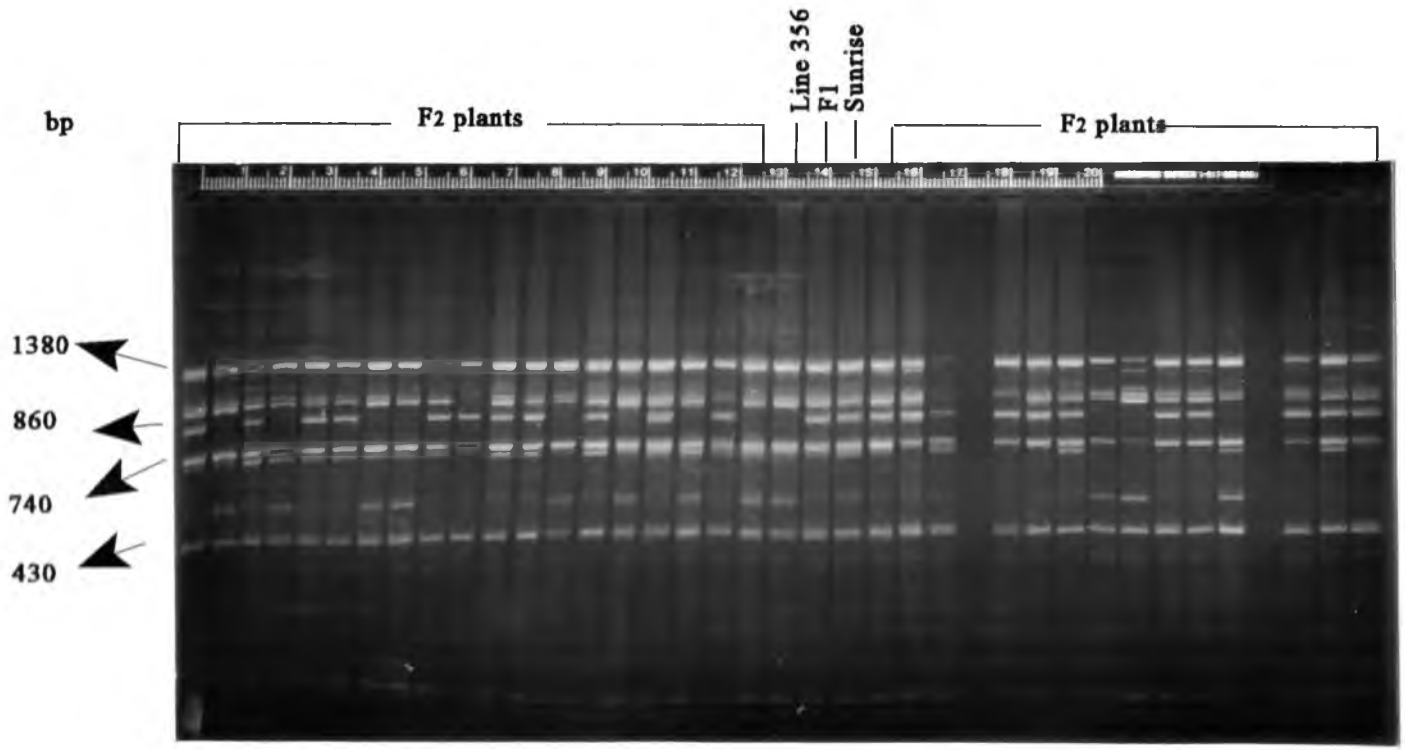


Fig. 3.1. segregation of a RAPD marker in Line 356 X 'Sunrise F_2 intercross population.

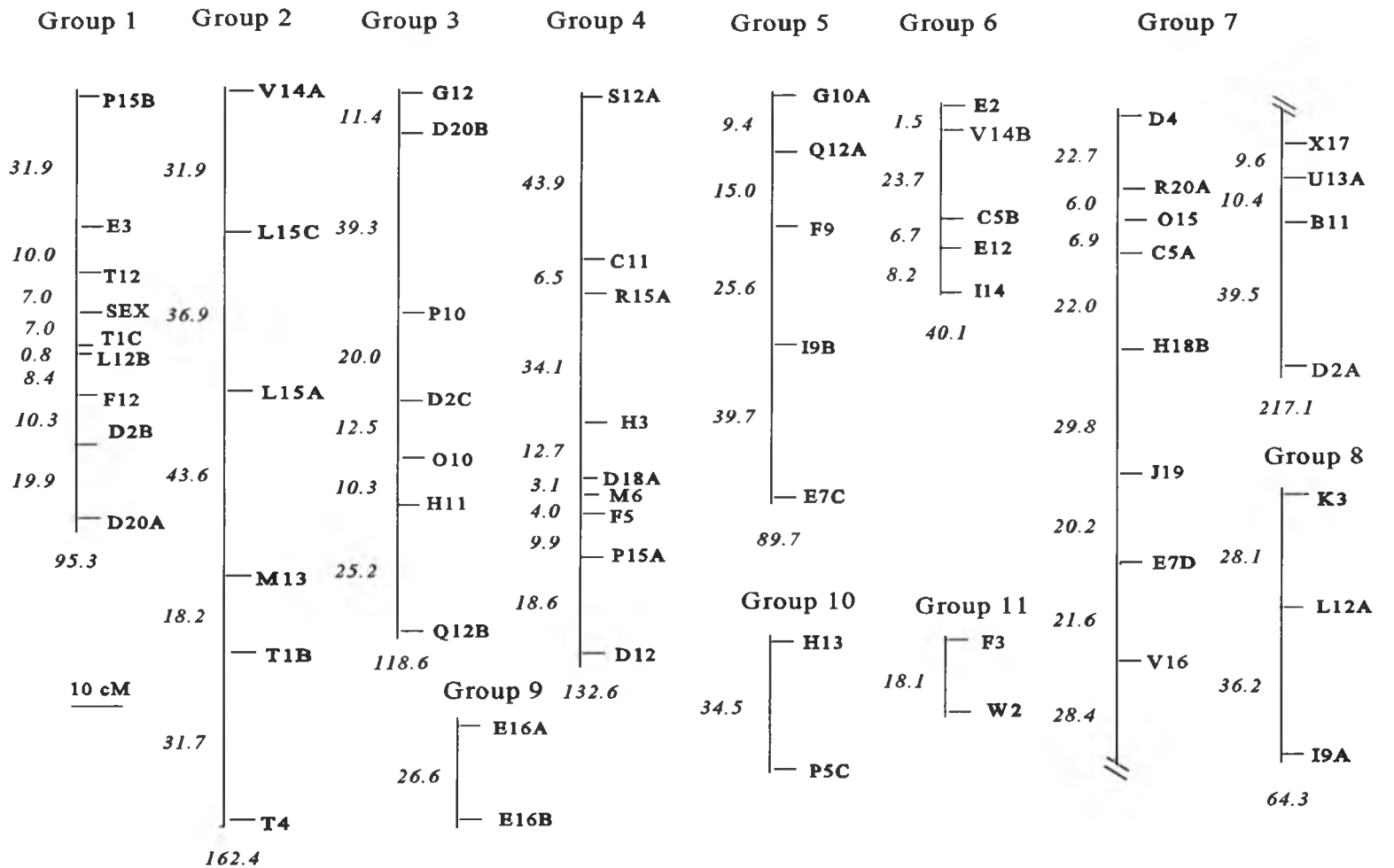


Fig.3. 2. Genetic linkage map of papaya. Marker names are indicated in block letters and the interval distances in cM are indicated in italics.

CHAPTER 4

Analysis of QTLs affecting plant vigor and precocity in papaya (*Carica papaya* L.).

4-1. Introduction.

Continuous variation in phenotype, observed in most of the commercially important traits in crop plants, has been a fascinating field of study since the rediscovery of Mendel's laws of inheritance. The pioneering work by Nilsson-Ehle (1909), Johannson (1909) and East (1916) provides a sound basis for QTLs, by attributing the continuous variation in phenotype to the action of many determining factors (polygenes), which follow Mendelian inheritance under the influence of environment. The elegant biometrical techniques that were developed later provided effective tools to estimate the number and mode of action of these factors or quantitative trait loci QTL)(Fisher, 1918; Mather and Jinks, 1971). However, these methods estimate average effects of the polygenic system and do not offer insight into the locations or individual contributions of QTLs. The early successful attempts to characterize QTLs by studying the Mendelian inheritance of morphological markers linked to QTLs emphasized the severe paucity of suitable morphological markers (Sax, 1923; Rasmusson, 1933; Thoday, 1961).

The recent discovery of DNA-based markers, including restriction fragment length polymorphisms (Grodziker, 1974) and randomly amplified polymorphic DNA markers (RAPD) (Williams et al., 1990; Welsh and McClelland, 1990) has provided the necessary tools to develop saturated genetic linkage maps suitable for locating and

characterizing individual QTLs. These markers rarely influence phenotype and enable construction of linkage maps that facilitate detailed genetic analysis. DNA marker-based genetic linkage maps are available for many important crop plants (Tanksley, 1989). The usefulness of such detailed maps in locating and characterizing QTLs has already been demonstrated (Paterson et al., 1988, 1991; Schon et al., 1993, 1994; Hays et al., 1993). Information on chromosomal location and action of individual QTLs is useful in selection and introgression of favorable genes into commercial cultivars.

We have developed a genetic linkage map of papaya based on RAPD markers. The map is based on an F_2 population of a cross between the Hawaiian cultivar Sunrise and breeding Line 356, an introduction from Florida. The map is 999 cM long, with 11 linkage groups representing over 70% of the expected papaya genome. In this paper, we present the result of our analysis of QTLs affecting plant vigor (plant height and stem diameter) and precocity, two economically important characters .

4-2. Materials and methods.

4-2-1. Parents and the mapping population.

The F_2 population of a cross between 'Sunrise' and Line 356 was used for QTL analysis. 'Sunrise' is a gynodioecious, late flowering, tall commercial variety of Hawaii, while Line 356 is a dioecious, early flowering, semi-compact selection derived from Florida introductions. The F_1 plant selected in 1987 by Dr. R.M.Manshardt, was

hermaphrodite and markedly heterotic with respect to growth parameters. A population of 100 F_2 plants, together with the parents, was grown out at Poamoho Experiment Station, University of Hawaii, for the present study.

4-2-2. Quantitative traits studied.

Plant height was measured as the distance in centimeters from the ground level to the tip of the growing point. Stem diameter was recorded 30 cm above ground level, and the same region was used for subsequent measurements. A total of five measurements were made of height and diameter, at intervals of 3 months, starting 4 months after planting. Data recorded for plant height, stem diameter, and growth rates during different periods were subjected to QTL analysis. Analysis of growth rates between measurements was undertaken to detect QTLs under seasonal environmental influence. Precocity was measured as the number of nodes above the cotyledonary node to the first flower-bearing node. Periods 1, 2, 3 and 4 refer to the periods between measurements: June-Sept, Sept-Dec, Dec-March and April-July 1992-93.

4-2-3. Genome composition.

The genotypes of F_2 individuals with respect to molecular markers, were generated by the computer program MAPMAKER/EXP (Lander et al., 1987). Regions of the genome that were homozygous for either parent were estimated using the flanking markers. Under situations where the homozygosity could not be estimated accurately, as is often the case with dominant markers, heterozygosity was assumed.

The smaller linkage groups with dominant markers in coupling phase were also assumed to be heterozygous.

4-2-4. QTL analysis.

The computer program MAPMAKER/QTL 1.1 (Paterson et al., 1988) was used to map and characterize QTLs. This program utilizes the maximum likelihood method and adopts the interval mapping technique to determine the chromosomal location of a QTL. The probability of the presence of a QTL at a particular location is expressed as a LOD score, which is the log of the odds that a QTL is present at a location to the odds that there is no QTL at that location. LOD scores are computed for the entire length of the map. The presence of the QTL at a location is declared when the LOD score exceeds a predetermined value.

The appropriate LOD threshold depends on the genome size and density of the markers genotyped. The higher the map density and genome size, the higher is the LOD threshold required to ensure an overall false positive rate of less than 5% (Lander and Botstein, 1989). Based on the calculations of Lander and Botstein (1989) for a sparse genetic linkage map (marker every 20 cM), a LOD threshold of 2.4 to declare a QTL is approximately equivalent to a significance level of $\alpha = 0.001$ for each individual test performed. The haploid chromosome number in papaya is $n = 9$ (Meurman, 1925), and the genome size is small (372 million base pairs) compared to tomato (1000 mbp)(Armuganathan and Earle, 1991) which has a total map length of

1400 cM. Since our map is primarily based on RAPD markers, we have adopted a more stringent LOD threshold of 3.0 to declare the presence of a QTL. The location of the QTL is indicated in an interval on the likelihood peak within a drop of LOD 1 (Ott, 1985) (Fig. 2.6 and 2.7). Multiple peaks within 40 cM distance were resolved by fixing a QTL and rescanning the likelihood surface for a LOD difference of 2.0. The effect of the QTL on phenotype was tested for dominant (d=a), additive (d=0) and recessive (d=-a) modes of gene action by constraining the QTL for each one of the above mentioned modes of action. A LOD likelihood difference of 1.0 was used to suggest the most probable mode of action of the QTL.

QTLs are named by the left flanking marker containing the likelihood peak followed by the linkage group number. The additive and dominance values indicate allele substitution effects of Line 356 in a 'Sunrise' background under the unconstrained or free genetic model.

4-3. Results.

4-3-1. Genome composition.

The frequency distribution of genome composition of F_2 individuals is presented in Fig. 4.1. The portion of the genome that was homozygous with respect to Line 356 and 'Sunrise' averaged 21.3% and 20.8% respectively. The portion of the genome that was heterozygous with respect to parental source varied from 30% to 90% with a mean of 57.9%. The sum of the regions homozygous for 'Sunrise' varied from under 1% to

50% of the total genome, while those homozygous for Line 356 varied from under 1% to over 70%. The total 'Sunrise' genome content in F_2 individuals ranged from 20% to 75% with an average of 54.01% (Fig. 4.2). The observed range and mean values of the parental genomes in the F_2 population are in conformity with those expected and previously reported in segregating population (Paterson et al., 1991; Keim et al., 1990).

4-3-2. Phenotypic variation and distribution.

Plant height.

The total increase in plant height among F_2 individuals during the experimental period ranged from 81 cm to 209 cm with a mean of 128.8 cm (SD 31.92 cm) (Fig. 4.3A). The total increase in height for 'Sunrise' varied from 180 to 220 cm with a mean of 201 cm (SD = 28) and for Line 356 from 60 to 100 cm with a mean of 88.3 cm (SD = 29). The distribution of final plant height in segregants ranged from 165 cm to 328 cm with a mean of 247 cm (Fig 4.3B). The mean plant heights of 'Sunrise' and Line 356 were 306 (SD = 27.9) cm and 178 cm (SD = 44), respectively.

Stem diameter.

The mean growth in stem diameter over the course of experiment was 9.7 cm (SD = 1.8) in 'Sunrise' and 7.6 cm (SD = 2.1) in Line 356. The mean growth of F_2 plants was 7.02 cm, with a range of 2.03 cm to 13.25 cm (Fig. 4.4A). Final stem diameters of F_2 phenotypes (Fig. 4.4B) ranged between 10.75 cm and 20.4 cm with a

mean of 14.37 cm. Both the growth in stem diameter and final stem diameter exhibited continuous variation in the F_2 population.

Node at first flowering.

The parents, 'Sunrise' and Line 356, showed a distinct difference in the number of nodes between the cotyledonary node and the first flower-bearing node. The semi-dwarf parent Line 356 was very precocious, flowering at about the 17th node, while 'Sunrise' bore the first flower at about the 36th node. In the segregating F_2 population, first flower bearing node ranged from the 15th to the 36th (Fig. 4.5).

4-3-3. Number of QTLs and their mode of action.

Plant height.

A total of 3 QTLs effecting plant height and growth during different periods were detected. The QTL parameters, likelihood maps and locations are presented in Table 4.1 and Fig. 4.6 and 4.7, respectively. Two of these QTLs, located on linkage groups 1 (sex-1) and 5 (Q12A-5), caused a reduction in growth rate and final plant height due to allele substitution by Line 356. The locus on linkage group 5 (Q12A-5) was active only in the first growth period. The QTL, Sex-1, was more consistent in influencing growth rate and plant height. This QTL was active during the 2nd and 4th growth periods, and affected the total increase and plant height. However, the mode, magnitude and direction of gene action, were not consistent. This locus increased height in the early stages (period 2). The possible mode of action was dominant or

additive. The same QTL caused reduction in growth rate during the 4th growth period with a recessive mode of action. The total increase in plant height and final plant height were also reduced by the effect of this QTL. The suggested mode of action was dominant or additive in influencing plant height, but there was no clear mode in influencing rate of growth. The third QTL is also located on linkage group 1 (D2B-1) and caused an increase in rate of growth (height) during the 3rd growth period. The phenotypic variance explained by these individual loci varied from 22% to 77%. The non-genetic variance ranged from 12% to 24%.

Stem diameter.

Four QTLs affecting the rate of growth in stem diameter and final stem diameter were detected. The biometrical parameters, likelihood maps and locations of these QTLs are presented in Table 4.2 and Figs. 4.6 and 4.7, respectively. The QTLs located on linkage groups 1 (T12-1 and Sex-1) and 3 (D2C-3) affect stem diameter in a positive direction. The locus Sex-1, increased stem diameter in the 2nd growth period, total growth and final stem diameter. The mode of action was consistent with additivity or dominance during the 2nd growth period, dominance or additivity regarding total growth, and dominance regarding final stem diameter. Locus D2C-3 had a positive effect on stem diameter in period 2, total growth in diameter, and final diameter with a recessive or additive mode of action. Locus T12-1 increased growth in the 3rd growth period and was consistent with an additive or dominant mode of action. The QTL on linkage group 4(S12A-4) affected growth negatively in growth period 2. The overall

effect of this locus on total growth was ambiguous with a large dominance (negative) and small additive (positive) effect. The phenotypic variance explained by the individuals QTLs ranged from 14% to 58% and the variance due to non-genetic factors ranged from 11% to 22%

Node at first flowering.

Two QTLs affecting node at first flowering were detected (Table 4.2 and Fig. 4.6 and 4.7). Surprisingly, parent Line 356, a precocious cultivar, had a QTL (T12-1) on linkage group 1, causing delayed flowering and the mode of action was consistent with recessivity. The other QTL (H13-10) located on linkage group 10 was responsible for early flowering with a dominant mode of action. The two QTLs T12-1 and H13-10 explained 20 and 12% of the total phenotypic variance respectively.

4-3-4. Phenotypic variance explained.

The percentage of the total phenotypic variance explained by the QTLs for each trait was estimated by fitting all the QTLS in the model. The QTLs influencing plant height explain about 64% of the total phenotypic variance observed in total growth in height and 42% of the total variance observed in final height. With respect to stem diameter, 52% and 37% of the total phenotypic variance observed in increase in diameter and final stem diameter, respectively, was accounted by the QTLs. About 30% of the phenotypic variance is explained by the two QTLs influencing node at first flowering.

The variance due to environment with respect to increase in plant height, final plant height, increase in stem diameter and final stem diameter is estimated to be about 20%, 26%, 25% and 28% of the total variance, respectively. This estimate is based on the variances observed in the parents. The variance estimate of the parent Line 356 was based on the third generation sib-mated population, which was still segregating for diameter and height.

4-4. Discussion.

We have detected three QTLs affecting plant height, four QTLs affecting stem diameter and two QTLs affecting precocity. The phenotypic variance explained by individual QTLs ranged from 12% to over 77%. These QTLs account for 79%, 56%, 69% and 51% of the total genetic variance observed in increase in plant height, final plant height, increase in stem diameter and final stem diameter, respectively. Two QTLs affecting node at first flowering explain about 30% of the total phenotypic variance. The suggested modes of action of these QTLs have also been indicated. The majority of QTLs studied (Table 4.1 and 4.2) did not indicate one clear mode of action. For all the traits studied factors have been detected in Line 356 that affect the phenotype in the opposite direction to its overall effect. The QTL analysis of growth rates during different seasons indicates the occurrence of environmentally sensitive or developmentally regulated QTLs influencing height and stem diameter in papaya.

Quantitative variation in phenotype is the cumulative result of many loci, each with small effect (Thoday, 1961; Lande, 1981); more often, a few of these loci account for a major portion of the observed genetic variance (Thompson, 1975; Edwards et al., 1987). The result of the QTL analysis on traits affecting vigor and precocity in papaya supports the occurrence of a few QTLs with major affects on phenotype. This does not preclude the occurrence of many other QTLs with minor affects. Since the discriminatory ability of our analysis is limited by population size (100 F_2 plants), QTLs with small phenotypic effects may not be detected. Apart from population size, the possible existence of closely linked QTLs, as suggested by Paterson et al. (1988), and the high LOD threshold (3.0) used in the present investigation have also contributed to an underestimation of the number of QTLs. Hence, the number reported here represents the lower limit, but the most significant QTLs.

The QTLs affecting vigor and precocity were non-randomly distributed. QTLs with major effects on phenotype for all the three traits studied were concentrated on linkage group 1, which also has the genetic factor for determining sex of the plant. Sex in papaya is determined by multiple alleles with the dominant homozygotes being lethal (Storey, 1938). Sex-related characters, like number of flowers and length of peduncle, are also hypothesized to be tightly linked to the sex locus (Storey, 1953). Occurrence of genomic regions with multilocus clusters or 'hot spots' affecting different traits, as observed in linkage group 1, have also been reported (Allard, 1988; Helentjaris, 1992) in other crops.

QTLs with positive as well as negative effects were detected in Line 356 in all the traits studied. Factors responsible for delayed flowering were hypothesized to be present in early flowering papaya cultivars (Nakasone and Storey, 1955), based on the occurrence of phenotypes exceeding the parental limits in a backcross trial involving early and late flowering parents. Occurrence of QTLs or factors for tallness in dwarf parent have also been observed in maize (Edwards et al., 1991). Factors or QTLs with effects opposite to the overall effect of the parents are reported in several crops (Tanksley et al.,1982; Weller et al., 1988).

Analysis of growth rates revealed the presence of environmentally sensitive or developmentally regulated QTLs affecting growth during different seasons, with no detectible effect on the final phenotype (Q12A-5, D2B-1 and T12-1). These were also the only QTLs active during their respective growth periods. The variation in magnitude of genetic effects of consistently expressed QTLs (Sex-1 and D2C-3) also indicate a large influence of environment on these traits. The contrasting effects of QTL (Sex-1) on plant height in different seasons suggest the presence of more than one closely linked QTLs with opposite effects. We have also shown the presence of a QTL at position T12-1, causing increase in plant height in a similar trial under diseased conditions (Chapter VI).

Occurrence of constitutive QTLs, active under varying environmental conditions, and environmentally regulated QTLs have been reported in annual crops

(Hayes et al., 1993; Paterson et al., 1991 and Schon et al., 1994). In a perennial plant like papaya, the seasonal fluctuations in environmental conditions are expected to have considerable effect over all growth stages of the plant. The results of the present analysis indicate that the response in growth of papaya to seasonal fluctuations in environment is the sum total of the genetic effects of regulated and constitutive QTLs. Information on the location of these QTLS should prove valuable in consolidating all the favorable QTLs in a common background. The merits of a marker based trait improvement program, especially for traits under environmental influence, has already been emphasized (Burr et al., 1983; Stuber and Edwards 1986; Soller and Beckmann 1988).

QTL analysis on growth rates in plant height and stem diameter detected two QTLs for diameter and height in linkage group 1. However, QTL analysis for final stem diameter and plant height (Fig 4.6) indicated the presence of one QTL each for stem diameter and one for plant height. The minor peaks (indicated by arrows) on the likelihood surface for final stem diameter and plant height, correspond to QTLs detected in different growth periods. The presence of QTLs in these regions have also been confirmed in a similar trial involving another 'Sunrise' X Line 356 population (Chapter VI). This clearly demonstrates the increased power of QTL analysis of growth rates in detecting environmentally sensitive QTLs and in effectively resolving multiple peaks. This approach should prove useful for increasing efficiency of QTL

mapping in perennial crops, that are subject to seasonal influences and have limited flexibility regarding population size.

This attempt demonstrates the overall suitability of a RAPD based genetic linkage map for QTL analysis. However, the mode of action of QTLs cannot be estimated accurately, especially in regions with several linked markers in coupling phase. Inclusion of two or three well placed co-dominant markers on each chromosome should greatly improve the accuracy of QTL analysis. Finally, the results of the present investigation are specific to the present location and we advise caution in extrapolating the results to different environmental conditions.

Table 4.1. The parameters of QTLs affecting plant height.

QTL	Period	LOD	%Var.exp	a	d	Mode
Q12A-5	1	4.76	22.0	-8.05	6.46	RA
Sex-1	2	11.23	44.0	15.92	6.66	DA
Sex-1	4	28.45	77.6	-54.28	57.17	R
Sex-1	growth	9.81	50.9	-33.27	73.18	DAR
Sex-1	height	5.67	29.0	-33.41	77.08	DA
D2B-1	3	5.83	56.0	8.6	-2.99	A

Foot note: The QTLs are indicated by the left flanking marker followed by the linkage group number. 'Growth' refers to total increase in height and 'height' refers to height at the end of the experiment. The growth period is indicated by numbers 1 to 4. The letters 'a' and 'd' denote additive and dominance effect due to substitution of a 'Sunrise' allele by a Line 356 allele. The mode of action is denoted by letters 'A','D' or 'R', which indicate additive, dominant or recessive mode of action respectively. Under situations where two possible modes of action are listed, the first letter indicates the most likely mode of action. For more details, refer to the text.

Table 4.2. The parameters of QTLs affecting stem diameter and precocity.

QTL	Period	LOD	%Var.exp	a	d	Mode
Sex-1	2	8.5	35.0	1.05	1.15	AD
T12-1	3	12.08	58.0	1.13	0.00	AD
Sex-1	growth	6.27	28.0	0.99	2.01	DA
Sex-1	diameter	6.32	27.0	0.54	2.32	D
D2C-3	2	3.72	16.6	0.71	-0.81	RA
D2C-3	growth	3.2	15.0	0.96	-1.25	RA
D2C-3	diameter	2.50	14.0	1.22	-0.33	RA
S12A-4	2	4.52	55.0	-0.23	2.48	DRA
S12A-4	growth	3.39	39.0	0.03	-3.11	RDA
Node at first flowering						
T12-1		3.50	20.0	4.10	-3.26	R
H13-10		3.08	12.3	-1.42	-1.62	D

The 'growth' refers to total increase in diameter and 'diameter' refers to stem diameter at the end of the experiment. For other information refer to table 4.1.

