Chapter 8
Avocado Genetics and Breeding

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8.1 Introduction

Avocado is highly heterozygous resulting in unpredictable progeny. Single gene mutations are unknown except for DNA markers. Avocado has only one seed per fruit and is characterised by heavy fruit drop, a long juvenile phase and a large tree size resulting in a substantial area required for a reliable assessment of hybrids. The advantages for the breeder are the wide genetic variation and the ease to vegetatively propagate the selected seedlings.

Avocado breeding programmes have been reported in California (Lammerts 1942, 1945; Schroeder 1960; Bergh 1961), Australia (Sedgley and Alexander 1983), South Africa (Du Plooy et al. 1992; Bijzet and Cilliers 1995), Mexico (Sánchez-Colin and De la Cruz-Torres 1992) and Israel (Lavi et al. 1991b). General reviews on avocado breeding were published by Bergh (1969), Bergh and Lahav (1996) and Lahav and Lavi (2002). The following chapter is based in part on the last two reviews.

8.2 A Short Taxonomic Description

Commercial avocado (*Persea americana* Mill.) belongs to the sub-genus *Persea* that also contains two other species, *P. schiedeana* (Nees) and *P. parviflora* (Williams). *P. americana* is a polymorphic species containing several separate taxa that are considered to be botanical varieties more commonly referred to as horticultural races (Scora et al. 2002). Botanical varieties that lie within *P. americana* include *P. americana* var. *drymifolia*, *P. americana* var. *guatemalensis* and *P. americana* var. *americana* (Bergh and Ellstrand 1986). These are commonly known as the Mexican, Guatemalan and West Indian (Lowland or Antillean) horticultural races

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respectively, based on their presumed centres of origin (Popenoe 1941). The number of chromosomes in avocado is $2n = 24$ (Garcia 1975).

There are no sterility barriers among the three races or among any taxa classified under *P. americana*. Hence, hybridisation occurs wherever trees of different races are growing in proximity, whether indigenously (Popenoe and Williams 1947) or under cultivation (Bergh 1969). 'Fuerte', the cultivar that long led production in California and most other Mediterranean/subtropical growing regions, is apparently a natural Mexican × Guatemalan hybrid, although with predominantly Mexican race characteristics. 'Hass', currently the dominant cultivar in most of these regions, is generally regarded as pure Guatemalan, but progeny produced by self-pollination indicate that it contains Mexican genes (Bergh and Whitsell 1974). Guatemalan × West Indian hybrids are currently the leading cultivars in Florida and look promising for future production in more tropical areas. For a detailed description of avocado taxonomy, see Scora et al. (2002).

8.3 History of Improvement

Two steps are usually involved in fruit tree improvement: selection of improved genotypes and their fixation by vegetative propagation. Avocado produces only sexual seeds and flowering dichogamy somewhat favours cross-pollination. Seedlings produced by a single tree (or cultivar) are extremely variable and in most instances have a prolonged juvenile period. The few selected seedlings, resulting from breeding projects that produce high yield of high fruit quality, must be vegetatively propagated as their sexual progeny have significant variation in fruit and tree characteristics. The first known grafting of avocado took place in Florida before 1900 (Ruehle 1963).

Selection of horticultural improved avocados occurred long before they were asexually propagated. Avocado seeds of varying antiquity (beginning about 7000 B.C.), excavated in Mexico (Smith 1966), indicated that selection for large fruit could have begun about 4000 B.C. However, this conclusion was essentially based on a comparison of the largest seeds at each level of the excavation. The number of seeds present was greater in the later deposits and sample size was strongly correlated with the largest individual seed size. Thus, Smith's data are compatible with selection for larger avocado fruits over the past several thousands years despite the absence of conclusive evidence. In addition, it seems reasonable that selection would also have occurred for a smaller proportional seed size as well as a larger fruit size (Popenoe 1919). However, there are no data to support this conclusion.

Extensive pre-Columbian avocado selection is evident from the high horticultural quality already present when Europeans first encountered the avocado. This was likely to have occurred through the laborious process of selection and propagation of superior cultivars by seed from small-fruited wild forms found in the forests of Mexico and Central America. Popenoe (1919) suggested selection methods as follows: cutting down the poorer seedlings, planting seeds from superior seedlings, selling choice fruit and thereby spreading the better types. It has been suggested that the smaller Mexican fruit size indicates less selection within that race (Chandler 1958). Further selection has occurred during the last century with superior cultivars being preserved through vegetative propagation (Popenoe 1952).