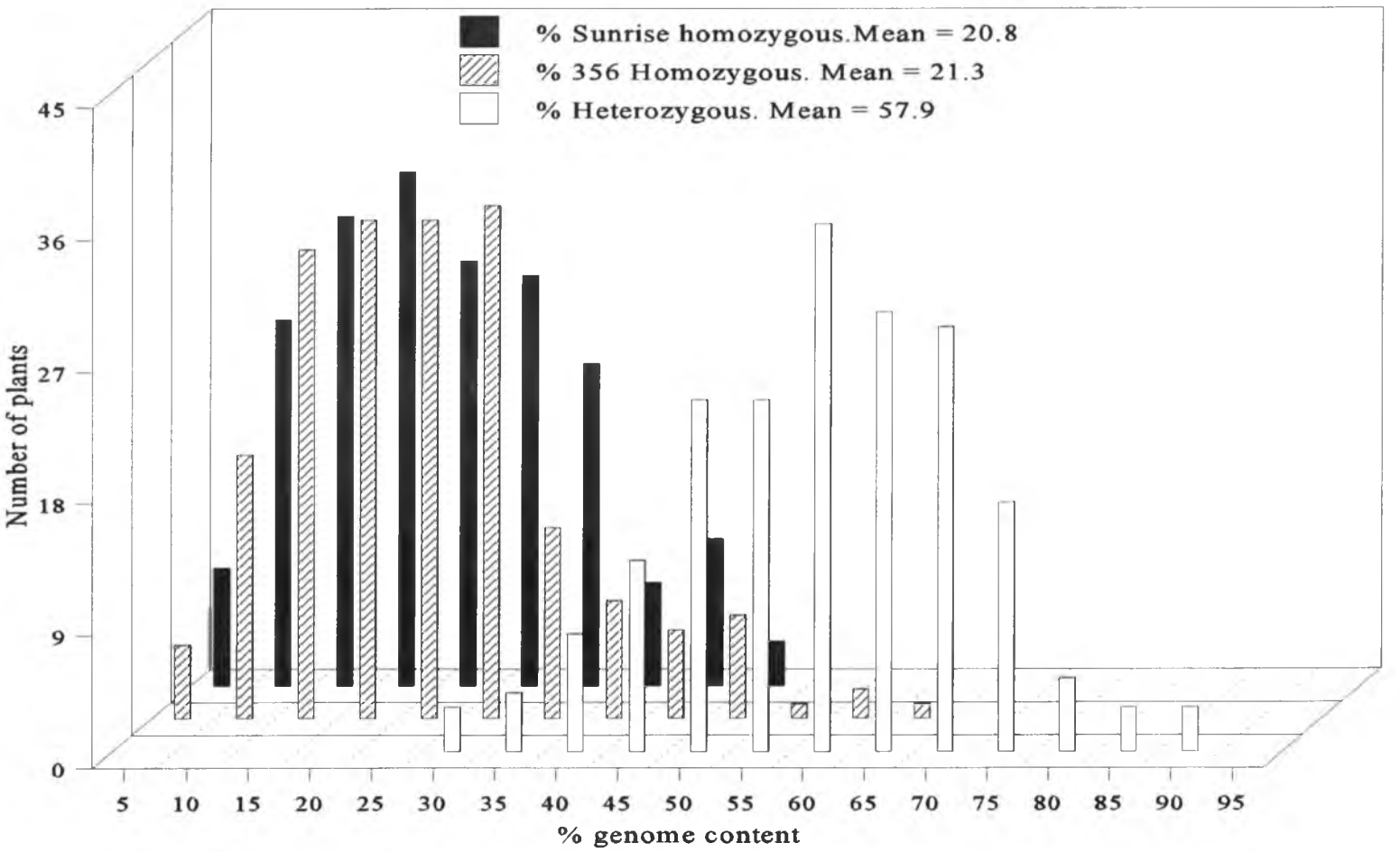


Fig. 4 1. The frequency distribution of genomic regions homozygous and heterozygous for Line 356 and 'Sunrise' in Line 356 X 'Sunrise' F_2 intercros population.

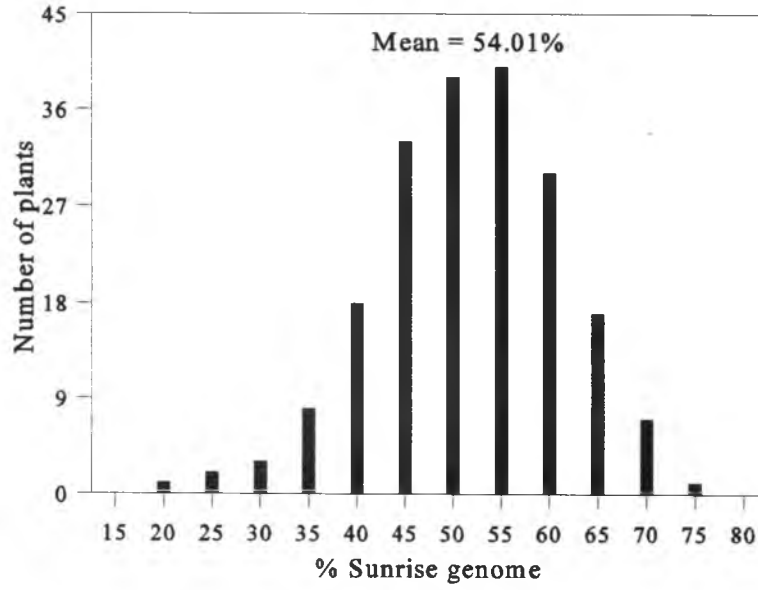


Fig 4.2. The frequency distribution of 'Sunrise' genome content in Line 356 X 'Sunrise' F_2 population

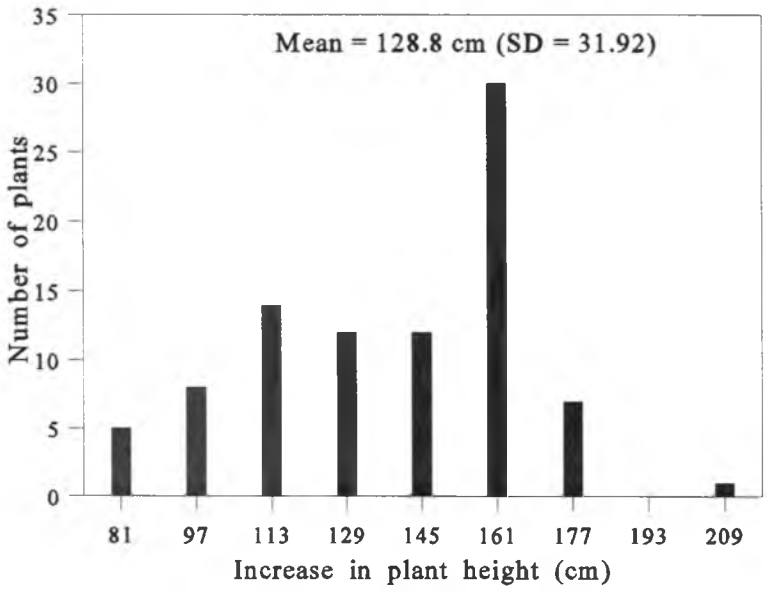


Fig. 4.3A

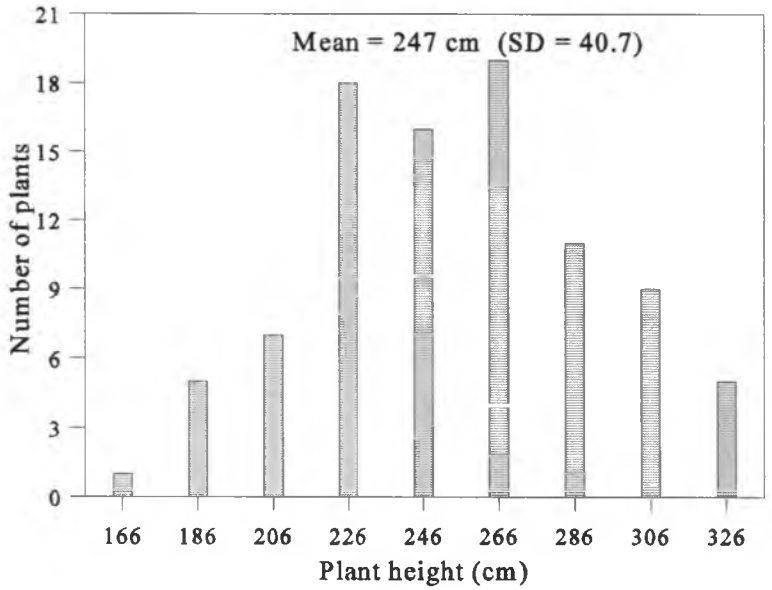


Fig.4.3B

Fig. 4.3A and B. The frequency distribution of increase in height and final plant height in Line 356 X 'Sunrise' F_2 population.

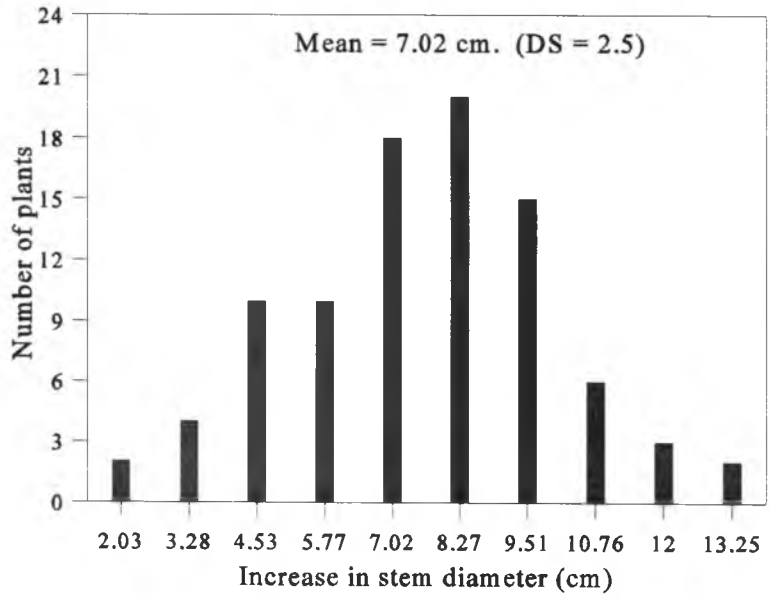


Fig. 4.4A

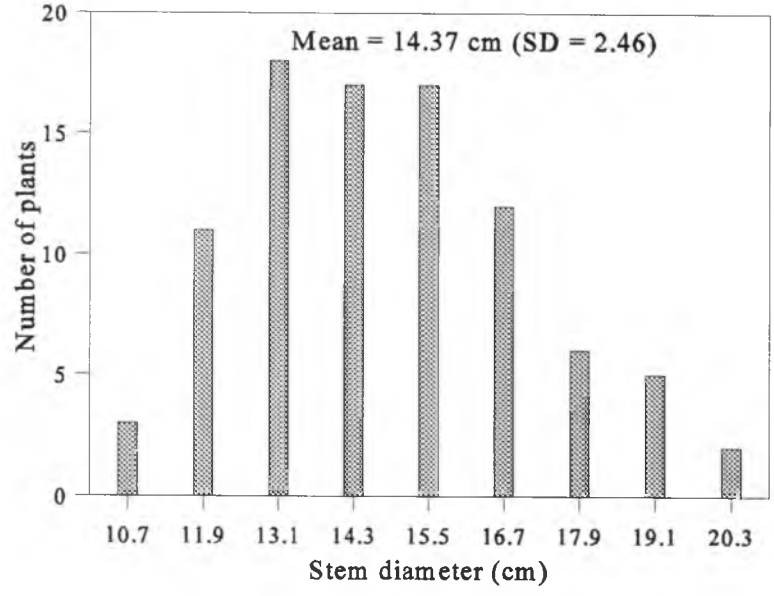


Fig.4.4B

Fig. 4.4A and B. Frequency distribution of increase in stem diameter and final stem diameter in 'Sunrise' X Line 356 F_2 population.

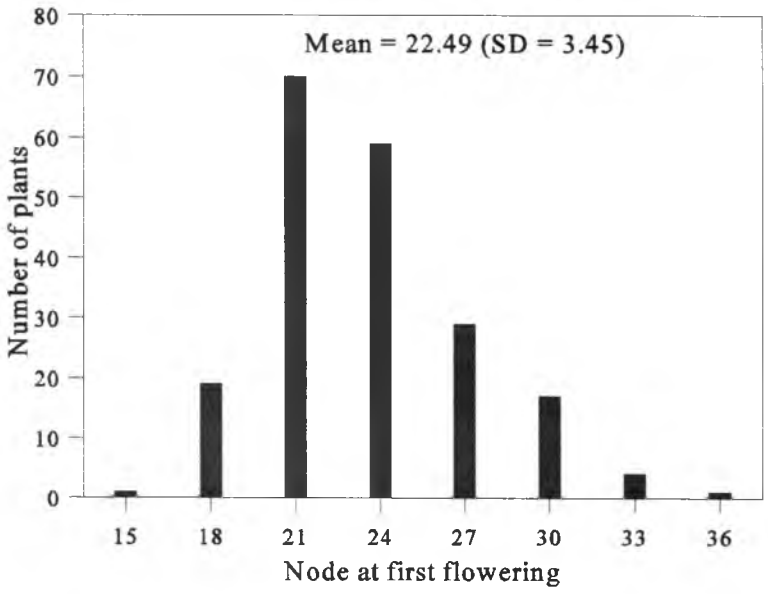


Fig. 4.5. The frequency distribution of node at first flowering in Line 356 X ' Sunrise' F_2 population.

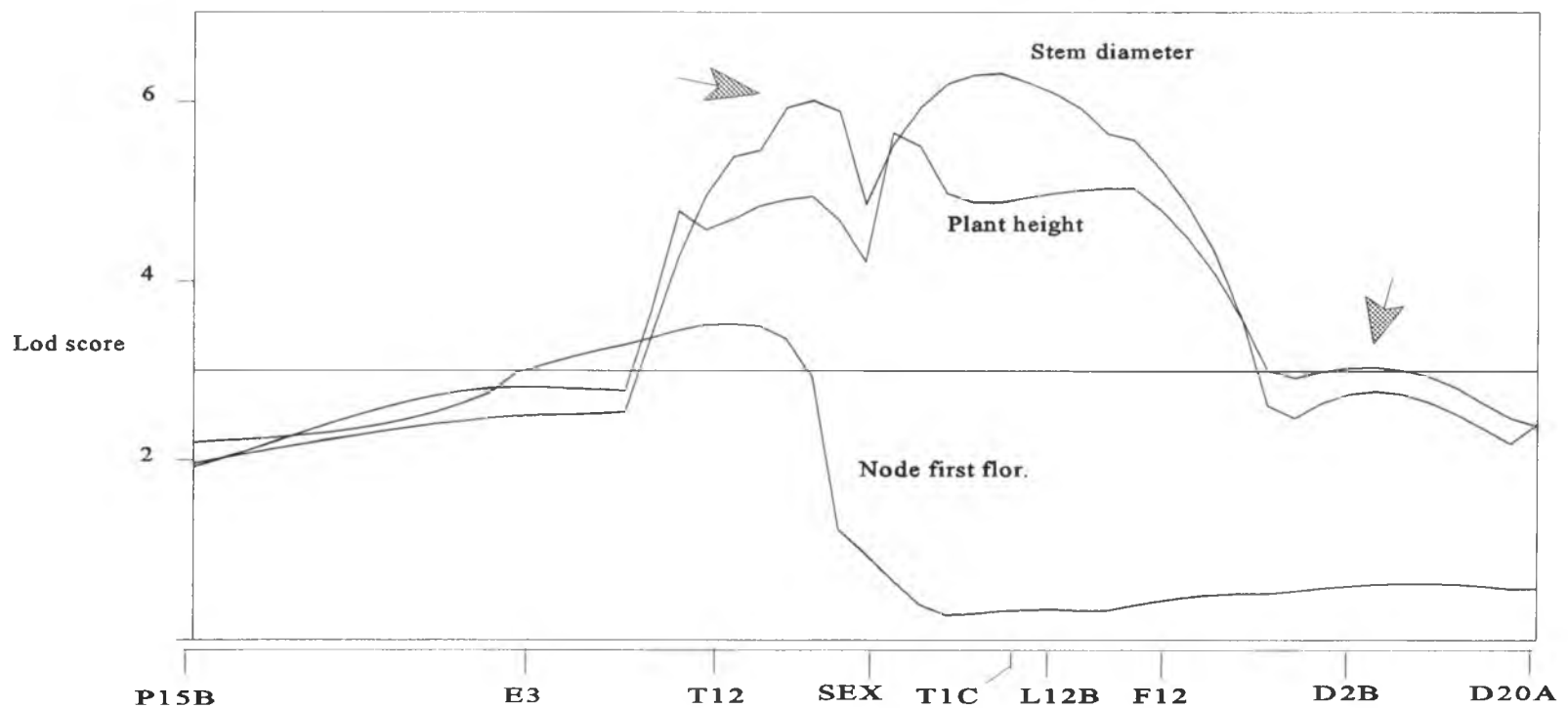


Fig. 4.6. QTL likelihood map for stem diameter, plant height and node at first flowering on linkage group 1. The arrows indicate the QTLs active during different growth periods.

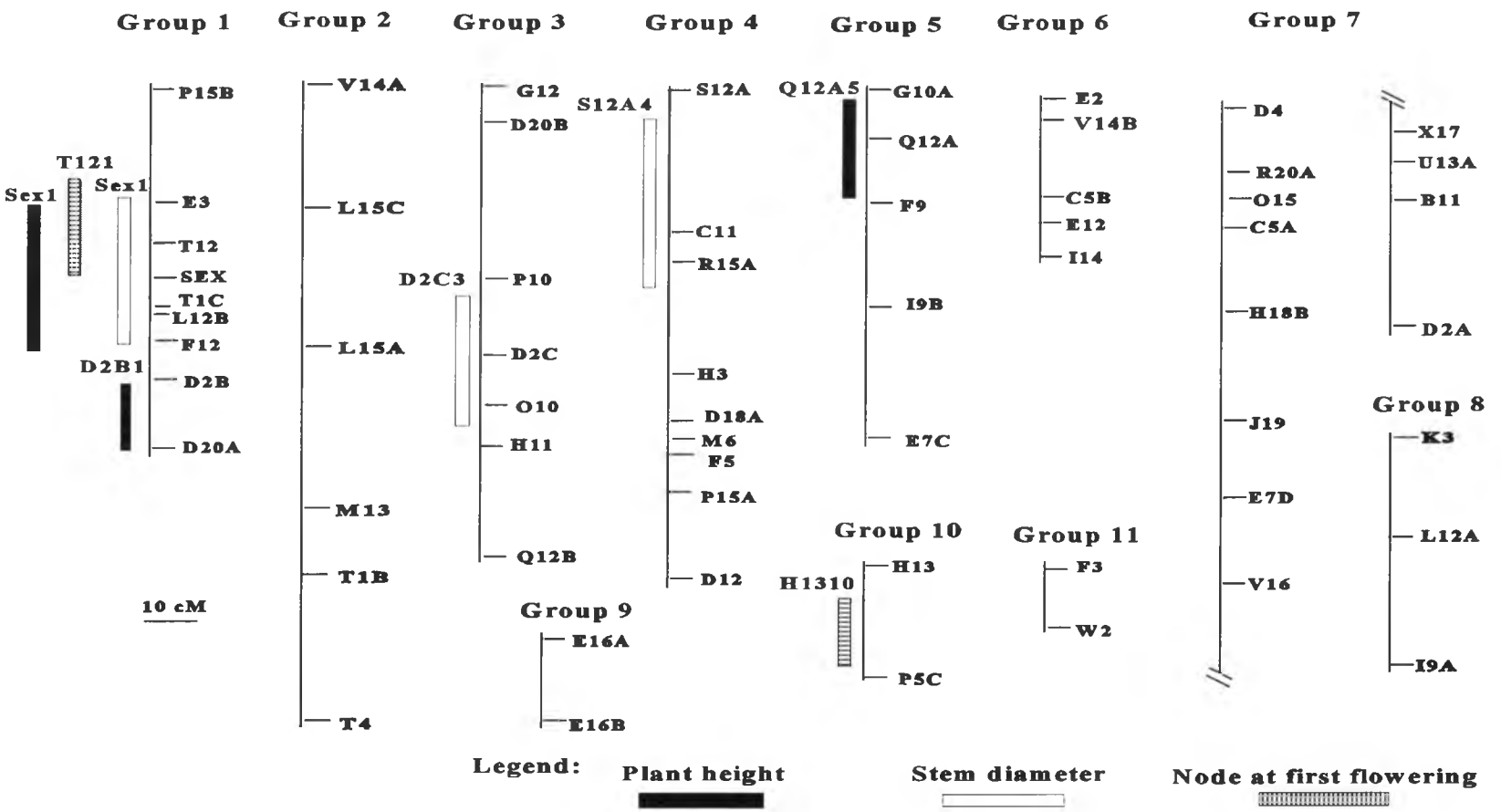


Fig. 4.7. Likelihood intervals of mapped QTLs affecting plant height , stem diameter and node at firsrt flowering in papaya. The bars indicate the likelihood peak region within a lod difference of 1.0. The letters on the bars indicate the designated names of the QTLs.

CHAPTER 5

RAPD map based analysis of quantitative trait loci affecting carpellody, yield and yield related traits in papaya (*Carica papaya* L.).

5-1. Introduction.

The majority of the commercially important yield and yield-related traits in crop plants exhibit continuous phenotypic variation due to the action of many genes or 'polygenes'. The currently available statistical techniques (Wright 1968; Mather and Jinks, 1971; Lande, 1981) for determining the genetic effects of these polygenes or quantitative trait loci (QTL) (Gelderman, 1975) do not provide information on their chromosomal location or individual effects on the phenotype. The application of statistical methods for identifying and characterizing QTLs (Sax, 1923; Thoday, 1961) has been limited by lack of suitable QTL-linked markers with discrete and neutral effects on the phenotype.

The recent development of molecular markers, particularly restriction fragment length polymorphisms (Grodziker et al., 1975) and randomly amplified polymorphic DNA (RAPD) markers (Welsh et al., 1990; Welsh and McClelland, 1990) has provided plant breeders with a potentially unlimited number of markers with which to determine the chromosomal locations and genetic characteristics of QTLs. These markers seldom affect phenotype and enable one to trace inheritance of a chromosomal segment in a segregating population. Within a relatively short span of only 15 years,

genetic linkage maps based on molecular markers have been generated in a number of crop and animal species (Tanksley, 1989). The potential of such linkage maps for detailed genetic analysis and dissection of quantitative traits has already been demonstrated in crop plants (Paterson et al., 1988, 1991; Schon et al., 1993, 1994; Hays et al., 1993).

Genetic linkage maps in which the QTLs affecting economically important traits have been defined and characterized, are a valuable breeding aid, especially in improvement of perennial crops like papaya (*Carica papaya* L.). The commercial cultivars of papaya in Hawaii ('Sunrise' and 'Kapoho') are gynodioecious inbred lines with superior yield and quality attributes. However, these varieties have some important deficiencies, among which is the seasonal production of deformed 'catfaced' or carpellod fruits and sterility in the progeny.

Carpellody is an unique phenomenon observed in hermaphrodite and male plants only. It is partial or complete conversion of the basal whorl of five stamens into the carpels, resulting in fruits composed of more than normal five carpels. Environmental conditions like cool temperature (Awada, 1958), high soil moisture, high relative humidity and high nitrogen levels have been shown to promote 'femaleness' resulting in carpellodic fruit production (Awada, 1953; Awada and Ikeda, 1957). Conversely, female sterility is caused by seasonal abortion of carpels, resulting in lack of fruits or production of deformed banana-like fruits composed of fewer than

the normal five carpels. More sterility is observed during warm seasons (Storey, 1941) and under conditions of nitrogen and moisture stress (Awada and Ikeda, 1957). The phenomena of carpellody and female sterility are controlled by independent sets of genetic factors with several gene pairs for each (Storey, 1953). The existence of genetic factors with an underlying quantitative nature of genetic factors controlling these traits has been shown by progressive reduction of carpellody and sterility to negligible levels in breeding trials (Hamilton and Ito, 1968). Currently, there is no information on the number or location of factors or QTLs affecting fruit number, fruit per node, carpellody or sterility.

In an attempt to identify and characterize QTLs affecting these traits, we have constructed a RAPD-based genetic linkage map of papaya with 60 dominant and 2 codominant markers. The map is 999 cM long with a mean interval distance of 20 cM, and represents over 70% of the expected genome size of papaya. In this paper we present results of our genetic analysis of QTLs affecting carpellody, sterility (fruit number), number of fruit per node and mean fruit weight based on a segregating F_2 population.

5-2. Materials and methods.

5-2-1. Parents and mapping population.

An F_2 segregating population of a cross between the Hawaiian cultivar 'Sunrise' and breeding Line 356 was used for the present investigation. The F_1 plant selected by

Dr. R.M. Manshardt in 1987 was hermaphrodite and displayed marked heterosis. A population of 100 plants was raised at Poamoho Experiment Station, University of Hawaii, for the QTL analysis. 'Sunrise' is a highly inbred gynodioecious, high yielding cultivar with negligible carpellody or female sterility. 'Sunrise' produces about 125 small to medium sized (500g) fruits per plant per year. The nodes often bear multiple fruits. Line 356 is a dioecious, semi-compact, slow growing cultivar. This selection does not bear multiple fruits per node.

5-2-2. Quantitative traits studied.

Carpellody.

Any fruit with more than five carpels were considered carpellod fruit. Carpellody is expressed as a percentage of total fruits produced per plant per year. Only hermaphrodite plants were used for recording this observation, because female plants produce no stamen and thus can not express carpellody.

Total number of fruits, fruits per node and fruit weight.

The total number of fruits per plant per year was recorded. Papaya plants bear an inflorescence in the axil of each leaf. The number of fruits per node was expressed as the total number of fruits per total number of leaves produced in a year. Fruit weight was derived as the weight of 5 fruits. Both hermaphrodite and female plants were used for recording these observations.

5-2-3. Genome composition.

The genome composition of the F_2 individuals was estimated based on the genotypes generated by the MAPMAKER/EXE program (Lander et al., 1987) . The flanking markers were used to estimate the regions that were homozygous for Line 356 or 'Sunrise'.

5-2-4. QTL analysis.

The computer program MAPMAKER/QTL (Paterson et al., 1988) was used to perform the QTL analysis. MAPMAKER/QTL is based on the maximum likelihood method and generates likelihood maps with LOD scores (log of odds) across the whole length of the genome. LOD scores above a suitable prefixed threshold, usually in the range between LOD 2 or LOD 3, indicate the presence of a QTL. A stringent LOD threshold of 3.0 was used in the present investigation to declare the presence of a QTL. The probable location of the putative QTL is indicated in a region on the likelihood peak within a LOD difference of 1.0 from the peak. The mode of action of QTLs is suggested by comparing likelihood maps of QTLs constrained for additive, dominant and recessive models. A LOD difference of 1.0 was used to suggest the most likely mode. Additional QTLs which did not exceed LOD 3.0 threshold, yet explained greater phenotypic variance in conjunction with a previously identified QTL, were identified by rescanning the entire genome after fixing the variation at the known QTL. The total phenotypic variance of a trait explained by all the QTLs was estimated by simultaneously fitting of all the QTLs in the model.

5-3. Results

5-3-1. Genome composition

The histogram of genome composition of the F_2 segregating population are presented in Figs 5.1 and 5.2. The genomic regions homozygous for 'Sunrise' varied from under 5% to 50% with a mean of 21.05%. The genomic regions homozygous for Line 356 ranged from under 10% to 55% with a mean of 22.2%. The mean extent of genomic heterozygosity in the segregating population was 56.75% and ranged from 30% to 85%. The mean 'Sunrise' genome content in the segregating population was 51.16% (Fig. 5.2). The means and the ranges, are in conformity with those expected, and previously reported in F_2 intercross populations of diploid species (Paterson et al., 1991; Keim et al., 1990).

5-3-2. Phenotypic variation.

Carpellody.

Frequency distributions showing the extent of carpellody among all (67) hermaphrodite plants (Fig. 5.3A) and among 50 hermaphrodite plants bearing carpellodic fruit (Fig. 5.3B) are presented. The extent of carpellody among the carpellod-fruit bearing plants ranged from 0.16 (1.5%) to 1.93 (85%). The results of QTL analysis performed on all hermaphrodite as well as carpellod-fruit bearing plants were similar.

Fruit number, fruit per node and mean fruit weight.

The frequency distributions for fruit number, fruits per node and mean fruit weight in the segregating population are presented in Fig. 5.4 and 5.5. The total number of fruits produced ranged from 53 to 170 fruits per plan per year with a mean of 105.7 (SD = 26.14). The number of fruits/node in the F_2 population ranged from 0.43 to 1.43 with a mean of 0.88 fruits/node (SD = 0.22). The mean fruit weight was 637 g (SD = 173.7) with a range from 377 g to 985 g. All of the above mentioned traits were normally distributed.

5-3-3. Number of QTLs and their mode of action.

The number of QTLs, their biometrical parameters, likelihood maps and likelihood intervals of location are presented in table 5.1, Figs 5.6 and 5.7. The QTLs are named after the left flanking marker of the interval containing the likelihood peak, followed by the linkage group number. The additive (a) and dominance (d) values refer to the affect of 'Sunrise' allele substitution by line 356 allele under an unconstrained (free) genetic model.

Carpellody.

The analysis involving all hermaphrodite plants and carpellod fruit bearing hermaphrodite plants, detected QTLs in the same location. The additive and dominance effects indicate the values under free model (Tab. 5.1). The QTLs Q12A-5 and D4-7, situated on linkage groups 5 and 7 respectively (Fig. 5.7), increased carpellody and

explained 56% and 61% of the total phenotypic variance respectively. However, the additive effect of D4-7 was negligible. The mode of action of these two QTLs (Q12A-5 and D4-7) was consistent with dominant or additive mode. The QTL X17-7, situated on linkage group 7 (Figs. 5.6 and 5.7), caused a reduction in carpellody and explained over 37% of the phenotypic variance. The suggested mode of this QTL was consistent with additive or recessive action.

Fruit number.

Three QTLs affecting total number of fruits produced per year were detected. Two of these QTLs (H3-4 and U13A-7) having LOD scores below 3.0, were detected by rescanning the intervals with a fixed QTL (E3-1). Locus E3-1, located on linkage group 1, explained about 18% of the phenotypic variance. Allele substitution by Line 356 at this locus caused a reduction in total fruit number. The mode of action of locus E3-1 was consistent with a dominant or additive mode. The QTL H3-4, located on linkage group 4, indicated recessive or additive mode of action by increasing the number of fruits due to allele substitution by Line 356. Substitution of a 'Sunrise' allele by a Line 356 allele at loci U13A-7 caused a reduction in number of fruits but no clear mode of action was found.

Number of fruits per node.

Two QTLs affecting number of fruits per node were detected. These loci are located on linkage group 1 (E3-1) and 7 (V16-7) (Fig 5.7), and cause a reduction in

fruits/node due to allele substitution by Line 356. Locus E3-1 was consistent with a dominant or additive mode of action and explains about 38% of the total phenotypic variance. Locus V16-7 did not have one clear mode of action and explained over 40% of the total phenotypic variance.

Mean fruit weight.

Three loci influencing mean fruit weight were detected on linkage groups 1, 2 and 7 (Tab 5.1, Figs. 5.6 and 5.7) The QTLs P15B-1 and V16-7 increased fruit weight due to Line 356 allele substitution, while locus T1B-2 caused a decrease in fruit weight. The QTL P15B-1 has an additive or dominant mode of action and explains 33% of the phenotypic variance. Locus V16-7 is consistent with a recessive or additive mode of action and explains about 54% of total phenotypic variance. The other QTL, T1B-2 has a dominant mode of action. This locus explains about 62% of the total phenotypic variance.

5-3-4. Total phenotypic variance.

The three QTLs influencing carpellody explain over 97% of the total phenotypic variance. Also the unexplained variance was found to be very low (0.009). A total of 52.2% of the observed phenotypic variance in total number of fruits per plant per year was explained by the three QTLs affecting total number of fruits. The two loci influencing number of fruits/node, accounted for 59% of the observed phenotypic

variance. Over 87% of the total phenotypic variance observed in mean fruit weight was explained by the three QTLs affecting mean fruit weight.

5-4. Discussion.

We have detected 3 QTLs each affecting carpellody, fruit number and mean fruit weight, and 2 QTLs influencing fruit per node. The phenotypic variance explained by the individual QTLs ranged from 18% to 62%. These QTLs also explained 97%, 52.2%, 59% and 87%, of the total phenotypic variance observed in carpellody, number of fruits per year, fruits per node and mean fruit weight, respectively. Over 95% of all these loci indicated more than one possible mode of action. Since the parents 'Sunrise' has negligible carpelody, and Line 356 show no carpellody (Line 356 is dioecious), most of the observed phenotypic variance in carpellody can be attributed to genetic variance.

The total variance explained with regard to the majority of the traits studied suggests the possible existence of many more QTLs with effects too small to detect in the present population (100 F_2 plants) using the mostly dominant RAPD markers. The use of a high LOD threshold (3.0) has also contributed to a possible underestimation of the number of QTLs. However, the results of our investigation on papaya seem to support the unequal distribution in magnitude of QTL effects, with only a few QTLs explaining the major portions of the phenotypic variance (Thompson, 1975;

Helentjaris,1992) as opposed to the existence of many QTLs with small and similar effects (Thoday, 1961; Lande, 1981; Weller et al., 1988).

The QTLs were non-randomly distributed (Fig. 5.7), with some genomic regions having loci affecting all of the traits studied (Fig. 5.6). The exact genetic composition of these regions can only be speculated on, due to the sparse map used in the current investigation. The seemingly pleiotropic effects expressed by these regions (V16-7 and E3-1) may also be due to the occurrence of multilocus clusters or hot-spots that have been reported in other crops (Allard, 1988; Helentjaris, 1992).

An interesting result is the presence of genetic factors in parent Line 356, with positive as well as negative effects on three of the four traits studied. There were two QTLs affecting carpellody, and one each affecting fruit number and fruit weight, that had effects opposite to the overall expected effect based on the phenotype of the parent. The presence of QTLs with such contrasting effects has been reported in other crops (Tanksley et al., 1982; Weller et al., 1988; Edwards et al., 1991).

Two QTLs affecting fruit number were detected, and, as expected, allele substitution by Line 356 caused a reduction in fruit number. These QTLs were also linked with QTLs responsible for higher fruit weight and lower fruit per node on linkage groups 1 and 7 (Fig. 5.7). This is consistent with the frequent occurrence of

larger fruits on plants with fewer fruits on the fruiting column and the resulting similarity in total yield in the segregating population.

A number of reports on seasonal occurrence of carpellody and sterility in hermaphrodite papaya (Storey, 1941; Awada, 1958; Lange, 1961; Arkle and Nakasone, 1984) give a general impression that these two traits are inter-related and may also have a common or closely linked genetic factors. However, Storey (1953) reported the existence of independent set of factors for carpellody and sterility, each with several genes. Individual trees may contain factors only for female sterility or only for carpellody or for both. trees having factors for both phenomena are characterized by sterility and carpellody during different seasons of the year.

The result of our analysis also confirm the presence of two or more major QTLs, affecting each of these traits in different regions of the genome except in linkage group 7. The likelihood intervals for loci X17-7 and U13A-7 affecting carpellody (X17-7) and fruit number (U13A-7) overlap in linkage group 7 (Fig. 5.6), and allele substitution at these loci by Line 356 alleles caused a reduction in carpellody and fruit number. This is consistent with a general low level of carpellody observed among plants producing lower number of fruits per year (<80).

Carpellody.

Although Line 356 is a dioecious selection with no expression of carpellody, there were two QTLs that increased and one QTL that reduced carpellody in the segregating Line 356 X 'Sunrise' F_2 population. This confirms the earlier hypothesis of the occurrence of factors for carpellody in female plants (Line 356 was used as the female parent) (Storey, 1953). These QTLs account for almost all the variance observed (96%).

Sex in papaya is decided by a single gene with multiple alleles (Storey, 1938; Hofmeyr,1938). It has also been hypothesized that this simply inherited locus in fact represents a complex of many tightly linked genes, which behave as an unit factor in heredity (Storey, 1953). The hermaphrodite and male types are dominant heterozygotes and the female is recessive homozygote. The hermaphrodite allele among the segregants used in the present population is contributed by the parent 'Sunrise', which has negligible or no carpellody. Since carpellody is observed only in hermaphrodite plants, it is logical to assume that the region bearing the hermaphrodite locus or a factor very tightly linked to this locus is required for expression of carpellody. This factor is necessary for expression of carpellody, but is not by itself alone sufficient for producing carpellody, since there are some F_2 hermaphrodites (and 'Sunrise' parent) which express no carpellody. Presumably this factor is critical for anther development, without which no carpellody can be observed. QTLs from Line 356 which modify carpellody can act only in the presence of this region bearing the hermaphrodite allele.

Despite the observed segregation of carpellodic and noncarpellodic fruit-bearing plants in the ratio of 3:1, we were unable to locate this region. Hence the absence of carpellody among 17 of the 67 hermaphrodite plants represent genotypes with ideal QTL composition.

This study is based on a specific cross and needs further confirmation by elaborate studies involving larger populations and different environments. Such an attempt may detect differences in magnitude of QTL affects or even new QTLs.

In summary, we have used a RAPD-based linkage map to detect major QTLs affecting all the traits studied. This also represents the first attempt on papaya. The findings of the present investigation are expected to aid in future attempts to understand the genome composition and organization in papaya. The results provide valuable guidelines for multiple trait improvement in papaya.

Table 5.1. The parameters of QTLs affecting carpellody, fruit number, fruits/node and mean fruit weight.

QTL	trait	LOD	%var.exp	a	d	Mode
Q12A-5	Carpellody	3.01	56.9	0.30	0.78	DAR
D4-7		3.10	61.0	0.001	-0.92	DA
X17-7		3.31	37.7	-0.50	-0.01	AR
E3-1	Fruit no	3.22	18.0	-8.17	-17.04	DA
H3-4*		(2.17)	12.5	11.77	-9.70	RA
U13A-7*		2.42	39.0	-8.60	-29.58	DAR
E3-1	Fruits/node	7.45	38.3	-0.13	-0.18	DA
V16-7		3.02	40.7	-0.17	-0.15	DAR
P15B-1	Fruit weight	4.41	33.2	170.05	-35.03	AD
T1B-2		3.97	62.6	-84.26	-242.00	D
V16-7		3.23	54.4	20.00	-258.90	RA

Foot note: The QTLs are indicated by the left flanking marker followed by the linkage group number. The letters 'a' and 'd' denote additive and dominance effect respectively, due to allele substitution by 356. The mode of action of QTL is indicated by letters 'A' for additive, 'D' for dominance and 'R' for recessive. Under situations where more than one mode is listed, the first letter indicates the most likely mode.
*These QTLs were identified by rescanning, with the fixed QTLs (refer text).

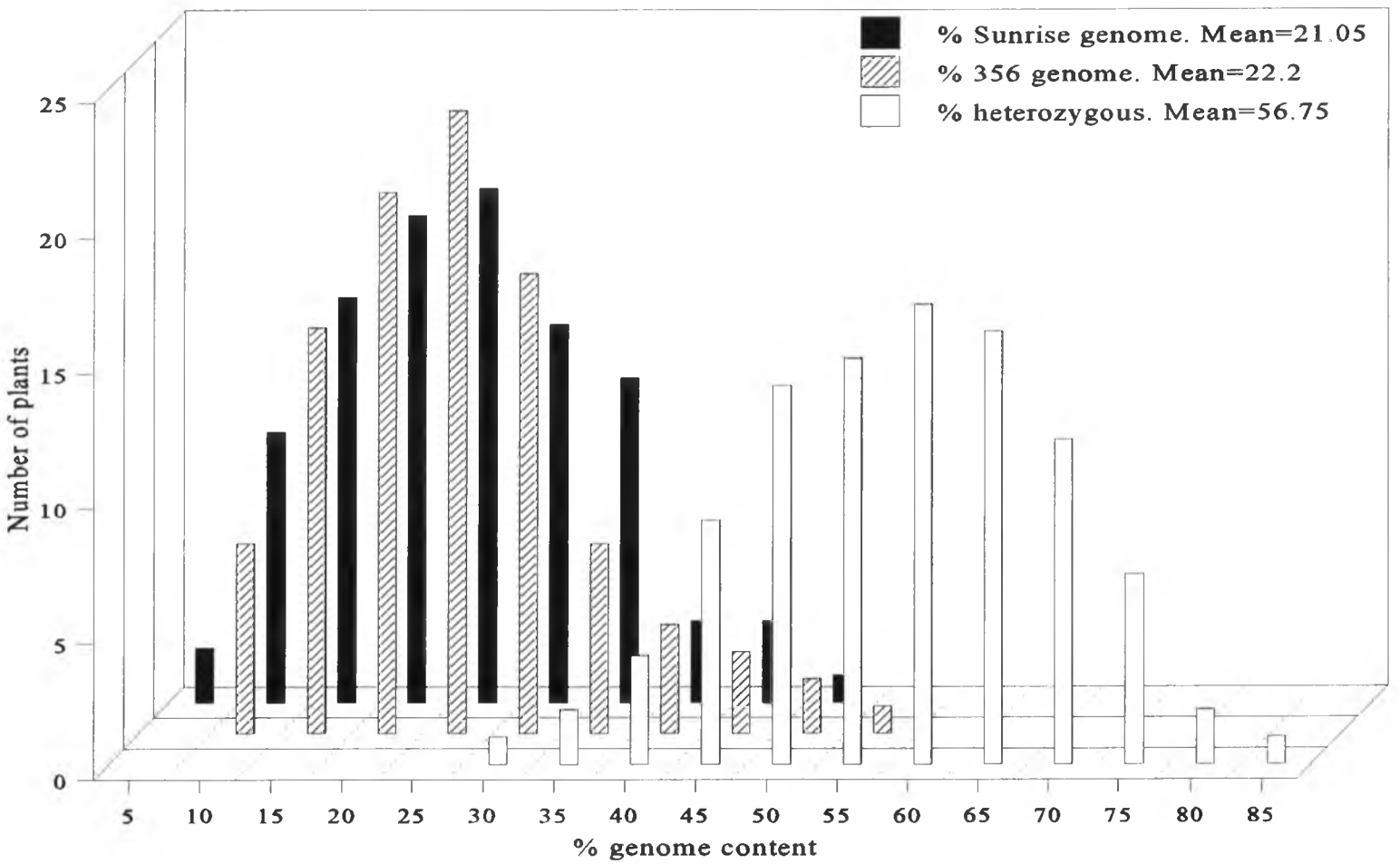


Fig. 5.1. The frequency distribution of genomic regions homozygous and heterozygous to Line 356 and ' Sunrise' genomes in Line 356 X 'Sunrise' F_2 intercross population.

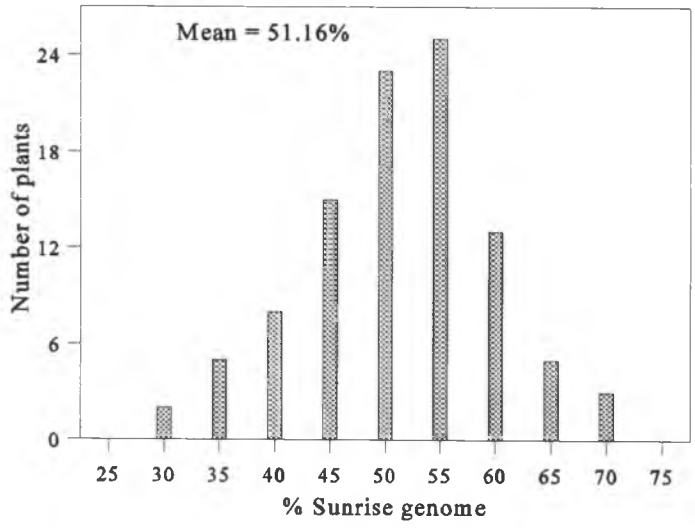


Fig. 5.2. The frequency distribution of % 'Sunrise' solo genome content in Line 356 X ' Sunrise' solo F_2 intercross population.

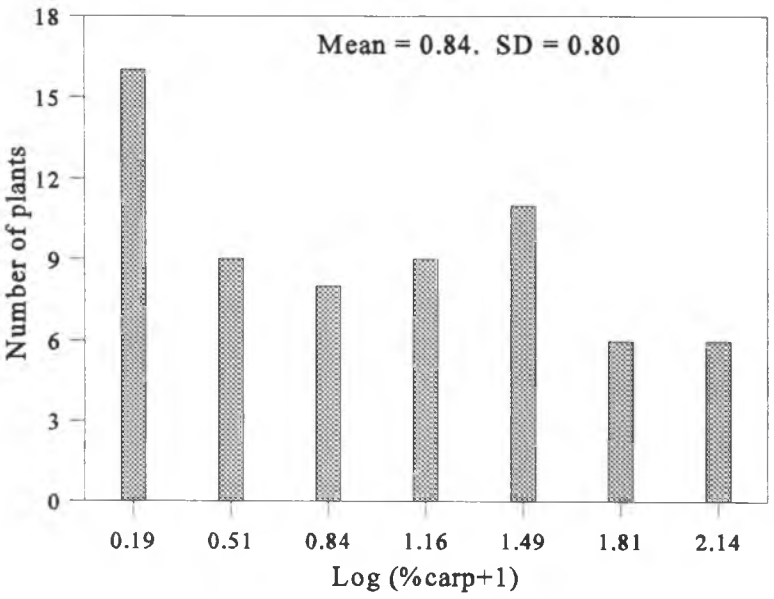


Fig. 5.3A

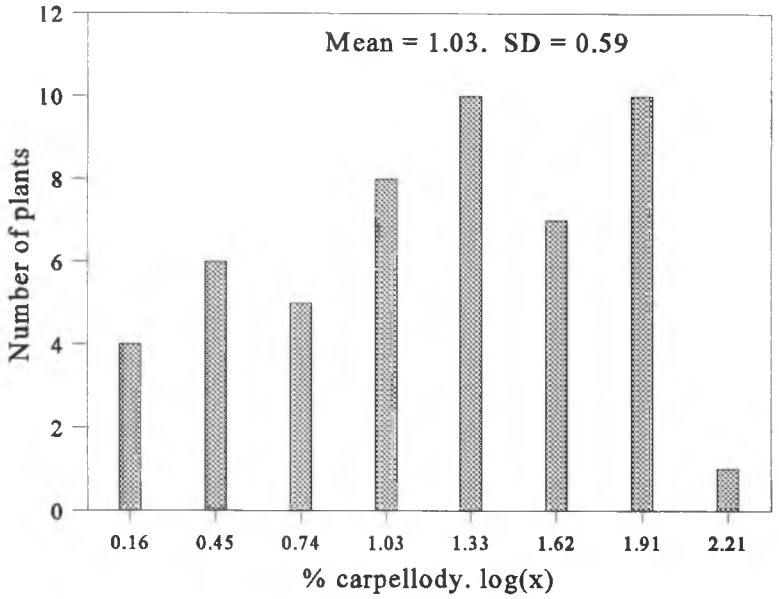


Fig. 5.3B.

Fig. 5.3. A and B. Frequency distribution of carpellody in hermaphrodite plants (A) and carpellod fruit bearing hermaphrodite plants (B) in 'Sunrise' solo X line 356 F_2 intercross population.

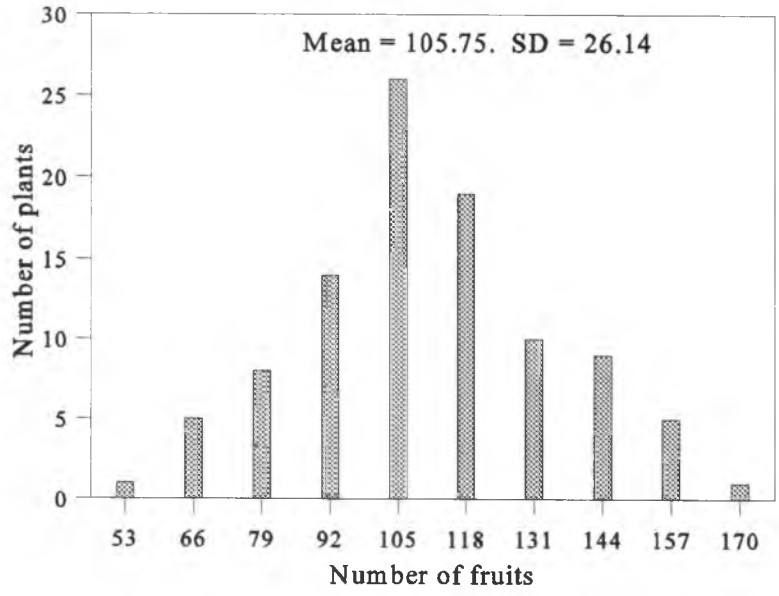


Fig. 5.4A

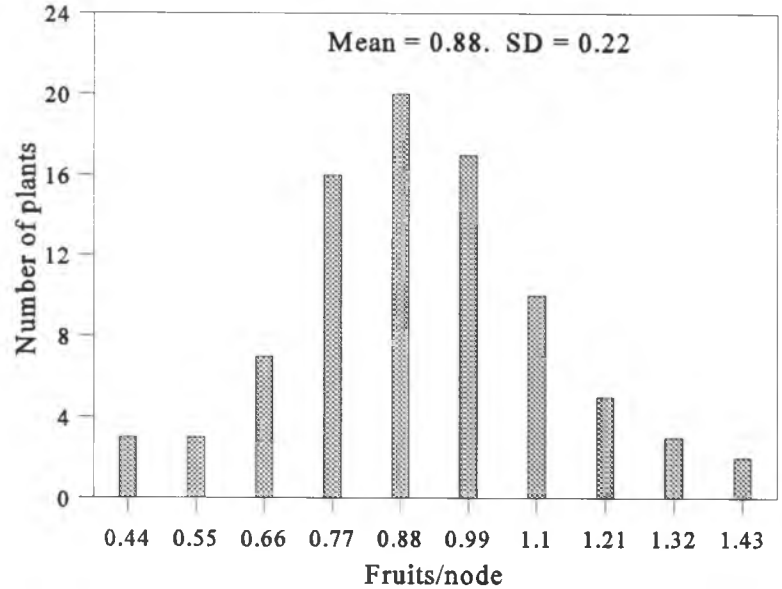
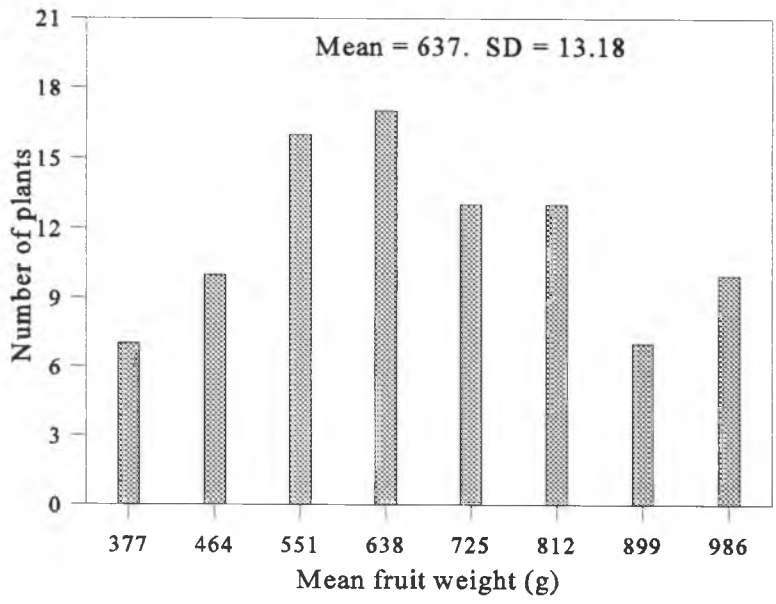


Fig. 5.4B.

Fig. 5.4A and B. Frequency distribution of fruit number (A) and fruits/node (B) in 'Sunrise' solo X line 356 F_2 intercross population.



Figt. 5.5. Frequency distribution of mean fruit weight (g) in 'Sunrise' solo X line 356 F_2 intercross population.

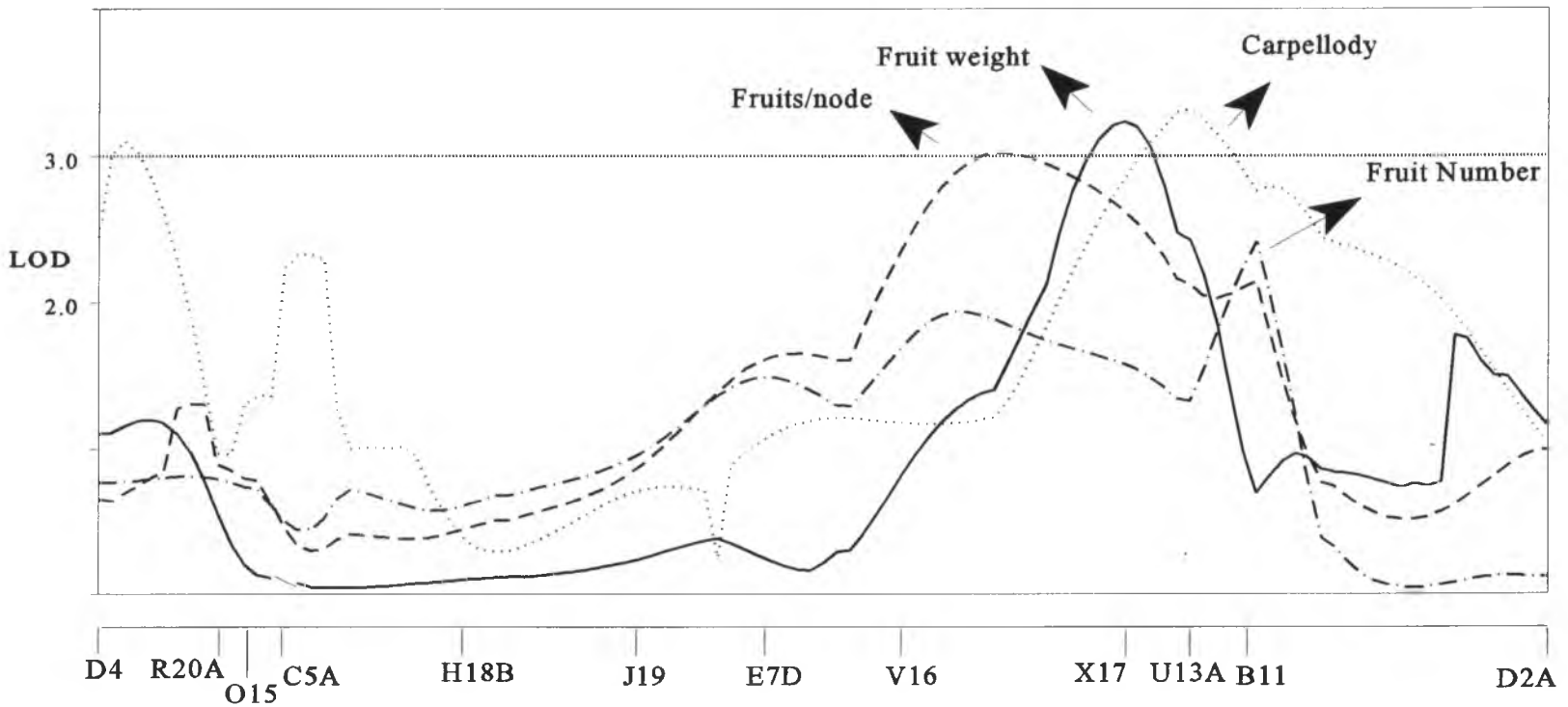


Fig. 5.6. QTL likelihood map for carpellody, fruit weight, fruit number and fruits per node on linkage group 7.

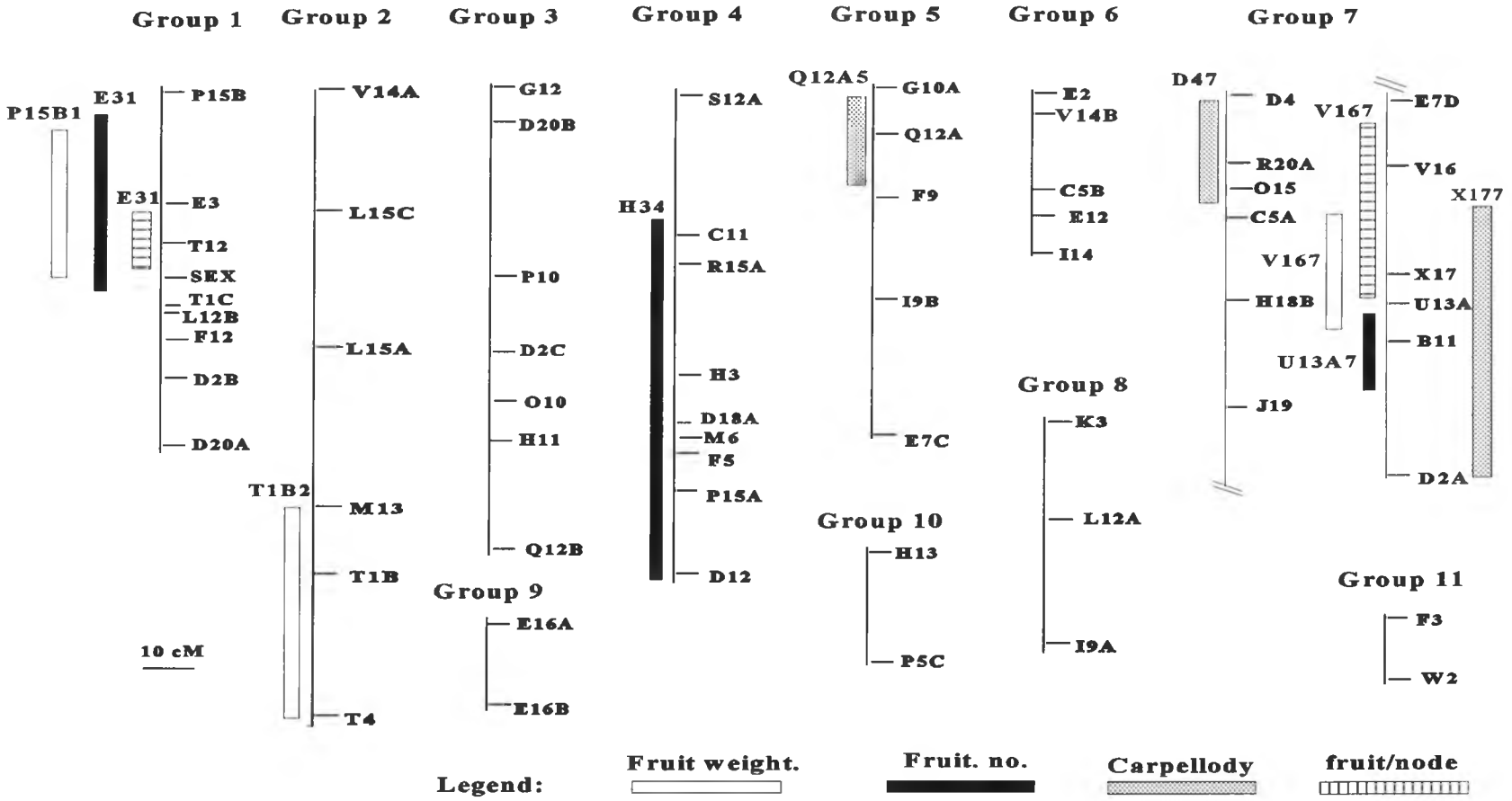


Fig. 5.7. Likelihood intervals of mapped QTLs affecting carpellody, mean fruit weight, fruits per node and fruit number in papaya. The bars indicate the likelihood peak region within a lod difference of 1.0. The letters on the bars indicate the designated names of the QTLs.

CHAPTER 6

Map-based analysis of resistance to papaya ringspot virus disease in papaya (*Carica papaya* L.).

6-1. Introduction

Papaya ringspot virus disease (PRV) is an important virus disease of papaya (*Carica papaya* L.) and in recent years has attained the status of a major disease causing severe economic loss in papaya-growing regions of the world. Papaya ringspot virus is a potyvirus (Purciful and Hebert, 1971) and is non-persistently transmitted by the principal aphid vector *Myzus persicae* (Namba, 1962; Cook and Milbrath, 1971).

High levels of resistance to PRV in other species of the family Caricaceae have been previously reported (Conover, 1964; Horovitz and Jimenez, 1967). Earlier attempts to introduce resistance into *Carica papaya* through interspecific hybridization have resulted in failure to obtain viable seeds (Sawant, 1958; Horovitz and Jimenez, 1967; Mekako and Nakasone, 1975). Cultivar tolerance to PRV disease in *Carica papaya* was for the first time identified in a dioecious introduction from Columbia (Conover, 1976) and improved to a useful level by breeding and selection (Conover and Litz, 1978, 1981; Zee, 1984). The tolerance to PRV disease is quantitatively inherited (Conover and Litz, 1976) and is readily transferred from Line 356, derived from Conover's material, to hybrids with Hawaiian commercial papaya cultivars (Zee, 1984).

Currently, Line 356 and 'Cariflora' (developed in Florida by Conover et al., 1986) remain the only sources of usable tolerance to PRV in the species *Carica papaya*. Despite the moderate success obtained with alternate crop protection strategies like cross protection (Yeh et al., 1988) and coat-protein mediated protection in transgenic plants (Fitch et al., 1992), the use of natural genetic resistance observed in cultivars provides the safest and most economical approach to crop protection (Browning, 1980). A knowledge of components of resistance and their chromosomal location is important, not only for understanding the resistance mechanism, but also in marker-assisted breeding programs for crop improvement.

Recent discoveries in molecular biology have presented plant breeders with DNA-based molecular markers, such as restriction fragment length polymorphisms (Grodziker et al., 1975) and randomly amplified polymorphic DNA (RAPD) markers (Williams et al., 1990; Welsh and McClelland, 1990), with which to develop saturated genetic linkage maps of plants. These markers occur in large numbers and seldom influence phenotype. The usefulness of molecular marker-based linkage maps in locating and characterizing qualitative traits and quantitative trait loci affecting disease resistance has already been demonstrated (Kreike et al., 1993; Freymark et al., 1990; Dirlewanger et al., 1994).

We have used a RAPD-based genetic linkage map of papaya to map quantitative trait loci affecting tolerance to ringspot virus disease in papaya. We have used a F_2

population of a cross between the PRV-tolerant Line 356 and the susceptible cultivar 'Sunrise'. The objectives of this study are to determine the location of and characterize the QTLs involved in PRV disease resistance.

6-2. Materials and methods.

6-2-1. Parents and the experimental population.

A F_2 experimental population was derived from the parents 'Sunrise' and Line 356. 'Sunrise' is a tall, gynodioecious commercial variety that is highly susceptible to PRV, while Line 356-3 is a semi-dwarf dioecious line with an useful level of tolerance to PRV. The F_1 plant with a high level of resistance to PRV and phenotypically similar to 'Sunrise' was selected in 1987 by Dr. R.M. Manshardt. A population of 100 F_2 plants and parents (10 each) was raised at the Wimanalo Experimental Station, University of Hawaii, for the present investigation.

6-2-2. Linkage map and genome composition.

We have previously constructed a genetic linkage map of papaya based on a F_2 intercross population of 253 plants. The map is 999 cM long with 60 dominant (RAPD) and 2 codominant markers in 11 linkage groups representing over 70% of the expected genome size of papaya. The map was generated by computer program MAPMAKER/EXP 3.0 (Lander et al., 1987) with a LOD score of 4.0 to group the markers into linkage groups. There are a total of 51 intervals in the map with a mean interval distance of 20 cM. The genotypes of the F_2 individuals used in the present

analysis were determined by GENOTYPE command. The regions homozygous for 'Sunrise' and Line 356 were estimated based on the flanking markers. Under conditions where the genotype could not be determined unambiguously, heterozygosity was assumed.

6-2-3. Disease inoculation.

The plants were mechanically inoculated 2 months after field planting of 5-month-old greenhouse-grown seedlings. Two leaves from the lower third of the canopy of each plant were inoculated. The virus inoculum was prepared by grinding 1 part infected leaf in 2 parts of 0.1 M (w/v) phosphate buffer at pH 7.5. Carborundum powder (300 mesh) was added to the grinding mix to improve fragmentation. The leaf surface was very sparingly dusted with carborundum dust and gently rubbed with a pestle dipped in the freshly made inoculum. Care was taken to avoid damage to the leaf. The inoculated surface was washed with deionized water 10 minutes after inoculation. The infection was confirmed 21-25 days later by visual symptoms and ELISA assay on apical leaves. Those that were not infected were reinoculated with virus.

6-2-4. Observations

Plant vigor.

Stem diameter and plant height were measured as indices of plant vigor. These observations were recorded at 3 month intervals starting from June 1992. Stem

diameter was recorded at 30 cm above ground level for the first observation, and the same region was used for the subsequent measurements. Plant height was measured as the height from ground level to the apical meristem of the plant. The gain in height and stem diameter during each period, total gain in height and diameter, final height and diameter were analyzed. Growth increaments were analyzed to avoid the confounding effect of growth before inoculation with PRV (a period of 5 months) and to identify environmentally sensitive QTLs. Growth periods 1, 2, 3 and 4 refer to the periods June-Aug, Sept-Nov, Dec-Feb and March-May 1992-93, respectively.

Symptom rating.

The virus symptoms assessed as a result of virus infection included leaf mosaic, leaf distortion, stem lesions, petiole lesions, fruit distortion and ring spots on the fruits. Disease symptoms were rated on a scale of 1 to 4 (Zee, 1984) with 1 = no symptom, 2 = mild symptoms, 3 = moderate symptoms and 4 = severe symptoms. Intermediate ratings (1.5, 2.5 and 3.5) were used in ambiguous situations. These observations were recorded 14 months after inoculation.

ELISA assay.

The double antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA) technique (Cook and Zettler, 1977) based on a polyclonal antibody to viral coat protein was used to assay the virus. ELISA assay was performed 14 months after inoculation. The microtiter plates with 96 wells were coated overnight at 4^0 C with 1

μg/ml of gamma globulin in 100 μl of pH 9.6 coating buffer (1.59 g of Na_2Co_3, 2.93 g $NaHCO_3$, 0.20 g of NaN_3 /1 l deionized water). The coated plates were washed three times for 3 minutes each with PBS-Tween (8 g NaCl, 0.2 g KH_2PO_4, 1.15 g, Na_2HPO_4, 0.2 g KCl, 0.2 g NaN_3 in 1 l water + 0.5 ml Tween 20) and blotted dry.

One part of tissue sample was ground in 5 parts (w/v) phosphate buffer at pH 7.5 (900 ml 0.25 M K_2HPO_4 + 100 ml 0.25 M KH_2PO_4 + 0.1 M EDTA). One hundred μl of extract was added to the coated wells and stored at 4° C overnight. The plates were rinsed three times with PBS-Tween and blotted dry. To these wells was added 100 μl of enzyme conjugate buffer (PBS-Tween, 2% PVP-Sigma 40T, 0.2% ovalbumin-Sigma A5503) containing gamma globulin conjugate at a dilution of 1:4000, and the microtiter plate was incubated at 37^0 C for 3 hours.

Enzyme-conjugate treated plates were then washed with PBS-Tween three times for 3 minutes each and 100 μl of substrate buffer (97 ml diethanolamine, 1 l water, pH 9.8) containing p-nitrophenyl phosphate (Sigma # 104) at the concentration of 0.5 mg/ml was added. The plates were incubated at room temperature for 20 minutes and the optical density was determined at a wavelength of 405 nm with a Biorad plate reader (Model 450). The positive and negative controls consisted of extracts from infected and uninfected leaves, respectively, from both the parents (4 wells each). Four wells in each plate were also used as buffer control.

We have attempted to use ELISA titer as a quantitative measure of disease resistance. Utmost care was taken in regard to uniformity in sampling and in assay conditions. Five leaf disks (30 mg), collected randomly from the third youngest leaf of each plant, were used for the assay. Each sample was replicated three times. Positive, negative and buffer controls were used in each plate. The assay was repeated on 25% of the total samples by randomly selecting samples to confirm the results. The ELISA titer was recorded 20 minutes after incubation with substrate as it resulted in maximum differences in ELISA titer between parents.

A replicated ELISA assay to test for the presence of compounds in Line 356 that interfere with ELISA titer was also carried out. Leaf extract from diseased 'Sunrise' was mixed with leaf extract from healthy 'Sunrise' and Line 356 in various proportions. Equal weights of leaf tissue (30 mg) was used for extraction. Each treatment was replicated three times.

6-2-5. Data analysis.

The QTL analysis was performed by interval mapping using MAPMAKER/QTL (Paterson et al., 1988). A LOD score of 3.0 was used to declare the presence of a QTL. The mode of action of a QTL was suggested by comparing likelihood maps of QTL constrained for additive, dominant and recessive models. A LOD difference of 1.0 was used to suggest the possible mode of action. The location of a QTL is indicated in a region within a difference of LOD 1 from the peak (Ott, 1980).

Additional QTLs influencing a trait were identified by fixing a QTL and rescanning the genome. Log transformation of ELISA titer ($A_{405}+1$) and total increase in stem diameter (cm+1) was performed to normalize the distribution. The QTLs are named after the left flanking marker, followed by the linkage-group number. The additive and dominance values refer to the effect of allele substitutions by Line 356 in a 'Sunrise' background under an unconstrained (free) model.

6-3. Results.

6-3-1. Genome composition.

The frequency distribution of genome composition among F_2 individuals is presented in Figs. 6.1 and 6.2. The regions homozygous for Line 356 varied from 5% to 65% with a mean of 21.00%. The genomic regions homozygous to 'Sunrise' varied from 5% to 50% with an average of 20.04%. The heterozygous genome content varied from 30% to 95% and the average was 58.96%. The 'Sunrise' genome content in segregating population ranged from 20% to 75% with an average of 49.45%. The observed range and mean values agree with the expected values in a F_2 population. Similar results have been reported in other crops (Paterson et al., 1991; Keim et al., 1990).

6-3-2. Phenotypic variation.

Plant vigor.

The histograms for distribution of increase in stem diameter [log(cm+1)], final diameter, increase in plant height and final plant height are presented in Figs. 6.3 and 6.4. The mean growth in stem diameter in Line 356 and 'Sunrise' under disease conditions was 4.06 cm (SD = 1.42) and 0.8 cm (CD = 0.20) respectively. This difference between parents was also apparent in final stem diameter of 10.9 cm in Line 356 (SD = 2.76) and 6.2 cm SD = 0.50) in 'Sunrise'. The mean increase in plant height in Line 356 and 'Sunrise' was 82.06 cm (SD = 9.8) and 67.03 cm (SD = 11.5) respectively. A mean final plant height of 188.7 cm (SD = 19) in 'Sunrise' and 171.8 cm (SD = 15.87) in Line 356 was observed. These statistics are based on seven observbations each of 'Sunrise' and Line 356.

The mean growth in diameter among F_2 individuals under disease conditions was 2.35 cm with a range from 0.75 cm to 7.25 cm. The stem diameters at the end of the experiment ranged from 5.99 cm to 13.57 cm and the mean diameter was 8.83 cm. The mean growth in plant height during the experimental period was 74.90 cm and ranged from 40.00 cm to 118.35 cm. The final plant height ranged from 131 to 244 cm with a mean of 182 cm. The mortality rates among Line 356, Sunrise and F_2 population were 0%, 58% and 34%, respectively.

Disease symptoms and ELISA titer.

QTLs with LOD scores greater than 3.0 were found for leaf mosaic and fruit distortion. The other symptoms studied did not give QTLs above this threshold. The F_2 symptom ratings ranged from 1.5 to 4 with a mean of 3.13 for leaf mosaic and from 1 to 4 with a mean of 1.93 for fruit distortion. The mean disease ratings for 'Sunrise' and Line 356 were 4.0 and 1.92, respectively, for leaf mosaic and 4.0 and 1.0, respectively, for fruit distortion, respectively.

Presence of compounds in extracts from healthy plants interefering with ELISA antigen-antibody binding has been reported in papaya species (Zee, 1984). A replicated ELISA assay on healthy, diseased and a mix of healthy and diseased extracts indicated presence of intereferring factors in healthy Line 356 leaf extracts which reduced the ELISA titer (Table 6.4). The mean ELISA titers of the 'Sunrise' diseased extract, 1:1 mix of 'sunrise' diseased and healthy Line 356 leaf extracts and 1:1 mix of 'sunrise' diseased and healthy 'Sunrise' leaf extracts were 0.213, 0.150 and 0.199 respectively.

PRV-infected 'Sunrise' and Line 356 show a non-overlapping ranges in ELISA titer (Fig. 6.5). The mean ELISA titer (based on four plants) in Line 356 and 'Sunrise' was 0.431 and 0.994, respectively (positive control). Low ELISA titers (A_{405} = 0.007 to 0.008) were observed with PRV-negative and buffer controls. The frequency distribution for ELISA titer among F_2 individuals is presented in Fig 6.5. The ELISA titers among PRV-infected F_2 segregants ranged from A_{405} equal to 0.037

to 1.147 with a mean of 0.61 (0.20). The distribution of ELISA titers in the F2 population was not expected to affect the results of the QTL analysis by interval mapping using flanking markers (Knott and Haley, 1992).

6-3-3. Number and character of QTLs.

Stem diameter.

A total of six QTLs affecting stem diameter at different growth periods were identified. The biometrical parameters and likelihood intervals of QTLs are presented in Table 6.1 and Fig. 6.6. The phenotypic variance explained by these QTLs ranged from 39 to 70%. Substitution of Line 356 alleles at four of these QTLs (D2B-1, T12-1, L15A-2 and L12A-8) had positive effects while substitution at other two QTLs (V16-7 and S12A-4) had negative effects on stem diameter. Substitution of Line 356 alleles at QTLs L15A-2 and L12A-8 caused an increase in stem diameter during the second growth period and the mode of action of both these QTLs was consistent with recessive action. Line 356 allele substitutions at QTLs V16-7 and S12A-4 caused a reduction in growth rates during the second and third growth periods, respectively, and were consistent with a dominant mode of action.

The two QTLs with consistent positive effect on stem diameter were both situated on linkage group 1. Substitution of the Line 356 allele at position D2B1 increased growth rates during the secnd and third periods, as well as total growth. However, the possible mode of action was recessive during the second period and

additive or dominant in the third period and overall. This QTL explained 40% of the total phenotypic variance observed in growth in diameter. QTLs D2B-1 and L15A-2 explained over 70% of the total phenotypic variance in the segregating population. Substitution of the Line 356 allele at T12-1 increased stem diameter during third and fourth periods as well as the final stem diameter. The suggested mode of action was additive or dominant in the third period and fir total growth, and additive in the fourth period. Over 45% of the observed phenotypic variance in stem diameter was explained by this QTL.

Plant height.

The QTLs affecting increase in plant height and final plant height, with their biometrical parameters and likelihood intervals, are presented in Table 6.2 and Fig. 6.6. Three QTLs individually explained 25 to 58% of the total phenotypic variance. Substitution of Line 356 alleles at QTLs T12-1 and L12B-1 increased plant height. Substitution of the Line 356 allele at position T12-1 increased plant height in the third and fourth periods, as well as overall. The suggested mode of action was recessive in the second period and additive or dominant during other periods. This allele from Line 356 caused a reduction in growth during the first growth period.

The increase in height in the fourth period and in final plant height were influenced positively by the substitution of the Line 356 allele at position L12B-1. The mode of action of this allele was consistent with dominant or additive action. The QTL

O15-7 was detected by scanning under a fixed QTL model and explained a greater portion of the phenotypic variance observed in growth in plant height (68%) and final plant height (69%) then did major QTLs alone.

Disease symptoms.

One QTL affecting leaf mosaic (S12A-4) and 3 QTLs affecting fruit distortion (L15C-2, S12A-4 and H13-10) were detected (Table 6.3 and Fig. 6.6). All of these QTLs were consistent with a recessive mode of action. Line 356 exhibited mild leaf mosaic (mean = 1.92) and no fruit distortion. However, substitution of Line 356 alleles into 'Sunrise' background at the above loci caused an increase in disease sympto severity.

ELISA titer.

A total of 6 QTLS influencing ELISA titer were detected (Table 6.3). Substitution of Line 356 alleles at three of these loci (D20B-3, I9B-5 and B11-17) resulted in lower ELISA titer under homozygous condition compared to heterozygous condition. The additive effects associated with each of these three alleles were very small. Substitution of Line 356 alleles at V14A-2 and K03-8 resulted in marginal reduction in ELISA titer under homozygous condition, however the heterozygous condition at these two loci resulted in much lower ELISA titer than homozygous condition. Substitution of Line 356 allele at QTL M13-2 caused a negligible increase in ELISA titer, however heterozygosity at this locus resulted in a substantial reduction in

ELISA titer. Lower ELISA titers were observed due to Line 356 allele substitution at three loci in homozygous condition while, the remaining loci showed lower ELISA titers under heterozygous condition. None of the six QTLs indicated any single mode of action. All the QTLs affecting ELISA titer were well distributed in the genome (Fig. 6.6). The extent of phenotypic variance explained by the individual QTLs was similar (78 to 81%).

ELISA titer had very poor correlation with stem diameter, leaf mosaic and fruit distortion (Table 6.5)

6-4. Discussion.

Analysis of the components of tolerance to papaya ringspot virus indicates the presence of multiple QTLs affecting each of the three components studied. We have detected and characterized nine QTLs affecting plant vigor (six for stem diameter and three for plant height), four QTLs affecting disease symptoms (one for leaf mosaic and three for fruit distortion) and six QTLs affecting ELISA titer. The number of QTLs detected suggests a complex mechanism of tolerance and is consistent with the quantitative nature of disease resistance to PRV reported earlier (Conover and Litz, 1978, 1981; Zee, 1984). The results of the genetic analysis of different components and the possible mechanism of tolerance to PRV disease in papaya are discussed below.

6-4-1. Plant vigor.

Vigor has been assessed by measuring rate of increase in height and stem diameter and final plant height and stem diameter. The vigor of the plant under disease conditions is an indication of disease tolerance (Cooper and Jones, 1983). The parents, 'Sunrise' and Line 356, show a marked difference in vigor under disease conditions, especially with regard to stem diameter. The drastic reduction in vigor observed in the susceptible parent, 'Sunrise', upon infection also appears to be detrimental for survival. This is evident by the high mortality rate observed in 'Sunrise' (58%) and in the F_2 population (34%). In the majority of cases, death of the weakened plant was due to secondary causes, and in all cases was associated with very low or no increase in stem diameter.

We were able to detect six QTLs influencing stem diameter under disease conditions due to the large difference in phenotype of the parents. Four of these QTLs (L15A-2, V16-7, L12A-8 and S12A-4) were active only during certain growth periods, but had detectible effects on the final stem diameter. The remaining QTLs (D2B-1 and T12-1), though more consistent in expression throughout the growth period, showed marked differences in magnitude of effect, and in one case (D2B-1) showed a difference in possible mode of action at different times. This suggests the influence of environmental conditions on growth in stem diameter. Occurrence of such environmentally sensitive and stable QTLs has been reported in annual crops (Hayes et al., 1993; Paterson et al., 1991). In a perennial plant like papaya, the combined effects

of environmentally sensitive and stable QTLs are bound to have marked influence on the phenotype.

Only three QTLs influencing plant height under disease conditions were detected. This was not surprising, as the difference in height between the tall parent 'Sunrise' and shorter parent Line 356 was not large under disease conditions. The QTLs explain 70% and 46% of the phenotypic variance observed in increase in stem diameter and final stem diameter, respectively. With regard to plant height, the QTLs explain about 60% and 69% of total variation in increase in plant height and final plant height, respectively. This suggests the possible occurrence of many more QTLs which escaped detection due to size of segregating population and the high LOD threshold (3.0) used in the present investigation. However, the results of our QTL analysis on papaya lend support to the presence of only a few QTLs with major effect on the phenotype (Thompson, 1975; Edwards et al., 1987) as opposed to many QTLs with small and similar effects (Lande, 1981; Weller et al., 1988).

Comparison of QTLs detected under disease and disease-free (Chapter 4) environments indicate the presence of several loci affecting stem diameter under disease conditions only. Though some loci were detected in both environments (T12-1 and S12A-4), there were four new loci affecting stem diameter under disease conditions only (L15A-2, V16-7, L12-8 and D2B-1). Substitution of Line 356 alleles at three of these loci affected stem diameter in a positive direction. This corresponds with

the high level of tolerance observed in Line 356. Similar occurrence of constitutive QTLs active in different environments and environmentally regulated QTLs have been reported in other crops (Hayes et al., 1993; Paterson et al., 1991 and Schon et al., 1994).

6-4-2. Disease symptoms.

Based on mild symptoms observed under PRV disease conditions, Line 356 is considered a tolerant parent. The tolerance is partly transferable to Line 356 X solo hybrids (Zee, 1984). Surprisingly, we were able to detect four QTLs (one for leaf mosaic and three for fruit distortion) for which the alleles from Line 356 increased the severity of disease symptoms. In particular, the effect of QTL S12A-4 in increasing leaf mosaic, fruit distortion and also in reducing growth in stem diameter (period 3) suggest the interrelatedness between these traits. This result is in contrast to other research which has showed that a dominant factor from Line 356 is responsible for suppression of fruit distortion in Line 356 X solo hybrids (Zee, 1984).

6-4-3. ELISA titer.

We have detected six QTLs affecting ELISA titer in a positive as well as negative direction. All the loci show very small additive and a large dominance effect.. Though three of these alleles from Line 356 produce a slightly lower titer under homozygous conditions, none of the six loci show a clear mode of action.

6-4-4. Genetic basis of tolerance.

Line 356 is classified as a tolerant selection based on its mild symptoms, better vigor and generally lower ELISA titer under PRV disease conditions. The genetic basis of tolerance in Line 356 is complex and quantitatively inherited (Conover and Litz 1976; Zee, 1984). The tolerance observed in Line 356 is also transferable to Line 356 X solo hybrids (Zee, 1984). The commercial cultivars of Hawaii ('Sunrise' and 'Kopoho') are highly susceptible to PRV disease exhibiting severe disease symptoms and poor vigor, often leading to death of the plants.

The objective of the present investigation is to identify and characterize the genomic location of factors or QTLs responsible for PRV disease tolerance in Line 356. Plant vigor, severity of disease symptoms and ELISA titer were used to measure the level of tolerance. Previous reports on the genetic basis of tolerance in Line 356 suggest the possible existence of several QTLs affecting each component (vigor and symptom expression) of tolerance Conover and Litz, 1978; Zee, 1984). The lower ELISA titer observed in the tolerant Line 356 may also suggest the presence of few major genes that either reduce the rate of virus multiplication or slow the spread of the virus. The QTLs affecting these components are possibly in genetic linkage and an improvement in one component (eg. vigor or symptom expression) would result in overall improvement in the level of tolerance to PRV disease in the progeny.

The result of QTL analysis indicate the presence of several QTLs affecting each component of tolerance. Three QTLs affecting height, six QTLs affecting stem diameter, four QTLs affecting symptom expression and six QTLs affecting ELISA titer were identified. These results confirm the quantitative nature of tolerance to PRV in Line 356. Four of the QTLs affecting stem diameter were unique to PRV disease environment. With regard to symptom expression, one locus affecting leaf mosaic and three loci affecting fruit distortion were identified.

An unexpected finding of the present investigation, contrary to the proposed model, is the lack of correlation between different components of tolerance (Table 6.5). This is also evident by the genomic distribution of QTLs affecting different components of tolerance (Fig. 6.6). Except locus S12A-4, which had overlapping QTLs affecting leaf mosaic, fruit distortion and stem diameter, all the other QTLs appear to be distributed in non-overlapping regions of the genome. This result clearly indicates a lack of relationship between different components of tolerance. This is in conformity with the frequent lack of consistent relationship between symptom severity and agronomic impact of disease observed in other crops (Khun et al., 1981; Culver et al., 1987; Brown et al., 1987).

Another surprising result is the presence of two QTLs affecting stem diameter and four QTLs affecting symptom expression that caused a reduction in level of tolerance due to allele substitution by Line 356. The origin of the parent Line 356 and

the lack of correlation between different components of tolerance to PRV offer a plausible explanation for the presence of QTLs in Line 356 (a tolerant parent) with an effects opposite to the expected overall effect. The parent, Line 356, used to produce the F_2 population is a dioecious line and was selected from a third generation sib-mated population. The improvement in tolerance with progress in generations was accomplished by crossing selected disease-tolerant male and female plants in each generation (Zee, 1984). Hence, Line 356 is not homozygous and may still be segregating for genes affecting various traits, including those that are involved in PRV disease tolerance and may still be carrying unfavorable alleles. Secondly, the selection for tolerance in Line 356 was based on symptom expression only. Due to the lack of correlation between different components of tolerance, the selection of Line 356 based only on symptom expression may not always result in selection of favorable alleles or elimination of unfavorable alleles with regard to other components of PRV disease tolerance (Stem diameter or ELISA titer), which is consistent with the findings of present investigation.

6-4-5. Mechanism of disease resistance .

The genetics of mechanism of resistance to viral disease in plants has been reviewed by Fraser (1990). In majority of the cases where the resistance is shown to be systemically effective (low virus multiplication), the genetics of resistance is associated with incomplete dominance. The resistance of Tm-1 gene for TMV virus in tomato has been shown to be fully dominant with respect to symptom expression, while virus

multiplication is inhibited to a greater extent in Tm-1/Tm-1 as compared to Tm-1/+ genotypes (Fraser and Loughlin, 1980).The resistance to BCMV (bean common mosaic virus) in *Phaseolus vulgaris* is shown to be recessive (Drijfhout, 1978) but the multiplication of virus in heterozygote is very strongly inhibited compared to the susceptible parent (Fraser, 1992). Resistance associated with overall inhibition of virus multiplication often exhibits gene-dosage dependence and is considered as a positive type mechanism of resistance (Fraser, 1990).

The term tolerance is a subjective description of disease severity in infected individuals that is assessed by the absence of symptoms or no loss in vigor (Cooper and Jones, 1983). Tolerance does not necessarily constitute an active response of the host against a virus disease. The result of analysis on ELISA titer (OD at A_{405}) in a F_2 population of a cross between Line 356 and 'Sunrise' has indicated the presence of six loci. Substitution by Line 356 allele at three of these loci result in lower ELISA titer under homozygous condition, while remaining loci show a lower ELISA titer under heterozygous condition. None of these loci show a clear mode of action. We have attempted to use ELISA titer as a quantitative measure indicating the load of viral coat protein in the plant. The parents, Line 356 and 'Sunrise' show a wide difference in mean ELISA titer (0.431 and 0.994 respectively), that correlates with symptom expression, leading us to the assumption that these correlated responses reflect genetic differences in the parents that determine the rate and extent of virus replication in the plant cells. However, we have also shown the presence of interfering compounds in

Line 356 responsible for a general reduction in ELISA titer independent of virus concentration (Table 6.4). This raises the possibility that ELISA may tell us nothing useful about virus concentration, and consequently PRV resistance, in the segregating F_2 population. However, the amount of reduction in ELISA titer attributable to interfering compounds is low (15 to 27%) and does not account for the wide variation observed in the segregating population (0.037 to 1.147). These results suggest the presence of factors in Line 356 responsible for suppressing virus multiplication, thereby by resulting in lower ELISA titer. Similar mechanism of 'suppressive virus resistance' or the ability of the plant to inhibit virus multiplication was reported by Moyer et al., (1985). Thus, the possible occurrence of factors for suppressive virus resistance in Line 356 resulting in lower ELISA titer, and their probable gene dosage dependent nature suggest the existence of a 'positive type mechanism of disease resistance to PRV in Line 356. Hence, the disease tolerance observed in Line 356 may be considered as resistance or resistance to virus and tolerant to disease (cooper and Jones, 1983).

In summary, the genetic analysis of tolerance to PRV disease in Line 356 has indicated multiple QTLs affecting various components of resistance. Stem diameter appears to be an important index of disease resistance. The QTLs affecting ELISA titer indicate a positive type mechanism of resistance to PRV. Information on location of QTLs is expected to be of valuable help in consolidating all the favorable QTLs for resistance in an individual. Further, in absence of a better source of resistance to PRV

in *Caric papaya*, a combination of native resistance with other strategies like cross protection and coat-protein mediated protection may prove effective in mitigating the loss caused by PRV disease. Finally, majority of the components of resistance studied are under considerable environmental influence, hence these results are specific to the present location and any extrapolation should be done with caution.

Table 6.1. The biometrical parameters of QTLs affecting Stem diameter.

QTL	Period	LOD	%Var.exp	a	d	Mode
D2B-1	2	6.61	68	0.56	-0.46	R
L15A-2	2	3.84	65	0.50	-0.51	R
V16-7	2	5.01	69	-0.43	-0.63	D
L12-8	2	3.62	70	0.60	-0.42	R
*T12-1	3	5.85	49	1.17	-0.45	AD
D2B-1	3	5.65	56	0.90	-0.24	A
*S12A-4	3	3.17	47	-0.57	-0.66	D
T12-1	4	5.0	38	0.58	0.00	A
D2B-1	growth	4.86	39	1.86	1.07	AD
L15A-2	"	1.34	56	0.09	-0.58	RAD
T12-1	diameter.	5.88	46	3.6	-1.63	AD

Foot note: The QTLs are indicated by the left flanking marker followed by the linkage group number. 'Growth' refers to total increase in diameter and diameter refers to final diameter at the end of the experiment. The growth period is indicated by numbers 1 to 4. The letters 'a' and 'd' denote additive and dominance effect due to substitution of Sunrise allele by 356 allele. The mode of actions are indicated by letters 'A','D' or 'R', which indicate additive, dominant or recessive mode of action. Under situations where two possible modes of action are listed, the first letter indicates the most likely mode of action. For more details refer text. The loci with stars were also active with similar effects under disease free conditions.

Table 6.2. The biometrical parameters of QTLs affecting plant height.

QTL	Period	LOD	%Var.exp	a	d	Mode
T12-1	1	4.17	25	-18.47	23.14	ADR
"	2	10.23	53	28.61	-23.07	R
"	3	8.81	56	8.43	0.53	AD
"	Growth	4.41	35	22.29	1.81	AD
*O15-7	"	2.26	45	5.38	-21.55	DAR
L12B-1	4	5.14	40	6.22	2.72	DA
"	Height	3.81	29	28.77	9.41	AD
*O15-7	"	2.93	58	7.4	-36.37	DAR

Foot note: The 'growth' refers to total increase in plant height, and height refers to plant height at the end of the experiment.* Refers to QTLs with LOD below 3, detected by rescanning the genome with a fixed major QTL. For other information refer to table 6.1.

Table 6.3. The biometrical parameters of QTLs affecting disease symptoms and ELISA titer.

QTL	LOD	%Var.exp.	a	d	Mode
Leaf mosaic					
S12A-4	3.47	42	0.53	-0.27	RA
Fruit dist					
L15C-2	4.51	74	0.92	-0.98	R
S12A-4	3.15	61	0.82	-0.93	R
H13-10	3.93	70	0.75	-1.22	R
ELISA titer					
V14A-2	4.05	80	-0.02	-0.310	DAR
M13-2	4.71	81	0.002	-0.301	"
D20B-3	4.38	80	0.02	0.435	"
I9B-5	4.12	80	-0.04	0.415	"
B11-7	3.94	80	0.05	0.448	"
K3-8	4.05	78	-0.02	-0.301	"

For other information refer to table 6.1.

Table 6.4. Effect of interfering compounds in healthy leaf extracts from Line 356 and 'Sunrise' on ELISA titer.

Treatments	ELISA titer (OD at A_{405})		
		Ratio 1:1	Ratio 1:3
PRV infected (cultivar 'Sunrise')	0.213 (0.191-0.253)	–	–
Healthy 'Sunrise'	0.001	–	–
Healthy Line 356	0.001	–	–
PRV infected+Healthy ' Sunrise' (Cultivar 'Sunrise')	–	0.2 (0.186-0.212)	0.15 (0.117-0.177)
PRV infected + Healthy Line 356 (Cultivar 'Sunrise')	–	0.15 (0.117-0.172)	0.12 (0.099-0.160)

The numbers in parenthesis indicate the range.

Table 6.5. Correlation of PRV symptoms and ELISA titer in Line 356 X 'Sunrise' F_2 population under disease conditions.

	ELISA titer	Leaf mosaic	fruit distortion	stem diameter
ELISA titer	1			
Leaf mosaic	0.05	1		
Fruit distortion	-0.02	0.05	1	
Stem diameter	0.02	-0.25	-0.15	1

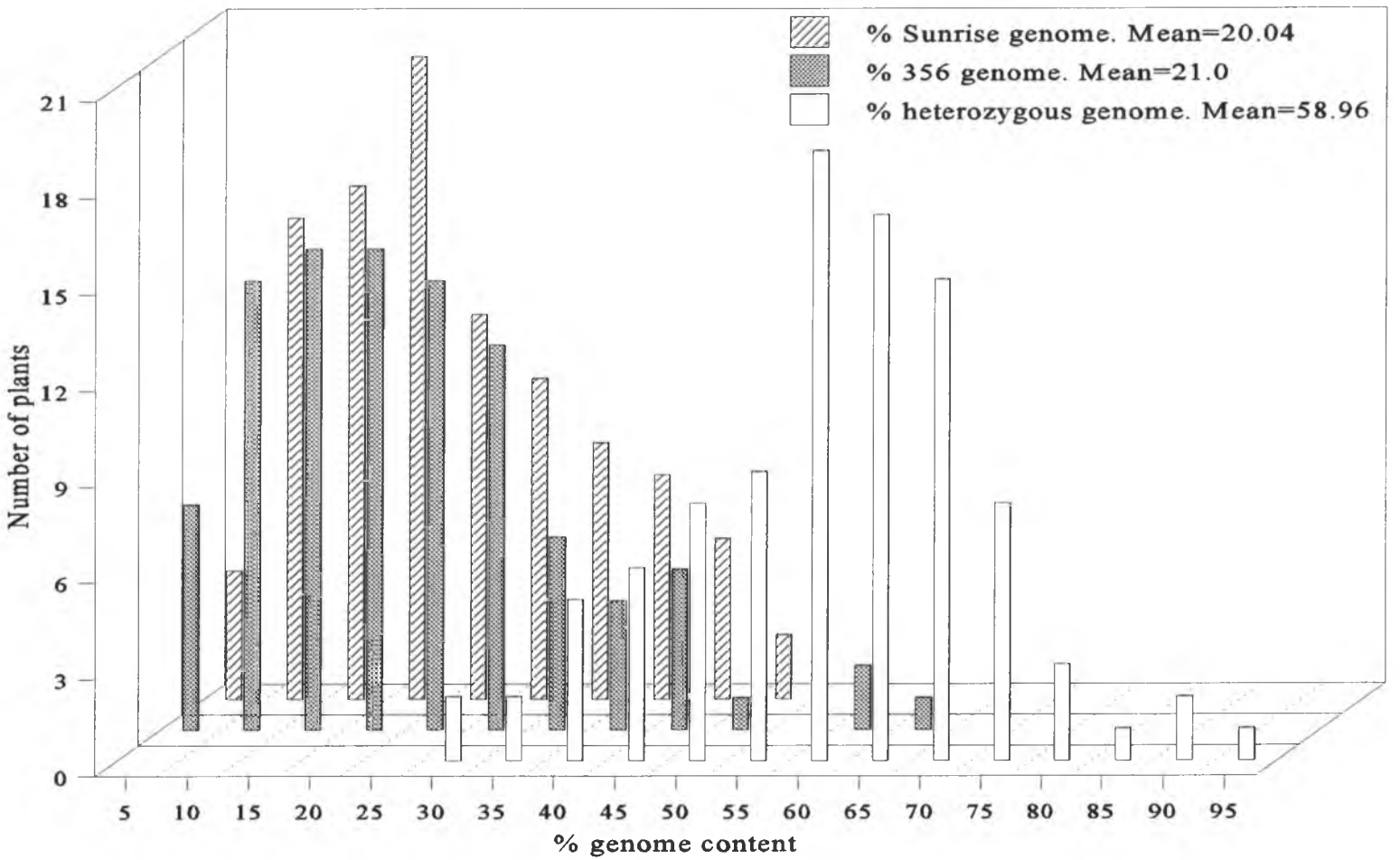


Fig. 6.1. The frequency distribution of genomic regions homozygous and heterozygous to Line 356 and 'Sunrise' genomes in line 356 X Sunrise F_2 intercross population.

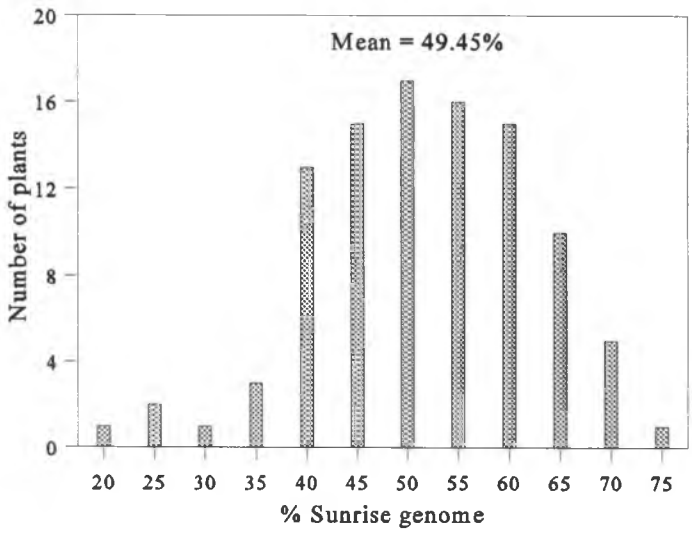


Fig. 6.2. The frequency distribution of percent 'Sunrise' genome content in Line 356 X 'Sunrise' F_2 intercross population.

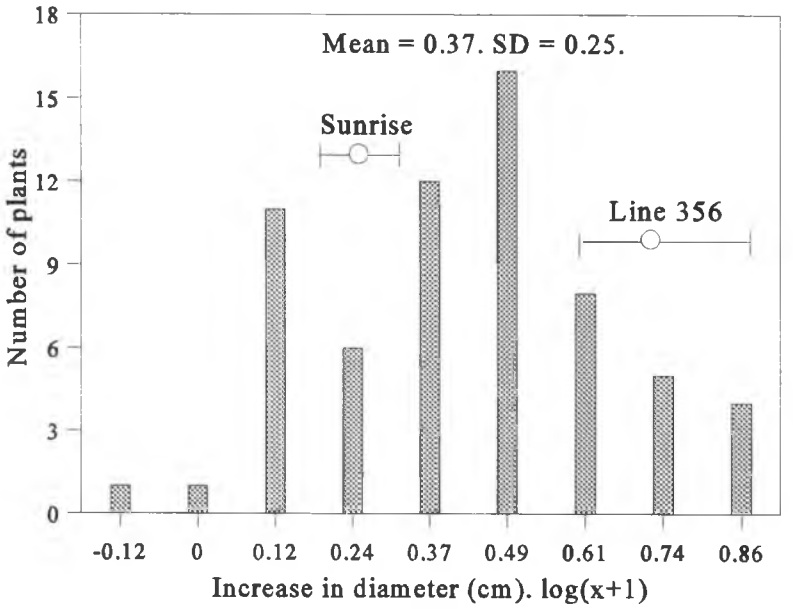


Fig. 6.3A

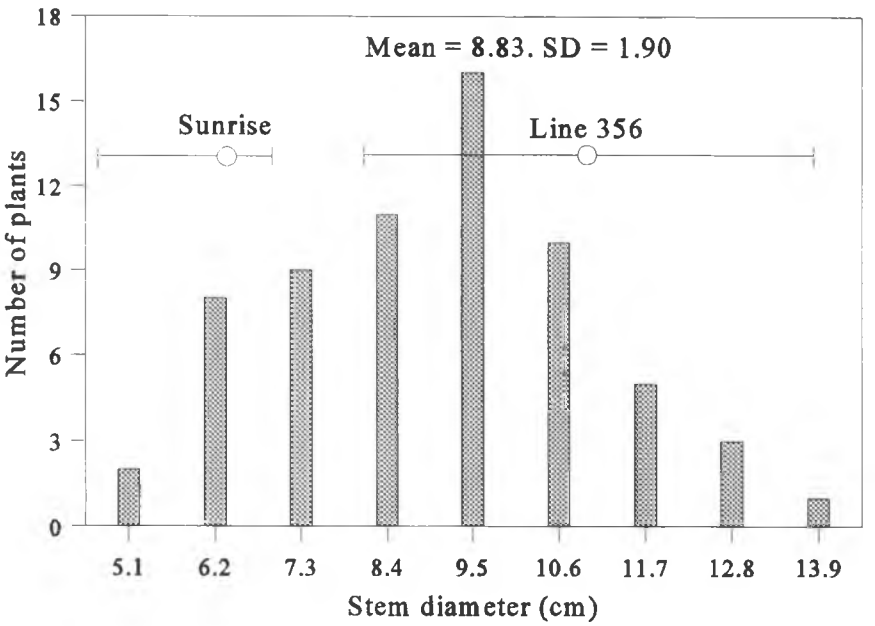


Fig. 6.3B

Fig. 6.3. A and B. Frequency distribution of increase in stem diameter and final stem diameter in 'Sunrise' X Line 356 F_2 population. The horizontal lines and open circles indicate parental range and mean, respectively.

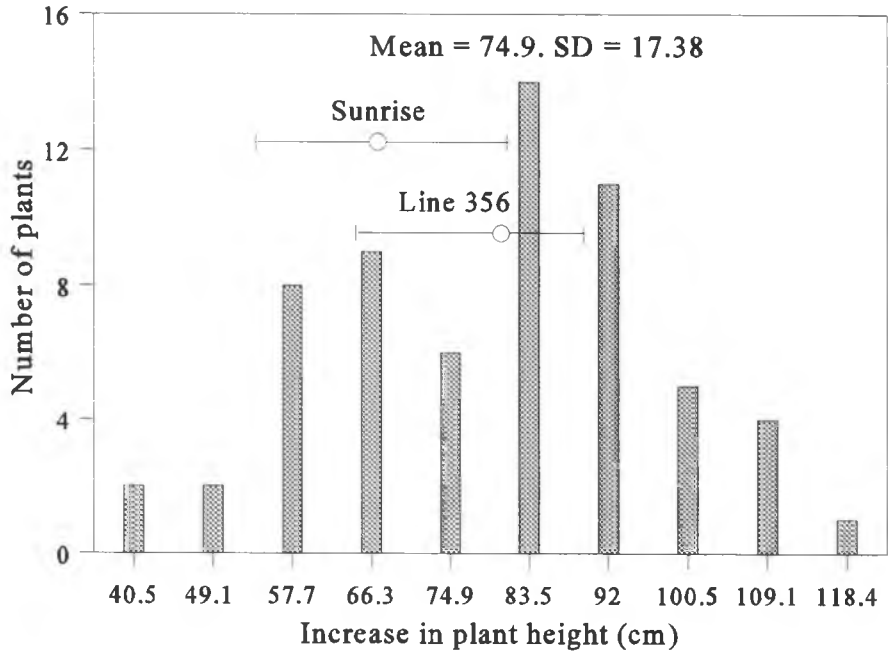


Fig. 6.4A

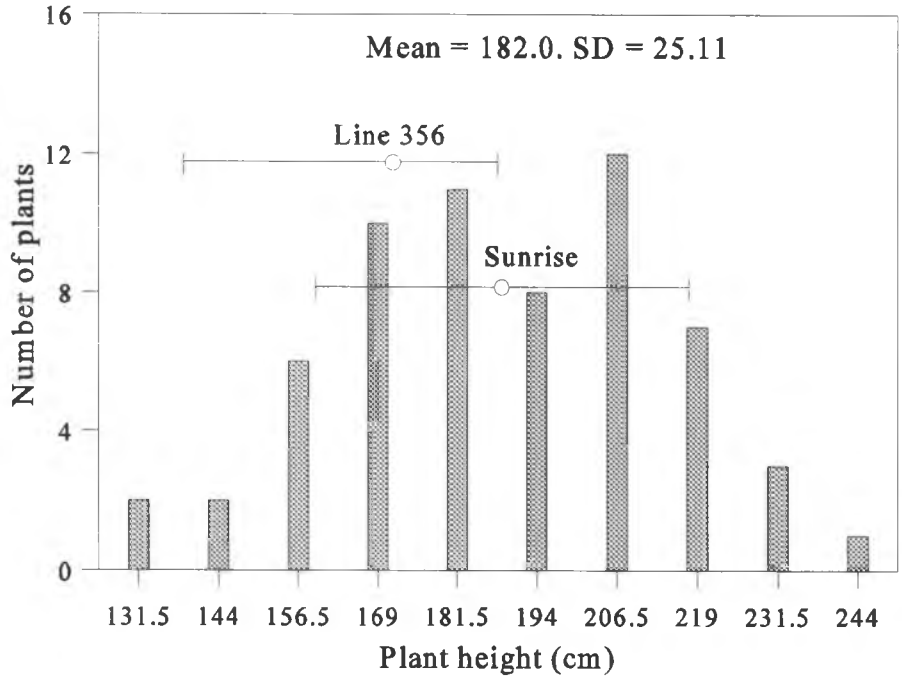


Fig. 6.4B

Fig. 6.4 A and B. Frequency distribution of increase in plant height and final plant height in 'Sunrise' X Line 356 F_2 population. The horizontal lines and open circles indicate parental range and mean, respectively.

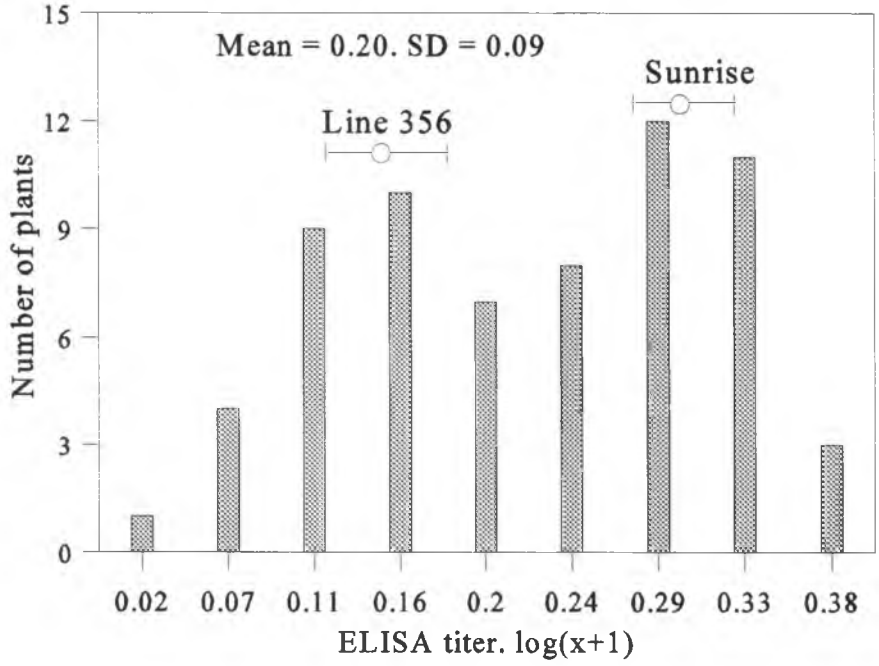


Fig. 6.5A

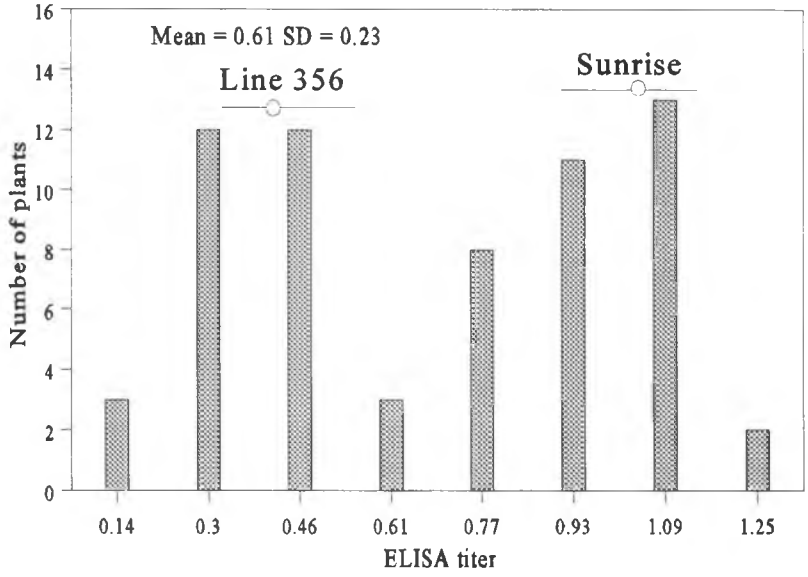


Fig. 6.5B

Fig. 6.5 A and B. Frequency distribution of ELISA titer in 'Sunrise' X Line 356 F2 population. The horizontal lines and open circles indicate parental range and mean, respectively.

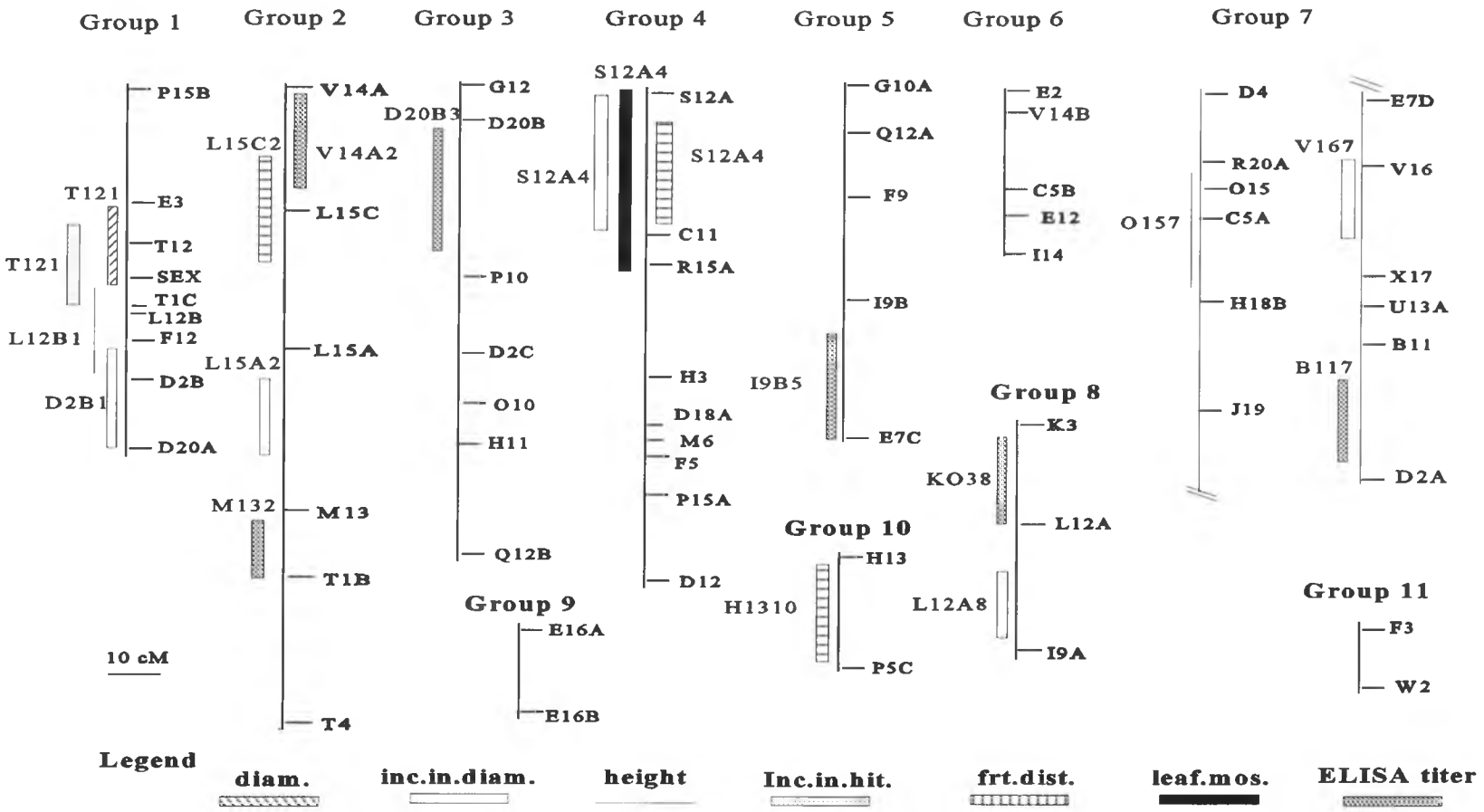


Fig. 6.6. Likelihood intervals of QTLs affecting height, diameter, disease symptoms and ELISA titer in 'Sunrise' X line 356 F_2 population. The bars indicate the likelihood peak region within a lod difference of 1.0. The letters on the bars indicate the designated names of the QTLs.

CHAPTER 7

Summary

Polymorphisms between 'Sunrise' and Line 356 were detected by using randomly amplified polymorphic DNA (RAPD) markers. A total of 596, 10-base oligomers were screened to obtain 96 polymorphisms. The extent of polymorphism observed (0.16/primer) between the phenotypically diverse cultivars is one of the lowest observed with RAPD markers among varieties of crop species. A total of 253 F_2 intercross segregants of 'Sunrise' and Line 356 cross were scored for 96 RAPD and 2 morphological markers (Sex and flesh color).

The segregation data was used to construct a linkage map using MAPMAKER/EXP program (ver 3.0). Seventy-four markers were grouped at a LOD score of 4.0. Sixty-two of these markers (61 RAPD and sex) were ordered into 11 linkage groups covering a total distance of 999.3 cM. Only one of the 61 RAPD markers, and sex of the plant were inherited codominantly. Over 80% of the RAPD markers showed the expected Mendelian segregation ratios. Seven linkage groups had 5 or more markers with linkage group 7 accounting for over one fifth of the total map length. The mean interval length is about 20 cM and over 75% of the intervals are within 30 cM. The *sex* locus is mapped to linkage group 1, within a marker bracket of 14 cM. The *sex* and the flanking markers (OPT12 and OPT1C) are consistent with the expected Mendelian ratios. The flanking markers are linked in coupling phase. Over abundance of female plants among the recombinants in this region compared to

hermaphrodite plants suggest the presence of the hypothetical lethal factor within the 14 cM marker bracket. These results strongly favor Storey's hypothesis on sex determination in papaya. The current practice of planting 3 plants per hill and subsequent thinning to keep one hermaphrodite plant at flowering (6 months) can be replaced by assaying for the flanking markers at seedling stage (2 leaf stage). The results clearly demonstrate the utility of RAPD markers in constructing a primary genetic linkage map of papaya.

QTL analysis

Analysis for vigor, precocity, carpellody, sterility, fruit weight, fruit number and fruit per node was performed on a population of 100 F_2 plants at the Poamoho Experiment Station, Oahu, Hawaii. The MAPMAKER/QTL program, based on maximum likelihood and interval mapping technique, was used. A LOD score of 3.0 was set to declare the presence of a QTL. Regarding vigor, defined as increase or rate of increase in stem diameter, the data on growth over three-month periods as well as annual growth were subjected to QTL analysis. Multiple QTLs affecting all the above mentioned traits were detected. The phenotypic variance explained by individual QTLS ranged from 18% to over 60%. The extent of total phenotypic variation explained by QTLs in the majority of the traits studied indicate the occurrence of a few QTLs accounting for the majority of the variation in papaya. Non-random distribution of QTLs is indicated by a concentration of several QTLs in certain map regions such as linkage group 1 and 7.

Analysis on growth rates enabled detection of QTLs that are environmentally sensitive, and which would not have been detected by the analysis of final phenotype (height or stem diameter at the end of experiment). It also resulted in better resolution of neighboring peaks in QTL analysis. Genetic factors with opposite effect to the overall phenotype of Line 356 were detected in the majority of the traits studied. A surprising finding is the occurrence of a QTL for delayed flowering in the precocious parent, Line 356. These results suggest that Line 356 is still segregating for several traits of economic importance.

Three QTLs affecting carpellody explained over 97% of phenotypic variance. This result coupled with the observed segregation with a 3:1 ratio of carpellod to non-carpellod fruit bearing plants suggests a possible interaction between a qualitatively inherited factor and at least 3 QTLs, referred to as modifying factors. The qualitative factor could not be mapped in the present analysis, perhaps due to its close linkage with hermaphrodite locus or the high LOD score (4.0) employed.

The genetic analysis of components of disease tolerance in Line 356 to papaya ringspot virus was carried out on 100 F_2 plants at Waimanalo Experimental Station, Oahu, Hawaii. All plants were inoculated mechanically with virus extract and the infection was confirmed by ELISA. Analysis of plant vigor indicated the occurrence of several QTLs contributing to growth in diameter and plant height under disease conditions not detected in uninfected plant. Stem diameter, in particular, is diagnostic

for survival of the plant. The severity of other disease symptoms were rated on a scale of 1 to 4. QTLs affecting leaf mosaic and fruit distortion were detected. All of these were consistent with recessive mode of action. The consistent wide differences in ELISA titer between infected 'Sunrise' and Line 356, and the range observed in the F_2 plants clearly indicates quantitative inheritance of ELISA titer. ELISA readings 20 minutes after incubation with substrate, performed on the third youngest leaf under controlled conditions were consistent and repeatable. QTL analysis detected several loci affecting ELISA titer. All had dominant or partially dominant modes of action. Among QTLs causing reduction in ELISA titer, some showed a greater reduction when heterozygous. The occurrence of QTLs causing a reduction in virus titer suggest the presence of a mechanism of "suppressive virus resistance" in Line 356. These results clearly suggest the presence of an active mechanism of resistance to PRV in Line 356.

Future line of work

The linkage map of papaya with 11 linkage groups, covering 1000 cM is incomplete. There is a need to saturate the map with more markers to cover the entire genome. Inclusion of 2 to 3 well placed RFLP markers per linkage group is needed to improve the map to perform fine scale genetic analysis of traits. Dominant markers (majority of RAPD markers) do not provide correct estimate of the genetic linkage (especially when linked in repulsion phase) and gene-dosage effects. Use of codominant RFLP markers results in better estimation of recombination and the genotype of the individual, thereby improving the accuracy of the map.

The results of the present investigation are expected to be valuable in formulating a strategy for crop improvement in papaya. The wasteful practice of supporting three plants per hill for first 6 months can be replaced by assaying for flanking marker at seedling stage to rise a population of preferred sex type, especially for research trials. The information on location and characters of QTLs under normal and disease-free conditions is helpful for multiple trait improvement aimed at introducing favorable QTLs in a common background within a reasonable time. With regard to PRV resistance, which demands immediate attention, an attempt is needed to further improve the disease resistance to PRV by combining the native resistance with other strategies like cross protection and coat protein mediated protection.

APPENDIX A
GENOTYPE OF F_2 PLANTS

```
Linkage group 1          Plant numbers
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000000
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
P15B ---------------------------------------------------------
E3   ---------------------------------------------------------
T12  ---------------------------------------------------------
Sex  HHHHHHAHAAAAHHAHHAAHHAAHHAAHHAHHHHHHHAHAHHAAHAHAHH
T1C  ---------------------------------------------------------
L12B ---------------------------------------------------------
F12  ---------------------------------------------------------
D2B  CCCCCCACCACCCCACCCCCCACCCC-CCCCCCCCCCACCCC-ACCCCCC
D20A ---------------------------------------------------------
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
15B  ---BDBBDDDDDBBDDD-DBDBDD-BDDBDDDDDD-DDDBD-BDDDDDDD
E3   ---CCCCCCCAACCC-CAACACAA-CCCCCHCAACCCCCCAACCCACAAA
T12  ---CCCCC-C-ACCCCC-CCCC---CCC-CACCACCCCCCC-C-CCCAAA
Sex  HHAHHHHHHHAAHHHHHHAHAHAAHHHHHHAHAAHHHHHHHHHHAHAAA
T1C  ---CCCCCCCACCCCCCCACACAACCCCCCACAACCCCCCCCACCCACAAA
L12B ---DDBDDDDDDDDD-DDDDDDDDDDDDDDDDDDDDBDDDDDDDDDDDD
F12  ---CCCCCCC--CCC-C-ACACAA-CCCCCACAACCCCCCC-CCCACAAA
D2B  CCACCC-CCCAACCCCCCCACCCHHCCCCCAACAACCCCCCCCACCCACAAA
D20A ---CCCCCCCACCCCCCCCACCCA--CCC-CACACCCCCCCCCCCCCACCAC
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111111
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
P15B DDDBDDDDDDDDDDDDDDHDBDDDDBDD-DDDDDB-HBDD-DDDDDBDDB
E3   CACCACCACCCCAACCCACCCAACACCCACCACCCCCCCAACCCCACCCC
T12  CACCACCACCACAACCCAACCAACACACCCCACC-CCCCC---CCACCCC
Sex  HAHHAHHAHHHHAAHHHAAHHAAHAHAHHHHAHHHHHHHHAAHHHHAHHHH
T1C  CACCACCACCCCAACCCAACCCACCCACCCCACCCCCACAACCCCACCCC
L12B DDDBDDDDDDDDDDDDDDDDDDDDBDDDDDDDDDDDDDDDDDDDDDD
F12  CACCACCACCACAACCCAACCCA-ACACCCCACC-CCCCA-CCCCACCCC
D2B  CACCACCACCHCAACCCAACCCACACACCCCCCCCCCCHAAACCCACCCC
D20A CACCACCCCCACAACCCAACCCCCCCCCCCCCCCCACCCC--CCCACCCC
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
P15B BBD--DDDDBDD-DDBDDDDD-DBDD-DDDB-DHDDBHDDHDBBDDDDBD
```

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E3    CCAACAACCCCACCCCACA-ACCC-CCCC-AAACAACCCCCCCCCAAACC
T12   CCAACAACCCCACCCCACC-C-CC-CCC-CA-CAA-CACCCCCCCACACC
Sex   HHAAHAAHHHHAHHHHAHAAHHHHHHHHHHAHHAHHAAHHAHAHHAHAHH
T1C   CCACCAACCCCCCCCCCCA-CCCCCCCCCCA-CAACCACCACACCACACC
L12B DDDDBDDDDDDDDBDDDDDDDDDDDDDDB-DDBDDDDDDDDDDDDDDDD
F12   CCAACAACCCCACCCCACA-C-C-CC-C-CA-CAACCACCACACCCCCAC
D2B   ACAACAACCCCHCCCCACA-CCCCCCCCCCCACCAACCACCACACCACACC
D20A CCAACAACCCC-CCCCACA-CCCCCA-CCCA-CCACCCCCCCACCACACC
-------------------------------------------------------------
      22222222222222222222222222222222222222222222222222222
      00000000011111111112222222223333333333444444444455555
      12345678901234567890123456789012345678901234567890123
-------------------------------------------------------------
P15B D-DBDDDBDDDBDDDDDDDBBDDDDBDDDDDDDDDDBDDDDBDDBDBBDDDDD
E3    CCACACCCCCCCCCCCCCCCCACCACACACACCCCCCCAACCACCAAACACCC
T12   CCACACC-C-HCCCCCCC-CCA-C--A--CAC-CCCCCAACCAC-AAACACCC
Sex   HHAHAHHHHHAHHHHHHHHHHAHHAHAHAHAHHHHHHHAAHHAHHAAAHAAHH
T1C   CCAAACC-CCACCCCCCCCCCCACCACACACCCCCCCCCCCACCACCAAACCCCC
L12B DDD-DBDDDDDDD-DDDD--BDDDDDDD-DDDDDDDD-DDDDDDDDDDDD-DD
F12   CCCCACC-CCCCCCCCCCCCCCACCACACCCACCCCCCCCCCCCACCACCCCCCC
D2B   CCC-ACC-CCCCCCCCCCCCCCACCACCCACACCCCCCCCACCCCCCCCCACCCCC
D20A CCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCCCCCCCCCCCC
```

Linkage group 2

```
-------------------------------------------------------------
      00000000000000000000000000000000000000000000000000
      00000000011111111112222222222333333333344444444445
      12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
V14A ---------------------------------------------------------
L15C ---------------------------------------------------------
L15A ---------------------------------------------------------
M13  ---------------------------------------------------------
T1B  ---------------------------------------------------------
T4   ---------------------------------------------------------
-------------------------------------------------------------
      00000000000000000000000000000000000000000000000001
      55555555566666666667777777777888888888999999999990
      12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
V14A ---CCCCAACCCAACC-CCCCCACCCAACCCCCCACCCC-CCCCACCCCC
L15C ---CCCCACCCCAA-CC-CCCAAC-CC--CCCC--CCCCAC-C-ACCCCC
L15A ---CCCCAACCAAA-AC---CAAC-CA--CCAC--CCCCCA-A-ACACCC
M13  ---DB-BDDBDDDDDDBDDDDDDBBDDDBBDDDDDBDDBDDBDBDDDDDB
T1B  ---DBBDDDBDDDDDBBBDDDDDBDDDDBBDDBDDBDBBDDDDBDDDDBB
T4   ----BDDDDD-DDDDBBDDDDDDDDDBDDBBDDBDDBDBBDBBDBDDDDBB
-------------------------------------------------------------
      11111111111111111111111111111111111111111111111111
      00000000011111111112222222222333333333344444444445
      12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
```

```
V14A CACCCACCACCCCCACACACCAAACAA-CCCAACC-CCCACCCCCAACCA
L15C CACCCACCACCCCCACACACCACACCCCCACAA-CCCCCACCCCCCCCCA
L15A ACCCCACAACCACCAAACACCACACCACAACAA-CCAAACAACCCAAACA
M13  DDDDDDDBDDBDDBDDDDDDDDDBDBBDDDDDDDDDDDDBDDDDDDBDDBBD
T1B  DDDDDDDBDDDDDDDDBDDDDDDBDBBDDDDDDDDDDDDDDDDDDDDDBD
T4   DDDDDD-DDDBBDDDBDDDDDDDDBBDDBDDDDDDDDBBDDBBDDBDDBBD
-----------------------------------------------------------
     11111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-----------------------------------------------------------
V14A ACCCC-CACCCACC-AACC-CCCCCCCCCCACACCAACCACACCACCCCC
L15C CCCCCCCCCACCCCCCACC-CCCCCCCCA-CCAACCCCCAAACCAC-ACC
L15A AAAAACAACACACACAACC-CCCCCCACA-CACACCCCCACACCAC-ACC
M13  DDDDDBBDBDBDDBDDDBD-DDBDDDDDBDDDDDBBBDBDDDDDDDDDDB
T1B  DDDDDBBDBDBDDBDDDBD-DDBDDDDDBDDDDDBBBDBDDDDDDDDDDB
T4   DDDD-DDDBDBDDDDD-DD-DDDDDDDDBDDDDDDDBDBDDDDDDDDDD
-----------------------------------------------------------------
     22222222222222222222222222222222222222222222222222222222
     00000000011111111112222222223333333333444444444455555
     12345678901234567890123456789012345678901234567890123
-----------------------------------------------------------------
V14A CCCCACC-ACCCCAACCCACCCCCAACCCACCCCAACCACCCCCCCAACC--CC
L15C CA--CACCCCCCCACCACCCCACCCAACCCACCCCACACCCCCCCCACAC-CC
L15A CA--CAACCCCC-ACCACCCAACCAAACACAC-CAACACCCCC--AACCC-AA
M13  DBDDDDBDBDDDDDDDBDDDBBDBDDDDDDDDDDDBDDBDDDDDBDDDDDDDBDD
T1B  DBDDDDB-DDDDDDDDDDBBDBDDDDDDDDDBDDDBDDDDDDDDDDDDDDDBDD
T4   DDDDDDD-DD-DDDDDDDDDDDDD-DDDDDDDDDDDDDDDDDDDBDDD-DDBDD
```

Linkage group 3

```
-----------------------------------------------------------
     00000000000000000000000000000000000000000000000000
     00000000011111111112222222223333333333444444444445
     12345678901234567890123456789012345678901234567890
-----------------------------------------------------------
G12  DDBDDBDDDDDDDDDDDDDDDDDDDDDDDDBDDDDDDDDDDDBDBDDBD
D20B ----------------------------------------------------
P10  ----------------------------------------------------
D2C  BBDDDBDBDBBDBDBBDDDDBBDBDD-DDDDDBDBDDBDBDDDDDDDDBD
O10  ----------------------------------------------------
H11  BBDDDBBDDDBDBBBBBDDBBDDDDDDDDDDDBDDDDDDBBDDDBDDDDD
Q12B ----------------------------------------------------
-----------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-----------------------------------------------------------
G12  DDDDDDDDDDBBBDBDDDBDDBDDDDBBDDDDBBDBDBDDDDDBDDDDBD
D20B ---DDDDDDDBBDBDDBBDDBD--BBB-DDBBBDBDBDDDBDBDDDDBD
P10  ---CA-CCAACCCAACACCCACCCCCCCCCAACCACACCACCACACCACA
D2C  BBBDD-BDDDDDDDDBDBBDDBDBDBBDDBDDBDDBDBBDDDDBBDDDDD
```

```
O10   ---BD-DDDDDDBDDBDBDDDBDBDBDBDDDDBDDBDBDDDD-BBDDDDD
H11   BDBBDBDDD-DDBDDDBDDDDDDBDDDDBDDDBDBBDBDDDBDBBBDDDD
Q12B  ---CACCCCCCCAACCCCCAACCAACCCCCAACCCCCCCCCCCCCCCACC
-------------------------------------------------------
      11111111111111111111111111111111111111111111111111
      00000000011111111112222222222333333333344444444445
      12345678901234567890123456789012345678901234567890
-------------------------------------------------------
G12   DDDDDDDDDBBBDBBDDDDDBBBBDDDBDDDBDDDDBDDDDDBBDDBDDB
D20B  DDDDDDDDDBBBD-BBDDDDBBBBDDDBDDDBDBDDBDDD--BBDDBDDB
P10   CCCCCCCCCCCCCACC-CAACCCCCCCCACC--CCAACCCCACCAC-CCCC
D2C   DDDBDDDDDDBDBDBDBBDDBDDBBDBDDDDBBDDDDBDDDDDBDDDBBDB
O10   DDDBDDDDDDBDBDBDBBDDBB-BDBBDDDDBDDDDDBD-DDDDDDDBBDB
H11   DDDBDDDDDDBDBDBDBBDDBBDBDBBDDDDBBDDDDBDDDDDDDDDBBDB
Q12B  CCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCAACCCCCCAAACCC
```

```
-------------------------------------------------------
      11111111111111111111111111111111111111111111111112
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-------------------------------------------------------
G12   DDDDDDDDDDDDDDDDDDDBBBDDDDB-DBDBDBBDBDDBDDDDDDDDDD
D20B  DDDDBDDDBDD-DDDDBBD-BBDDDD-BDBD-DBBB-DDDDDDDDDDDDD
P10   CCCACCCCCCCCCCCCACCACCCCCCCCCCAACCCCCCCACCCCCCCCCCCC
D2C   DDDDBDDDBDBDDDDBDDD-DDDDDDDDBDDDDDBDDDDDBBDDDDDDDDD
O10   DDDDDDDDBDBDDDDDDDD-BDDDDDDBDDDDBBDDDDDBBDDDDDDDDD
H11   DDDDBDBDBDBDDDDDDDD-BD-DDDDBDDDDBBDDDDDBBDDDDDDDDD
Q12B  CCCCCACCCCCCCCCCCAA-CCCCCCCACCAACCCACAACCCCCAAACCACA
-------------------------------------------------------
       22222222222222222222222222222222222222222222222222
       00000000111111111122222222223333333333444444444455555
       12345678901234567890123456789012345678901234567890123
-------------------------------------------------------
G12   DDBBDDDDDDDDDDDDDBBDDDDDDDDDDBDDDDDDBDDDBDDDBDBBBDDDD
D20B  D--BDBBDDDDDDBDDDBBDDDDDDDDDDBDDDBDDBDDDBDD-BDD-BDDDD
P10   CCC--CC-C-CCCCCCCCCCCCC-----------------------------
D2C   BDD-DDD-DDDDDDBDDBDDDDDDDBDDDDDDDBDDDDDBBDDDBDBBBDDDB
O10   BDDDDDD--DDDDDBDDBDDDDD-DBDDDBDDDBDDBDDBBDDDBDBBBDDDD
H11   BDDDDDDDDDDDDDBDDBDDDDDDDBDDDDDDDBDDBDDBBDBDBDBDBDDD
Q12B  CCACACC-CCCCCCCAACCACCCCCCCACACCCCACAACCCACCCCCCCCACC
```

Linkage group 4

```
-------------------------------------------------------
      00000000000000000000000000000000000000000000000000
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-------------------------------------------------------
```

```
S12A ----------------------------------------------------
C11  DDBDDBDDDDDDDDDDDDDDDDDDD-DDDBDDDDDDBBDDDDDBDDDBD
R15A ----------------------------------------------------
H3   BDBDDDBDDDBDDDDDDDDDDBDDDB-DDDDDDDDDDDDDDBDDDDDDBD
D18A CCCCACCCACCCCCAACCCCCCCCCCC-CCCACACACACCCCCCCCCACCCC
M6   ----------------------------------------------------
F5   CCCCACCCACCCCCAACCCCCCCCCCC-CCCACCCACACCCCCCCCCACCCC
P15A ----------------------------------------------------
D12  ----------------------------------------------------


----------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
----------------------------------------------------------
S12A ---DBDDDDBBBDDBDD-BDDBDDDBDB-BDBDBBDDDBBDDDDDDDDBD
C11  DDDDBDDDDBDDDDDDDBDDDDDDDBDBDBDBBDBBDDDBDDBDDDDBDD
R15A ---ACACCCCCCAAAAACCCCCCCACACCCCCCCCCCCCCCCCCACCCAC
H3   DBDDD-BBDBDDDDDDDDDDDDDDBDBDDBDDBDDDDDDBDDDDBDD
D18A CCCCC-CCCCCACCAAAACCCCACACACCCCCCCCCACACCACAACCCAA
M6   ---HH-BBHBHAHHAAAAHHHHAHAHAHHHHBHHHBAHABBABAABHBAA
F5   CCCCCCCCC-CACCAAAACCCCA-ACACCCCCCCCCACACCACACCCCAA
P15A ---CCCCCCCCACCAHCCCCAC-C-CACCCCCCCCCACACCCCACCCCAA
D12  ---CCCCCCCCACCCACCCCCCACCCACCCCCACCCACACCACACCCCAC
----------------------------------------------------------
     11111111111111111111111111111111111111111111111111
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     12345678901234567890123456789012345678901234567890
----------------------------------------------------------
S12A BBDDDDDBBBBDBDDBDDDBDDBDDBDDBDBDBBDDDDDD-BDBBBBDDD
C11  DDBDDDDDDBDDBBDBDDBBDDDDDDDDBDDBDDDDDDD-DDD-DDDBBD
R15A CCCACCAACCCACCCCCCCCACCCCCCCCCACCCACAAACCCCCCCCCCC
H3   DBBDBDDDDDDDDBBDDDDDDDDDDDDDDDDDDDBDDDDBDDDDDDBBDB
D18A AACCCAAAACC-CCCCCCCCACACCHACACA-CCCAAAAACCCCCCCCCC
M6   AHBHHAAAAHHHHBBBHHHHABAHHHAHAHAHHBHAAAAHBHHHHHBBHB
F5   ACCCCAAAACACCCCCCCCCACACCCACACACCCCAAACCCCCCCCCCCC
P15A ACCCCAAAHCACCCCCCCCCACACCAACCCACCCCAAACCCCCCCCCCCC
D12  CCCCCAAAACACCCCCCCCCCCCCACACCCCACCCCCCCCCCCCCCCCC
----------------------------------------------------------
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     12345678901234567890123456789012345678901234567890
----------------------------------------------------------
S12A DDBDDDBDDDDDDD-DDBD--DD--D-BD-D-DDBBDDDBDDDBDBDDDDB
C11  DDDDBBBDDDDDD-DDDBD--DDDDDDBBDDBDBBBDDDBDDBDDDBDDB
R15A AACACCCCCCACCACCCCCCCAAAACACCACCACCCACCCCACCCCCACC
H3   DDDDDBBDDDDDDDDDDBBD-DDDDDBDBDDDDDDDDBDBDBDDDDBDDDD
D18A AACHCCCACCCACAACCCAACAAAACCCCCCACCCCCCACCACCCCACAC
M6   AAHAHBBAHHHAHAAHBBA-HAAAABHBHHHABHHBHBABHAHHHBHHAB
F5   AACACCCACCCCCACCCCC--A-AA-C-CCCACCCCCCACCACCCCCCAC
```

```
P15A AACACCCACCCCCCCCCCCCC-ACCCCCCCC-CCCCCCACCACCCCCCAC
D12  ACCACCCACCCCCCCACCC-CCCCCC-CCCCCCACCCCACCCCCCCCCCC
-------------------------------------------------------------
     22222222222222222222222222222222222222222222222222
     00000000011111111112222222223333333333444444444555
     12345678901234567890123456789012345678901234567890123
-------------------------------------------------------------
S12A B-DDBBBDDBDBBBDDDDDDDDDDDD-D-DBD-DDDDDDDBDDB-DB-DD-DD
C11  BDDDBDBBDDDBBBDDDDDDBDDDDDDDBDBBB-BDDDDDDDDDBDDDDBDDD
R15A CCCACCC-CACCCCAAACCACACCACCCCCCCCCCAAAACCCCCCACCCCCCA
H3   DBDDDDB-DDDDDBDDBDDDDDDDDDDBDBBDDBDDDDDBDBDBDBDDDDDD
D18A CCCCACC-CACCCCCCCACACACAACAACCCCA-CAAAAACCHACACCCAA-C
M6   HBHHAHB-HAHHHBHHBAHHHAHAHHAABHHBHHBAAAAAHHHABAHHHAHHH
F5   CCCCACCCCACCCCCCCAACCACACCCACCCCCCCAACAACCCACACCCACCC
P15A C-CCACCCCACCCCCCCAHCCACACCCACCCCCCCCHCAACCCCCACCCCCCC
D12  CCCCACCCCACCCCCCCAACCACACCCACCCCCCCCACAACCCCCACCCCCCC
-------------------------------------------------------------
```

Linkage grouop 5

```
-------------------------------------------------------------
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-------------------------------------------------------------
G10A DDDDDDDDDDDDD-DDBDDDDDDBDB-DBDDDDDBDBDDDDBDBDDDBDD
Q12A -------------------------------------------------------
F9   DDDDDDDDDDBDDDDBBDDDDDDBDD-DBDDDDDBDDHDDDDDBBDDDBD
I9B  ACCCCCCCCCCCACCCC-CACCACCCC-ACACCCCC-CACCCCCCCCCACCA
E7C  -------------------------------------------------------
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888999999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
G10A DBDDBBDDDDDDDDBBBDDDDDBDDDBDDDBBDDDDDDDDDDDDDDDDDD
Q12A ---DDBDDDDDDDBBBBBDDDBDDDBDBDBBDDDDDDDDDDDDDDDBDD
F9   DBDBDBDDD-DDDDBBBBBDDD--DDDDBDBDDDDDDDDDDBDBDDDDBDD
I9B  CC-CC-CCACCCCCCCCCCCCACCAACCCCCCCCCCCCCCCACACCCCAC
E7C  ---CCCCC-CCCCCC-CACCCCAC-AACCCCCCCCCCCCCCAACACCCCAC
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111111
     00000000011111111112222222223333333333444444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
G10A BDBDBDDBBDDBDDDDDDDBDBBBDDDDDDDDDDDDBDDDDDDDDBBDDD
Q12A BDBDBDDBBDDHDDDDDDDBBHBBDDDDDDBDDDDDBDDDDDDDDBBDDD
F9   BDBDBDDBDBDBBDDDBDDBBBBBDDDDDDBBBDDDBDDDDDD-DDDDDD
I9B  CACACCCCCCACCCCCCCCCCCCACCCACACCCCCCACCCACCA-CCCCCC
E7C  CCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCACCC--CCCCCCCA
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111112
```

```
     5555555556666666666777777777788888888899999999990
     1234567890123456789012345678901234567890123456789O
-------------------------------------------------------
G10A DBDDBDBDDBDBDBDDBDD-DDBBDBBDDDBDDDDDDDDDDDDDDDDDD
Q12A DBDDBDBDDBDBDBDDBBD-DDBBDBBDDDDDDDDDDDDDDDDDDDDB
F9   DDDDBDDDDDBBBDB-DBBB-DD-BD-D-DDDDDDDDDDDDDDDDDDDB
I9B  CACCCCCCCCCCCCCCCCC-AACCCCCCCACCCCACCCCCCCCCACCCCC
E7C  ACCCC-CCCCCCCCACCCA-CAC--C-CCCC-CCCCCCCC--CACCCCAC
-------------------------------------------------------
     2222222222222222222222222222222222222222222222222
     0000000001111111111222222222233333333334444444445555
     1234567890123456789012345678901234567890123456789123
-------------------------------------------------------
G10A DDDDDDDDDBDDDDDDDBDDDDDDDDDDDDDDDDDDDDDDDDDBB--BBDDD
Q12A DDDBDDD-DBDDDBDDDBDDDDDDDDBDBDDDDHHDDDDDDDDDBBDBBBDDD
F9   DDDBDDDDDBDDDBDDDBDDDDDDDDBBBBDHDHHDDDDDDDHDBDD-BBBDD
I9B  CCCCCACACCCCACACCCAACCCCCCCCCCCCC-CAAACAACCACACCCCCCA
E7C  CAAAACCCCCCCACCCCCCCCCCCCCCCCCCCCCAAAAACCCACCCCCCACA
-------------------------------------------------------
```

Linkage group 6

```
-------------------------------------------------------
     0000000000000000000000000000000000000000000000000
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-------------------------------------------------------
E2   -------------------------------------------------
V14B -------------------------------------------------
C5B  ACCCACCAAACCAACACCACACACCC-CCACCCCCCCCCCCCCAAACCCC
E12  DDDDDDDDDDBDDDDDBDDBDDDBDB-BDDDDDDDDDBDDDDDDDDDBB
I14  ACCCACCAAACCAACCCCACACACCC-CCACCCCACCCCCCCCCAAACCCC
-------------------------------------------------------
     0000000000000000000000000000000000000000000000001
     5555555556666666666777777777788888888899999999990
     1234567890123456789012345678901234567890123456789O
-------------------------------------------------------
E2   ---DDBDD-D-B-DDDDDD-DDDD-DDDBDDBDDDDDDDDB-DBDDBDBD
V14B ---CCCCACCACCACCCCCCCCCCCCCCCCCCCCCCACCA--ACCCACCCC
C5B  CCCCCCCCCACCCCACACCACCCC-CCCCCC-CCCCACCACCCC-CCCCCC
E12  BDDDDBDDD-DDBDDDDDDBDDD-DDDDBDDDDDDDDDDDBDDBDDBDBD
I14  CCCCC-CCCCCCCCACCCCCCCCCCCCCCCCCCCCCCACCACCACCCCCCCC
-------------------------------------------------------
     1111111111111111111111111111111111111111111111111
     0000000001111111111222222222233333333334444444445
     1234567890123456789012345678901234567890123456789O
-------------------------------------------------------
E2   DDDDDDBDBDDDDDBBBDDDDDDDDDDDDBDDBDBDDDBDDD-DDDDBDDDD
V14B CCCCCCCCCCAACCCCCCCCAACCCCCCAC-ACCCCCA--ACCCAACCCCCCC
C5B  CCCCACCCCAACCCCCCCCAACCCCCACCCCCCCCCAACAC-CAA-CCCCCA
E12  DDDDDDDDBDDDDDDDDDDDDDDDDDDDBDDBDBDDDBDD-BDDDB-DDDD
I14  CCCCACCACAACCCACCCAACCCCCCACCACCCCCAACACCCACCCCCCCC
```

```
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
E2   DDDDDDDDBBDDBBDDDDD-DDBD-B-DDDBDDDDDDDBDBDBBDDBBBB
V14B ACCCA-CACCCCCC-AACC-AACCCCAACCCCCCCCCCCCACCCCACCCCC
C5B  ACCCACAACCCACCCACAC--ACCCCCAACCCCCCCCCCACACCCCCCCCC
E12  DBDDDDDDDDDDDBBDDDDD--D-DD-D-DDDBDDDDDDBDDDDDDDDDDD
I14  ACCCACAACCCACCCAAAC-CACCCCAACCCCCCCACCCCAACACAAAACC
-------------------------------------------------------------
     22222222222222222222222222222222222222222222222222222
     00000000011111111112222222222333333333344444444445555
     12345678901234567890123456789012345678901234567890123
-------------------------------------------------------------
E2   BDDDDBD-BDDDDDDBDDDDDDDDBDDBDDDDBDDDDDDBDDDDBDBDDDDDD
V14B CCC-CCCCCAAACCCCACCACCCCCCACCCCCCCCCCAACCCAACCCCCACCCC
C5B  CCACCCCCCCCACCCC-CCCCCCACACCCCCCCCCCCCACCCCAACCCC-CAACC
E12  BDDDBBDDBBDDDDDDDDDBDDDDDDDDDBDBBBDDDBBBDDDDBBD-DDDDD
I14  CACCCCCCCCCACCCCCCCCCCAAAACCCACCCC-CCACCCCCCCCCCCACAACC
```

Linkage group 7

```
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000000000
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
D4   CCCACCCCCCACACAACCCCCACCAC-AACCCACACAACCCCCCCCCCCC
R20A -----------------------------------------------------
O15  -----------------------------------------------------
C5A  CCAACCCCCACCACCACCCCAACACA-ACCACCCACCCCCCCCCCCCCCC
H18B CC-AAACCACCACCCCCCCCACACA-ACC-AACACCCCCCCCCCCCCAA
J19  -DDDDDDDDDDDDDBDDDDBDDDDDD-DDDDDDDDDDDDBDBBDDBBDDDD
E7D  -----------------------------------------------------
V16  -----------------------------------------------------
X17  -----------------------------------------------------
U13A -----------------------------------------------------
B11  -----------------------------------------------------
D2A  DDDDDDDBDDDBDBDDBDBDDDDDDD-DDDDDDDDDDDBBBBBDDDDBDB
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
D4   CCCCAAACCAACCCCC-CAACC--CCCCCACCCCCCCCACCACCCAACACA
R20A ---CA-CCCCACCCCCCCAACCACCCCCCCCCCCACCAACAACCCCCACCCA
O15  ---CAACCCCCCCCCCCCAACCACCCCCCCCCCCACCAACCACCCCCACCCA
C5A  CCCCAACCCCCCCCCCCCACACCA-CCCCAC-CCACCCACCCCCC-CACCCA
H18B CAACAACCCCCACCCCCCAAAACCACCCCCCACCCCACCAAACAACCCCACACA
J19  DDDDD-DBDBDDDBDDDDDDDBBDDDBDDDDDDDDDDDBDDDDDDBDDDDD
E7D  ---C--CC-CCC-C--C-AACCAA-CCCCCCCCAACCAAAC--CACCCAAA
```

```
V16   ---BDDDBDB--DDDDDDDDBDDDDDDDDDD--DDDDDBDDDDDDDBD--
X17   ---DDD-BDBDDBBDDDBDDBBDDDBDDDDDBDDDDDDDBDDDDBDBDDD
U13A  ---DD-DBDB-DBDDDD-DDBB-D-BDDDDDBDDDDDDDBD-DDBDBDDD
B11   ---DDDDD-BD--BD-DDDDBDDD-DDD-DDBDDDDDDDDDBDDBDBDDD
D2A   BDDDDDDDDBDDBBDDBBDDBBDDDDDBBDDBBDDBBDBDDDDBBDBBBD
-----------------------------------------------------------
      11111111111111111111111111111111111111111111111111
      00000000011111111112222222222333333333344444444445
      12345678901234567890123456789012345678901234567890
-----------------------------------------------------------
D4    CCCCCCCAACAACCCCCCCCCCACCACCCCACCAACCCA-CACACCCAAC
R20A  CCCCCACAACCCACC-CACCCCCCCACCCCACCCACCCCCCCACC-CACC
O15   CCCCCACAACCAACCCCACCCCCACACCCCACCCACCCACCCCACCCACC
C5A   CCCCCACAACCAACCCCACCCCCACACCCCACCCACCCAACCCACCCACC
H18B  CCCCCACAACCACCAACACCACCACACACCAACCACCCAACCCAAAAACC
J19   DDBBDDDDDDBDDDDDDDBDDDDDDDDDDBDBDBDBDBBDDDBDDDDDBB
E7D   CCCCCCCCCCCCACACACAACCAC-CCCC-CAC-CCC-C----CCCCCCCC
V16   BDDBDDDDDDBDDDBDDDD-D-DDBDDDDDDDDDDDD-------------
X17   BDDDDDDDDDBDDDBDDDDDDDDDBDDDDDDBDDDDDDDD-DBDD-BDBB
U13A  DDDDDDDDDDDDDDDBDDDDDDBDDBDDDDDDBDDDDDDDDDDBDDDBDBB
B11   DDDDDDDDDDDDDDDBDDDDDDB-DBDDDDDDDDD-DDDDDD-BDBDBD-B
D2A   DDDDDDDDDDBBDDBBBDDDDBBDBDDDBDBDBDBBBBDBDBBDBDBDBB
-----------------------------------------------------------
      11111111111111111111111111111111111111111111111112
      55555555566666666667777777777888888888999999999990
      12345678901234567890123456789012345678901234567890
-----------------------------------------------------------
D4    CACACCCCCACACACAACC-AACACCCCCCCCCACCCCCCCCACCCCCAA
R20A  CACACCCAA-CCCCCAACC---CACC-CCCCCCACCCCCCCCCCCCACAC
O15   CACACCCCAACACCCAACC-CACACCCCCCCCCACCCCCCCCCCCCCACAC
C5A   CACACCCCAACACCCAACC--ACAACCCCCCCCCACCCCCCCCCCCCCCA
H18B  AAACCCCCAACACCAAACC-CACACCCCCCCCCACCCCCCCCCCCCCACAC
J19   BBDDDBDDDDDDDDDDDDB-BDBDBDDDDBDDBDDBBBDBDDBDDDDDDD
E7D   CCCAC-CCCAAACCCCACC-C-C--C-CC-C-CACCCCCA--C---CCA-
V16   ---BDBBDD-DDDDDDDDD-DDBDBDDDBDDD-DBBBDDBBDBD-DDDDD
X17   BDDDDBBDDDDDBD-DBBD-D-BDBBDDBDD-BDDDBDDBBDBDDDDDDD
U13A  -DDDDBBDDDDDDBDDDD--D-BDBBDDDDD-BDBBBDD-BDBDDDDDDD
B11   BDD-DDBDDDDDDDDDDDD-D----DDD--D-BDBBBDDBDDBDDD-DDD
D2A   BDBBDDBBDDBDDDDDDBB-DDDDBDDDBBDDBDDDDDBBDDDDDDDDD
-----------------------------------------------------------
      22222222222222222222222222222222222222222222222222
      00000000011111111112222222222333333333344444444445555
      12345678901234567890123456789012345678901234567890123
-----------------------------------------------------------
D4    CCCCCCCCACCACAAACCCCCCCCACCAACCCCACACACAACCCCCCACCCA-
R20A  CCCCCCC-ACCCCCAACCCCCCCACACCAACCCCAC-CACAACC-CCC-CC-AC
O15   CCCCCCC-ACCCACAACCCCCCCACACCAACCCCACACACAACCCCCCCCCAAC
C5A   CCCCCCCCCACCCACA-CCCCCCACACCAACCCCACACACAACCCCCC-CCAAC
H18B  CACCCCCCACCAACAACCCCCCCAC-CCAACCCCCCCACACAACCCCCCCACCAAC
J19   BDDDDDDB-DBDDDDDDDDDDBDBDDDDDDDDDDBD-DDBDDDDDDDDDDDDDDDD
E7D   C--ACCCCACCC-CCCCCCCCCCACACCAACCCCACACACACCCACCCCCCAC-
```

```
V16  DDDDDDD-DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
X17  DBBD-DD-DBDDDDDDDDBBDBDDDBDDDBDDBDDDDDBDDBDDDDBBBDDDD
U13A DDDDBDD--BDDDDDDD-BDDBDDDBDD-BDDBDDDBD-DDBDDDDBBBDDDD
B11  DDDDBBDDDBDDDDDDDDBBDBDDDBDD-BDDBDDD-DBDDBBDDDBBBD-DD
D2A  DBDDBBD-DBDDDDDDDDBBDBDDBBBDDBBBBBBDBBBDBBBDBBBBBBDDB
-------------------------------------------------------------
```

Linkage group 8

```
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000000
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
K03  -------------------------------------------------------
L12A -------------------------------------------------------
I9A  BBDDDBDBDDBDDDDD--DDBBDDDD-BDDBBDDD-DDBDDBDDDDDDBD
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
K03  ---BB-D-DBDBDDDDDDDBDBDDDBBDDDBDDDDBB-DDDDDDDDBBBD
L12A ---BBBDBDDDBDDDDDDDBDBDDDBDB-BDDDDDDDBDDDDD-DDDDBD
I9A  DD-BB-DBDBDBBDDDDDDDDDDDDDBDDDDDDBDDDBBDDBDBDDDBDBB
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111111
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
K03  DDDDDDDBDDDDBDBDDDDBBBD-BDDDBBBBDBDBDBDD--BDDDBBBD
L12A DDDDDDDDBDDDDDDDDDDDBDDDBDDDBDDDDBBBBDDBDDDDDDDDD
I9A  DDDDDDDDBDDDDBDDDBDDBDDDBDDDBDDDDDDBDDDDDDDDDDDDD
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
K03  BDDBDDDDDD-DDDDDDDD-DDDBDDBDDDDBBBDDDDBDBBDBDDDDBB
L12A BDDBDDDDDDBDDDDDDDDBDDDBDDBDDDDBBBDDDDBDBBDBDDDDDD
I9A  BDDDDDDBBDDDDDDDDDD-DDDDDDBDDDDDDBBDBDDBBBBBDDDDD
-------------------------------------------------------------
     22222222222222222222222222222222222222222222222222
     00000000011111111112222222223333333333444444444555
     12345678901234567890123456789012345678901234567890123
-------------------------------------------------------------
K03  BDDDDDB-DDDDB-BDDDDBBBDDDDDDBDDDD-DDDDDDDBD-BDD-DDDDD
L12A DDDBBDDBDDDDD-DDDDDBBD-DDDDD-DDDDBDDD-DDDBDDBDDDDD-DD
I9A  BDDDBDDDDDDDDDDDDDDBDDDDDDDBBDDDB-DDDDDDDBBBDDDDDD-BD
```

Linkage group 9

```
-------------------------------------------------------------
```

```
     0000000000000000000000000000000000000000000000000
     0000000011111111112222222222333333333344444444445
     1234567890123456789012345678901234567890123456789
-----------------------------------------------------------
E16A BBBDDBDDBDBDBBDBBDDBDBDDBB-BBBDDDBDDDDBBBDDDBBBBDD
E16B DDDDDDDDBDBDBDDBBDDBDBDDDBB-BBBDDDBDDDDDDDDDDBBBDDD
-----------------------------------------------------------
     0000000000000000000000000000000000000000000000001
     5555555556666666666777777777788888888899999999990
     1234567890123456789012345678901234567890123456789
-----------------------------------------------------------
E16A BBDDDBBDDDBBDDBBDBBDBDDBDDDDDDBBDDBBDDDDBDDBBDDDDD
E16B BBDDDBDDDDBDDDBBDBDDBDDDDDDDDDDBDDDDDDDDDDDDBDDDDD
-----------------------------------------------------------
     1111111111111111111111111111111111111111111111111
     0000000011111111112222222222333333333344444444445
     1234567890123456789012345678901234567890123456789
-----------------------------------------------------------
E16A DBBDDBBDDBDDDBDBDDDDBDBDBDBDDDDBDBBBBDB-BBBBDBDD-B
E16B DBBDDBDDDDDDDBDBDDDDDDBDDBDDDDDDDDDBDDB-BDBBDDDD-D
-----------------------------------------------------------
     1111111111111111111111111111111111111111111111112
     5555555556666666666777777777788888888899999999990
     1234567890123456789012345678901234567890123456789
-----------------------------------------------------------
E16A DBBDDDDDDBDBBDBBBDD--BBDBDBDDDDDBBBBDBDDBBBDDBDDDD
E16B DDDBDDDDDDDBBDDDBDD--BDDBDDDDDDDBBBBDDDDDDBDDBDDDD
-----------------------------------------------------------
     2222222222222222222222222222222222222222222222222222
     0000000011111111112222222222333333333344444444445555
     1234567890123456789012345678901234567890123456789123
-----------------------------------------------------------
E16A DDDBDDBDBDBDBBDBBDBDBDDDDDBD-BDDD-BBDBDDDBBBDBB-DDB--
E16B DDDDDDBDBDDDDDDBDDBDBDDDDDBD-DDDD-DBDDDDDDBBDBD-DDB--
-----------------------------------------------------------
```

Linkage group 10

```
-----------------------------------------------------------
     0000000000000000000000000000000000000000000000000
     0000000011111111112222222222333333333344444444445
     1234567890123456789012345678901234567890123456789
-----------------------------------------------------------
H13  A-CCCACACACCCCCACCACACCCCA-CCCCCCCCCAACC-CCCCACCCC
P5C  -----------------------------------------------------
```

```
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
H13  ACCAC-CAACCCCCCCCCCACCACCACCCACACACCCACAAACAACCCCC
P5C  ---CCCCCCCCACCACCACACCCCAACC-CCACCCCCCCCCAAC-AAACCC
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111111
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
H13  CCACCACCCACCACACCAACCCCCCCCCCCCCACCACACACACAACAACC
P5C  CCCCCCCCCACCCCCCCACCCCCCCCCACCCCCACCCCCCA-ACAACCACC
     ----------------------------------------------------
-------------------------------------------------------------
     11111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
H13  ACCACACAAACACCCCCCC-ACCCCCCCCCAAACCCACCCCCCCCCACCCAC
P5C  CCCCCCCCCCCCCCCCCCA-CCCCCCCCCCACCCCCCCCCCCCCCCACCCAC
-------------------------------------------------------------
     22222222222222222222222222222222222222222222222222
     00000000011111111112222222222333333333344444444445555
     12345678901234567890123456789012345678901234567890123
-------------------------------------------------------------
H13  CCCCCCC-CCCCCCC-ACCACCCCCCAC-CCCC-CCCCCCACCCCCCCCCCAC
P5C  CCCCCCCCCCCCCCCCAACCACCCCACACCCCCCCCACCCCCACCACCCCCCCC
-------------------------------------------------------------
```

Linkage group 11

```
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000000
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
F3   CCCCCACCCCCCCCCCCCACCCAACC-CCCCAAACCCCCCACCCACCCAC
W02  ---------------------------------------------------
-------------------------------------------------------------
     00000000000000000000000000000000000000000000000001
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
-------------------------------------------------------------
F3   CCCACCCCA-AACAACACCCCAC-CCCAACAAACACCCCCCCCCCCCACCC
W02  ---DBDDBDBDDDDDDD-DDDDDDBBDDDDDDDDDDDDDD-DDDDDDDDDDD
```

```
------------------------------------------------------------
     11111111111111111111111111111111111111111111111111
     00000000011111111112222222222333333333344444444445
     12345678901234567890123456789012345678901234567890
------------------------------------------------------------
F3   CACCCCACCCCCCCACCCCACCCCACCCCCCCACCCACACAAACACACCAC
W02  DDBDDDBDDBDD-DDDBD-DDBBDBDDBDDDDDDBDDBBDDDDBDDDDDDD
------------------------------------------------------------
     11111111111111111111111111111111111111111111111112
     55555555566666666667777777777888888888899999999990
     12345678901234567890123456789012345678901234567890
------------------------------------------------------------
F3   CCCACCCACCCCACAACCC-CC-AC-C-AACCCACCCAACCACACCCAAC
W02  DDDD-DBDDDBDDBDD-B---DDDDDBDDDBBBDD-DDDDDDBDDBBDDD
------------------------------------------------------------
     22222222222222222222222222222222222222222222222222
     00000000011111111112222222222333333333344444444445555
     12345678901234567890123456789012345678901234567890123
------------------------------------------------------------
F3   CACACCCCACCCCCACCCCCCCCCCACCCCCCCCCCCCCCCCCCCACCCAAA
W02  DDBDDDDDDDDDDD--D-DDDDDDBDD-DBDDDDDDDDDDBDBDBDDDDDDDDD
------------------------------------------------------------
```

Appendix B

Poamoho population (100 plants from 54-153)

Node at first flowering																		Pl.no.
27	19	27	21	22	26	23	18	21	17	27	23	23	19	28	19	32	28	1-18
20	21	24	29	36	19	31	30	22	21	26	15	26	28	24	29	18	26	19-36
28	22	28	24	20	19	23	22	16	18	19	19	25	23	21	18	24	19	37-54
16	19	25	17	25	22	27	21	27	17	33	22	28	21	17	25	22	26	55-72
20	24	21	28	18	25	25	26	26	26	25	28	28	26	27	28	25	27	73-90
29	23	25	30	21	22	26	-	28	24									91-100
Stem diameter-1 (cm)																		
5	7	5.7	9	11.5	7.5	7	9	6.8	8	6.3	7.5	4	8.5	6	9	7	6.5	1-18
10.8	8	7.8	4	6	7	6	6	8	7	6.9	7.5	7	6.5	8.5	6.5	6.6	7	19-36
7	10.8	7.2	5.5	7	9	10	8.8	9	9.8	8	5.5	9.8	7	6	7.3	8		37-53
8.6	9	9.1	8	4.1	6.4	4.3	5	7.7	6.2	9	4.9	4.3	6.7	10				54-68
9.8	5	7	8	6.5	7.5	7.6	8.6	8	9	8.8	6	4.5	5	6.6	6	7.6		69-85
6	9	8.3	7	8	4.3	7.8	8.8	8	9	8	8.8	6.3	6.1	5.8	7.3			86-100
Stem diameter-2																		
7.2	9.9	8.2	10.5	15.5	9.5	8.5	10	9.8	9.7	9	9.1	6.8	9					1-14
10	11.9	9.1	8.1	13.2	-	12.5	6.3	9.5	7.9	7.9	8	10.8						15-27
8.2	11	10.6	9.5	9.9	12.9	8.2	8.6	8.5	9.8	14.2	10.5							28-39
8.4	9.3	10.3	11	9.9	12.2	11.5	11	8.3	13	8.6	7.3	7.9						40-52
9.5	11	9.5	10	11.5	-	9	7	7.9	9.5	9	11.8	8.5	6	9.7	11			53-68
11	8	11.3	7.9	8.9	9.9	10.8	12.9	9	9.7	8.9	7	7.2	9	7.3				69-83
8.8	7.1	10.2	9.9	10	9	7.2	10.1	9.2	9	12.3	10	9.6	10.2					84-97
11.3	7.6	11.6																98-100
Stem diameter-3																		
11.3	12	12.9	13	17.5	11	12.1	10.7	11.8	13.6	14	12	12						1-13
10.3	16.2	13.7	12.6	9	-	-	15.5	12.8	13.6	10.5	12.6							14-45
11.6	14.1	10	17.3	12	10.5	14.2	14.2	10.2	12.3	12.2								46-36
14.5	14.5	11	11	13	16.6	13.7	12.9	12.6	11.7	11	12							37-48
14.5	13	12	10	13.9	14	-	10.5	14	-	13.7	11	11.5	13.7					49-62
14	15.6	11.3	7	15	17	12	9.7	16.3	-	12.2	10.7	15.3						63-75
16.8	10	13	10.4	12.8	11	11.5	11	14.1	10	14.3	11.3	11						76-88
12.8	11.9	12.2	12.7	9.3	18	14	11.3	16.8	16	11.4	14.5							89-100
Stem diameter-4																		
12.3	13.7	14	14.6	18.6	12.2	14	10.8	12.2	15.7	16.4								1-11
14.1	13.5	11.8	17.6	14	14.5	9.2	14.3	-	-	15.0	14.6							12-23
12.8	13	13	16.4	11	19.5	14	11.2	16.2	15.8	11.2	15							24-35
14.2	16.1	15.8	11	12.3	14.4	18	14	15.8	13.6	11.5								36-46
12.2	13.5	15	14	13.5	10.6	15	16.2	-	11.6	15.2	-	15						47-59
11.8	12.2	15	15.8	18	12	-	16.5	18.5	12.5	10.5	18	-						60-72
14	10.8	18.6	19.2	11	15	11.2	14.2	12.2	13.2	12.5	16							73-84
10.9	16	12.2	11	14	12.3	13.3	13.7	9.8	21	15.8	13							85-96
18.7	18.1	-	14.1															97-100
Stem diameter-5																		
12.3	13.9	14	15	19.3	12.7	14.5	11.2	13	15.8	16.6								1-11
14.5	13.5	12	18	14.9	15	10	-	-	-	15.1	15	12.5	14.5					12-25
14	16.8	11	-	14.2	12	17	16.1	11.7	15.5	14	16.5	16						26-38
11.7	12.8	14.5	18	14.3	16.3	15	12	12.8	14	16	14	14						39-51

11 15.2 17 - 11 16.5 - 15.2 12 12.9 15 15.3 18.2 13 52-65
- 16.6 17.4 13 10.6 18.1 - 14 11.2 18.3 19.5 11.3 15 66-78
11.3 14.5 12.5 13.2 12.3 16 10.9 16 13 11.2 14 12.2 79-90
13.8 13.7 10 22.5 16 12.5 19 18.5 - 14.1 91-100

Plant height-1(cm)

101 141 141 171 194 120 142 148 112 141 121 117 77 1-13
118 110 93 136 121 162 112 90 84 121 135 116 98 121 14-27
114 81 119 92 103 151 90 118 119 96 147 110 92 130 28-41
147 173 130 124 150 92 88 129 153 118 133 122 110 42-54
118 200 135 86 118 79 87 117 102 113 82 81 102 143 55-68
152 137 115 134 133 121 108 120 135 114 81 79 121 96 69-82
82 107 123 131 118 107 123 91 104 133 107 106 102 126 83-96
91 110 95 77 97-100

Plant height-2

139 186 156 217 265 170 180 187 147 181 165 147 110 1-13
131 163 115 183 159 226 - 123 123 172 155 161 122 160 14-27
146 115 162 128 154 200 145 151 143 144 198 142 123 28-40
175 190 238 177 166 179 121 113 161 188 148 159 170 41-53
152 114 182 188 - 154 117 120 154 135 143 101 118 147 54-67
182 190 181 155 181 172 178 162 194 161 140 116 128 68-80
175 164 126 136 172 173 156 155 169 125 154 168 139 81-93
169 141 174 148 179 128 122 94-100

Plant height-3

179 243 216 271 322 223 252 216 175 229 228 200 159 1-13
172 223 153 249 196 - - 154 181 237 195 209 176 218 14-27
181 172 190 157 210 252 216 209 210 205 244 171 168 28-40
224 264 283 224 187 202 120 152 198 268 208 187 227 41-53
223 - 206 228 - 211 173 160 218 198 204 151 150 218 54-67
255 216 206 201 - 233 198 205 249 195 185 150 180 68-80
224 202 155 191 213 243 184 195 233 190 196 216 168 81-93
255 191 237 217 217 158 158 94-100

Plant height-4

204 274 234 292 335 245 274 229 188 260 256 225 185 1-13
186 240 175 274 212 270 - - 204 256 208 227 197 245 14-27
198 188 204 175 229 278 245 224 250 232 285 188 190 28-40
266 288 300 255 202 214 155 172 208 286 236 204 248 41-53
254 - 226 259 - 238 195 175 244 220 232 164 - 245 278 54-68
232 230 242 - 258 218 240 280 218 208 165 210 242 69-81
225 178 215 230 268 202 210 258 208 222 238 176 295 82-94
213 260 238 244 - 178 95-100

Plant height-5

212 295 250 318 370 266 295 246 208 280 280 244 198 1-13
195 264 190 305 238 - - - 226 272 225 248 216 274 218 14-27
- 225 190 246 305 264 246 264 260 290 200 208 288 306 28-41
325 288 222 230 165 190 224 312 144 214 268 278 - 244 42-54
280 - 266 224 186 264 232 258 186 - 258 304 244 248 55-70
258 - 280 234 258 314 228 218 176 224 260 238 184 232 71-84
236 282 222 224 278 224 240 250 186 324 236 276 262 85-97

264 - 190 98-100

Normal fruits Period-1

9 6 15 15 25 24 23 38 43 31 10 23 13 17 17 54 8 40 1-18
34 - 63 4 19 22 9 13 38 36 1 59 52 24 26 7 13 22 32 19-37
14 88 17 36 12 63 29 78 69 51 16 40 15 8 30 12 25 - 38-55
37 17 - 17 22 36 23 8 14 29 37 15 12 42 26 6 22 16 56-73
51 32 24 15 23 31 3 24 35 6 9 14 19 37 47 9 7 23 2 74-92
39 8 21 23 5 47 - 54 93-100

Carpellod fruits Period-1

6 37 0 7 0 8 0 0 0 0 0 0 0 9 0 0 19 0 - - - 0 0 0 14 1-25
2 2 0 - 0 0 5 24 28 9 0 0 40 0 18 0 0 0 0 0 0 0 0 0 0 26-49
0 0 0 0 0 - 0 24 - 0 0 0 0 0 0 0 0 0 0 0 0 0 - 0 0 0 50-75
0 1 1 0 0 1 0 0 0 9 0 0 0 0 0 12 0 0 0 7 0 0 1 0 0 76-100

Normal fruits Period-2

14 11 44 31 26 27 27 34 37 38 16 18 29 27 46 42 12 1-17
20 - - - 43 46 12 14 19 50 24 - 36 36 35 30 28 30 10 18-36
47 16 24 24 49 41 38 14 29 10 35 26 33 46 29 21 36 37-53
32 - 17 14 - 27 38 57 49 36 20 30 - 32 26 40 25 0 - 54-72
36 26 32 18 6 22 32 29 35 14 16 14 20 35 17 31 27 38 73-90
12 37 22 22 40 29 45 26 - 57 91-100

Carpellod fruits Period-2

15 10 0 1 2 0 0 0 0 0 0 2 1 0 0 0 11 0 - - - 0 0 0 1-24
12 1 - 0 - 0 0 1 6 6 6 0 0 22 0 4 0 0 0 0 0 0 0 0 0 0 25-49
0 0 0 0 0 - 6 17 - 3 0 0 0 0 0 0 0 0 0 0 0 0 - 0 0 0 50-75
0 0 1 0 0 0 0 0 0 2 0 0 0 11 0 2 0 0 0 2 0 0 0 - 0 76-100

Normal fruits Period-3

0 3 24 18 30 15 22 15 19 29 19 25 26 22 33 27 0 11 - 1-19
- - 36 32 18 0 25 28 28 - 27 23 22 9 4 0 26 24 1 20-38
23 10 32 45 19 26 26 7 19 24 16 27 46 23 35 26 - 20 39-56
1 - 19 23 21 41 32 49 24 - 37 30 26 13 31 - 31 16 38 57-75
23 19 29 14 18 11 35 25 19 16 29 14 16 19 26 13 17 76-92
12 39 19 26 25 38 - 26 93-100

Carpellod fruits Period-3

18 20 13 12 2 6 7 0 0 7 0 1 1 4 2 0 27 0 - - - 2 6 2 1-24
25 14 7 0 - 0 0 2 36 6 26 0 2 26 0 18 0 0 0 5 0 0 0 25-47
0 0 0 0 0 0 4 - 8 28 - 15 0 0 4 0 0 0 - 3 4 0 0 0 - 48-72
0 0 1 0 8 11 0 0 10 5 0 1 0 0 0 0 8 1 8 0 0 0 7 2 6 73-97
0 - 0 98-100

Normal fruits Period-4

3 0 6 3 23 7 19 23 28 27 12 9 13 12 7 34 0 25 - - - 1-21
34 9 11 0 5 15 18 - 36 24 17 1 4 0 20 28 0 17 3 28 22-41
21 24 15 47 21 18 12 28 38 14 17 11 10 - 17 2 - 17 42-59
20 21 14 6 19 43 - 17 13 20 22 7 - 9 19 16 30 4 8 26 60-79
15 11 25 8 11 - 20 5 10 11 11 17 11 14 32 13 19 18 20 80-98
- 41 99-100

Carpellod fruits Period-4

14 28 8 15 3 6 5 0 0 2 1 7 0 2 1 0 24 0 - - - 0 0 5 1-24
19 6 10 0 - 0 0 7 29 5 25 0 0 19 0 15 0 0 0 2 0 0 0 25-47

0 0 0 0 0 0 1 - 7 24 - 7 0 0 3 1 0 0 - 2 4 0 0 0 - 0 48-73
0 0 0 5 4 0 4 9 0 0 0 - 0 0 0 2 0 12 0 0 0 6 0 3 0 - 74-99
0 100

Fruit weight(g)

806 706 551 442 818 718 676 495 501 782 966 916 723 1-13
627 593 617 832 660 670 447 - 482 428 635 784 949 14-26
841 377 - 482 612 749 509 558 548 604 955 649 423 27-39
527 670 641 424 913 572 638 578 904 614 498 526 509 40-52
822 964 - 808 537 - 792 575 386 914 559 691 978 - 53-66
830 846 358 463 746 - 354 756 766 550 445 726 496 67-79
555 344 473 422 770 - 573 424 304 725 585 777 624 80-92
312 963 548 642 674 926 - 519 92-100

Number of nodes Period-1

27 32 27 35 42 36 34 33 33 25 36 25 - 35 35 28 31 33 1-18
- - - 28 36 27 34 35 39 34 - - 34 33 42 33 30 38 40 19-37
39 34 32 36 34 34 42 40 41 32 36 36 26 36 31 19 37 38-54
- 35 37 - 32 27 34 30 34 42 31 - 37 36 32 28 42 - 31 55-73
34 39 44 - 34 36 31 36 35 31 30 - 28 34 38 27 31 34 74-91
32 34 39 34 31 32 40 - 36 92-100

Number of nodes Period-2

35 40 40 39 39 39 41 33 33 52 41 45 - 36 38 39 42 38 1-18
- - - 39 40 40 39 36 41 28 - - 33 47 41 38 37 44 46 19-37
48 31 38 41 41 36 49 32 28 30 36 40 43 41 27 60 41 - 38-55
32 35 - 39 40 38 45 38 43 37 - 44 41 35 31 37 - 39 56-73
32 48 32 - 36 35 41 37 41 38 42 - 50 36 41 50 41 42 74-91
40 33 42 36 47 44 43 - 37 92-100

Number of nodes period-3

20 21 22 22 25 26 25 22 15 26 27 19 - 24 23 20 22 18 1-18
- - - 27 27 21 22 26 26 23 - - 18 26 27 24 21 24 25 19-37
20 18 18 27 27 23 23 21 16 21 27 20 21 19 23 24 27 - 38-55
21 23 - 22 18 20 24 23 29 24 - 26 18 21 15 26 - 26 16 56-74
24 30 - 23 19 21 23 24 22 20 - 23 23 17 22 23 23 19 75-92
14 22 28 22 23 25 - 24 93-100

Number of nodes period-4

24 31 26 25 31 31 27 23 22 28 33 30 - 23 24 29 30 28 1-18
- - - 27 28 23 24 27 26 20 - - 26 24 25 26 24 31 33 19-37
32 19 24 24 24 20 34 29 20 19 21 23 26 21 16 34 34 38-54
- 31 28 - 30 24 22 27 23 27 27 - 23 27 20 25 29 - 24 55-73
22 40 32 - 26 25 31 22 29 22 25 - 28 24 25 27 23 33 74-91
23 14 32 24 25 24 30 - 33 91-100

Waimanalo population (100 plants. Numbers 154-253)

Stem diamter-1 (cm)

9.0 8.0 9.0 6.0 8.5 8.3 8.0 7.0 9.0 7.0 6.3 7.3 10.2 1-13
9.0 6.5 6.1 9.0 7.1 6.0 7.2 5.5 9.0 6.5 4.8 5.0 8.0 14-26
6.2 7.0 8.2 8.5 7.0 7.5 6.5 6.6 5.5 7.0 6.0 8.5 7.5 27-39
5.5 7.0 8.2 8.0 9.2 9.0 9.5 7.0 7.0 7.0 6.0 6.3 5.5 40-52
7.5 7.0 8.0 7.0 9.8 7.2 8.2 6.1 6.0 7.3 7.0 8.0 4.8 53-65
6.0 6.0 7.5 7.0 7.2 8.2 6.0 9.0 6.0 8.0 6.0 6.0 7.0 66-78

6.0 6.5 7.0 8.0 4.5 7.3 8.0 7.8 7.8 8.0 7.0 6.2 7.3 79-91
6.0 5.5 5.5 6.6 7.0 5.2 7.0 7.2 6.5 92-100

Stem diameter-2

9.1 - 9.3 6.0 7.0 9.0 9.0 7.0 8.5 7.0 - 7.2 10.5 7.6 1-14
6.7 7.0 9.2 7.2 6.0 6.6 5.0 9.3 6.5 4.5 6.0 8.5 8.0 14-27
7.0 8.8 8.2 7.0 7.5 7.2 6.5 6.5 7.0 6.3 8.5 7.4 5.9 28-40
8.0 8.1 7.6 9.3 8.7 9.3 6.8 8.0 6.8 6.2 7.0 6.1 8.0 41-53
7.5 7.6 7.0 8.0 7.3 9.0 7.0 6.0 9.1 7.2 8.0 - 6.0 54-66
6.9 8.0 6.5 7.2 8.3 6.6 9.0 6.3 8.2 6.0 6.0 7.2 7.0 67-79
6.8 7.1 8.0 5.5 7.2 8.3 8.0 8.0 8.0 6.9 7.3 7.5 6.0 80-92
- 5.8 7.2 7.8 6.0 7.0 8.3 7.3 93-100

Stem diameter-3

9.1 - - 5.8 8.3 - 8.1 7.0 - 7.0 - 7.0 10.9 - 6.8 7.0 1-16
9.3 7.2 - - - 9.7 6.6 - 5.9 8.9 8.6 6.9 8.9 8.5 - - 17-32
7.9 7.0 6.5 - 6.6 8.6 8.3 5.9 9.5 8.3 - 9.3 - - - 8.6 33-48
6.9 6.1 6.9 6.2 8.0 8.0 7.4 8.0 - 7.3 8.5 7.0 - 10.5 49-62
7.6 7.9 - 5.9 8.2 8.9 - 7.1 8.3 7.0 8.9 6.3 8.1 - 63-76
6.0 7.0 7.0 7.0 7.9 8.0 7.0 - 8.4 - 8.0 8.0 7.0 8.4 77-90
8.0 6.0 - 6.1 7.1 9.2 6.2 7.6 8.3 7.3 91-100

Stem diameter-4

8.9 - - 6.0 9.5 - - 8.3 - 7.8 - 7.5 12.5 - 7.0 - 9.1 1-17
7.9 - - - 10.6 7.3 - 7.2 10.2 8.3 - 9.6 9.5 - - 8.2 18-33
8.0 - - 7.0 8.9 10.6 5.8 11.2 9.0 - 9.9 - - - 9.9 6.9 34-49
6.3 - 6.1 9.2 9.0 8.0 10.0 - 7.6 9.0 8.1 - 13.8 9.0 50-63
9.2 - 6.1 10.7 10.7 - 7.3 9.0 7.0 10.0 6.5 8.5 - 6.5 64-77
7.1 7.5 8.5 8.3 9.0 9.0 - 9.9 - 8.1 9.0 7.2 9.2 9.2 78-91
6.0 - 6.5 7.2 11.2 6.5 9.0 9.0 8.0 92-100

Stem diameter-5

9.0 - - 5.8 9.8 - - 9.0 - 8.2 - 8.0 13.3 - 7.0 - 9.0 1-17
8.0 - - - 11.1 8.0 - 8.0 11.3 8.5 7.0 9.8 9.9 - - 18-32
8.5 - - - 7.5 8.5 11.1 5.7 12.0 9.9 - 9.7 - - - 10.7 33-48
6.7 6.3 - 6.0 9.8 9.1 8.0 10.2 - 7.5 9.0 9.0 - 16.0 49-62
9.0 10.0 - 6.0 11.0 11.8 - 7.3 9.3 6.8 10.9 6.3 9.0 63-75
- 7.0 7.3 8.0 9.0 9.0 9.1 9.5 - 10.0 - - 9.1 7.3 9.2 76-90
9.8 6.0 - 6.5 7.2 12.0 6.3 9.1 10.0 8.6 91-100

Plant height-1

130 132 120 118 087 102 100 130 114 090 090 117 135 1-13
115 103 104 115 111 088 109 084 110 084 100 097 097 14-26
084 095 122 130 090 091 096 100 092 115 065 126 096 27-39
101 106 099 112 132 113 134 086 095 106 098 115 083 40-52
113 123 124 100 138 102 110 092 100 089 091 128 086 53-65
103 097 120 087 115 120 090 134 101 121 105 102 087 66-78
114 099 110 120 106 141 123 110 110 131 109 092 096 79-91
101 079 096 097 125 117 111 115 106 92-100

Plant height-2

154 - 129 138 106 121 103 151 126 100 - 129 114 125 1-14
129 128 127 123 095 112 092 134 96 103 114 113 119 15-27
120 151 151 103 107 116 117 117 124 111 140 121 121 28-40

138 121 121 149 120 150 099 125 129 110 132 102 142 41-53
146 149 117 142 116 127 117 113 122 108 156 - 120 133 54-67
143 095 136 142 105 159 120 142 120 130 100 148 118 68-80
131 151 139 168 144 122 127 158 135 119 111 112 - 117 81-94
112 152 136 140 145 135 95-100

Plant height-3

163 - - 150 117 - 104 165 - 112 - 138 182 - 139 129 1-16
142 137 - - - 147 109 - 128 130 134 133 174 173 - - 17-32
125 123 123 - 129 162 145 130 164 143 - 164 - - - 33-47
152 137 116 138 109 161 166 161 140 - 127 134 129 - 48-61
150 124 169 - 133 157 170 - 148 158 119 179 127 154 62-75
- 140 106 157 139 153 168 168 - 160 - 132 178 146 76-89
141 134 124 - 136 127 182 147 159 157 149 90-100

Plant height-4

176 - - 155 133 - - 185 - 132 - 149 207 - 153 - 148 1-17
154 - - - 167 125 - 147 150 152 - 198 197 - - 145 18-33
143 - - 144 170 165 144 182 163 - 187 - - - 172 143 34-49
131 - 117 178 183 180 158 - 138 145 150 - 171 140 50-63
192 - 145 182 196 - 155 181 129 208 135 174 - 155 64-77
116 171 159 170 187 185 - 178 - 139 200 157 148 155 78-91
139 - 146 135 201 158 176 176 168 92-100

Plant height-5

184 - - - 147 - - 202 - 154 - 166 225 - 168 - - 173 1-18
- - - 186 141 - 166 175 170 - 215 218 - - 166 - - - 19-36
164 - 184 - 203 182 - 202 - - - 197 160 141 - 123 202 37-53
207 202 170 - - 162 172 - 195 155 213 - 161 208 218 54-68
- 172 200 - 235 146 197 - 179 126 197 176 184 210 208 70-83
- 199 - - 220 170 168 173 155 - 154 162 225 170 198 84-98
192 192 99-100

ELISA titer (OD at A-405nm)

0.672 - - - 0.999 - - 0.913 - 0.894 - 0.893 0.196 - 1-14
0.264 - - 0.965 - - - 0.398 1.045 - 0.281 0.277 0.618 15-27
- 0.129 0.979 - - 0.443 - - - 0.544 - 0.906 - 0.486 28-41
0.230 - 0.662 - - - 0.906 1.203 0.455 - 0.375 0.290 42-53
0.934 0.796 0.942 - - 0.698 0.073 - 0.943 0.949 0.785 54-64
- 1.003 0.907 0.735 - 0.432 0.376 - 0.761 0.476 0.037 65-75
- 0.349 0.416 0.296 0.875 0.950 0.943 1.147 - 0.698 76-85
- - - 0.395 0.158 0.282 0.989 - - 0.181 0.314 - 0.784 86-98
0.154 0.423 99-100

Plant numbers used for analysis and field layout numbers.

Plant No.	Field No.	Plant No.	Field No.	Plant No.	Field No.	Plant No.	Field No.
054	A1	091	D10	129	I8	167	1-26
055	A2	092	D15	130	J1	168	2-4
056	A3	093	D19	131	J3	169	2-5
057	A4	094	D20	132	J4	170	2-7
058	A5	095	D21	133	J5	171	2-8
059	A6	096	E4	134	J7	172	2-9
060	A7	097	E5	135	J8	173	2-11
061	A8	098	E8	136	K1	174	2-13
062	A9	099	E9	137	K2	175	2-14
063	A10	100	E12	138	K3	176	2-16
064	A11	101	E13	139	K5	177	2-18
065	A12	102	E16	140	K6	178	2-21
066	A13	103	E18	141	K8	179	2-23
067	A22	104	E19	142	L2	180	2-26
068	B1	105	F1	143	L3	181	2-28
069	B7	106	F2	144	L5	182	3-2
070	B11	107	F3	145	M1	183	3-5
071	B12	108	F4	146	M2	184	3-6
072	B13	109	F6	147	M6	185	3-7
073	B17	110	F7	148	N1	186	3-8
074	B19	111	F8	149	N2	187	3-10
075	B21	112	F11	150	N3	188	3-12
076	B22	113	F12	151	O4	189	3-14
077	B23	114	F14	152	P1	190	3-17
078	C6	115	F17	153	P6	191	3-20
079	C7	116	G1	154	1-1	192	3-21
080	C10	117	G5	155	1-2	193	3-24
081	C11	118	G7	156	1-4	194	3-25
082	C13	119	G8	157	1-7	195	4-1
083	C14	120	G9	158	1-8	196	4-2
084	C15	121	G16	159	1-15	197	4-3
085	C17	122	H1	160	1-16	198	4-4
086	C19	123	H2	161	1-18	199	4-7
087	C20	124	H3	162	1-19	200	4-8
088	C22	125	I1	163	1-20	201	4-9
089	D5	126	I2	164	1-21	202	4-10
090	D7	127	I3	165	1-23	203	4-11
		128	I4	166	1-24	204	4-12

Plant Numbers used for analysis and field layout numbers. (cont.)

Plant No.	Field No.	Plant No.	Field No.	Plant No.	Field No.	Plant No.	Field No.
205	4-13	219	5-14	233	6-15	247	7-14
206	4-16	220	5-15	234	6-17	248	7-15
207	4-18	221	5-16	235	6-19	249	7-17
208	4-20	222	5-18	236	6-20	250	7-20
209	4-22	223	5-21	237	6-21	251	7-23
210	4-24	224	5-23	238	6-24	252	7-26
211	4-27	225	5-24	239	6-26	253	7-27
212	4-28	226	5-25	240	6-28		
213	5-2	227	5-28	241	7-1		
214	5-4	228	6-4	242	7-5		
215	5-6	229	6-7	243	7-8		
216	5-8	230	6-8	244	7-9		
217	5-9	231	6-11	245	7-10		
218	5-11	232	6-12	246	7-13		

CHAPTER 8

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