The selection procedure in avocado is illustrated by the Rodiles orchards near Atlixco (Mexico) where a seedling population is said to be non-variable (Anderson 1950). For generations, the Rodiles family planted seeds from the finest available local avocados, resulting in thousands of fruit bearing trees with high quality. 'Fuerte', long the world's leading cultivar, was developed in this orchard as was 'Puebla' that also was a leading California cultivar for many years (Kellogg 1971). In contrast, the authors of this chapter have always encountered great variation among seedling populations (Lavi et al. 1993b). A most zealous programme to improve the avocado industry by introducing scions of superior seedlings growing in indigenous habitats was centred in Guatemala and culminated in 1917 with the introduction to California of 'Nabal', 'Benik' and several other Guatemalan race cultivars. The marketing results were regarded as disappointing as at that time it was not realised that the preference of Californian consumers was for a much smaller fruit than is preferred in the tropics. Most of the introductions produced large fruit while others cropped poorly under California conditions or lacked other desirable characters. Nevertheless, some of these introductions made genetic contributions to the development of present day cultivars. Popenoe (1951) selected a few additional seedlings from the Rodiles orchard (predominantly Mexican types), looking for a wide germplasm for Central American avocado production. In addition, sporadic introductions of seeds from superior types in Central America and in adjoining regions widened the genetic background of commercial cultivars in California, Florida and elsewhere (Bergh 1957). All current important Florida cultivars were selected from locally grown seedlings produced from open-pollination. These were predominantly West Indian and (more recently) West Indian × Guatemalan hybrids. Several tropical areas have also advanced their regional avocado industries by selecting and vegetatively propagating superior local seedlings. All major current commercial cultivars in California (with the exception of 'Fuerte') are local random selections.

8.4 Breeding Objectives

We distinguish between "Specific" and "General" objectives. The first refer to situations where the breeder has a specific objective such as introducing a specific resistance gene like resistance to fire-blight in pears or seedlessness in citrus. Such objectives are usually found in the breeding of well-established crops where good cultivars already exist and there is a need to improve them. "General" objectives are those characterised breeding of "exotic crops" where the breeders and growers are interested in cultivars that are better than the current available ones but have no major restrictions regarding the fruit characteristics of the new cultivars.

Avocado breeders have 'General' breeding objectives and are interested in high quality fruit with long shelf life and high yield and are not restricted to a specific colour, shape and size in which producer and/or consumer preferences change with time (however, the American breeders prefer the 'Hass' colour and shape). The following are the current mainstream objectives:

8.4.1 Rootstocks

8.4.1.1 Phytophthora Root Rot

Avocado rootstock selection and breeding was reviewed by Ben-Ya'acov and Michelson (1995). Adequate resistance to *Phytophthora cinnamomi*, which causes root rot, is the most desired trait throughout the avocado world. Extensive attempts to hybridise avocado with *Persea* species of sub-genus *Eriodaphne* that are resistant to the disease have failed. An alternative approach was suggested by Witjaksono (1997) and by Witjaksono and Litz (1998) who developed protoplast fusion and somatic hybridisation methodology to assist with the provision of *Phytophthora cinnamomi* resistance and salinity tolerance in avocado. The last available report on this approach is Litz (2005). Thus, through this technology sexual and graft incompatible species as *P. borbonia*, *P. caerulea* and *Machilus* spp. may be hybridised with *P. americana* (Pliego-Alfaro et al. 2002). The assumption of this approach is that cytoplasmic genes control the resistance. At this stage, regeneration of somatic hybrids is the limiting factor. Limited resistance to Phytophthora root rot is known in certain lines of avocado and the closely related *P. schiedeana*. A few selections from them are important, especially in California, and have been clonally propagated to maintain the resistance level. 'Duke' and its derivatives ('Duke 7', 'Barr-Duke' and 'D9') and 'Thomas' are the most important ones (Newett et al. 2002; Pegg et al. 2002). For decades, Zentmyer (1972) and his successors have been searching in Mexico and Central America for wild sources of root rot resistance. The Mexican-race 'G6' achieved some commercial use. Another import, 'Martin Grande' (G755), evidently a cross between *P. schiedeana* and a Guatemalan-race tree (Ellstrand et al. 1986), has a comparatively high level of resistance but usually produces low-yielding trees due to excessive vigour.

The South African random seedling 'Dusa', of Guatemalan-Mexican origin, is reported (both in South Africa and California) to be significantly more root rot tolerant and more productive than 'Duke 7' (Kremer-Kohne and Mukhumo 2003).

Zentmyer et al. (1965) reported Guatemalan rootstocks to be more sensitive than Mexican rootstocks to *Dothiorella* and *Verticillium* wilt. Ben-Ya'acov and Frenkel (1974) found significant differences in sensitivity to *Verticillium* wilt among different West Indian rootstocks, while Tsao et al. (1992) found that some rootstocks tolerant to *P. cinnamomi* are sensitive to *P. citricola*, a serious pathogen of the avocado. No resistance to *Dematophora necatrix* is known.

8.4.1.2 Salinity

Resistance to salinity is greatest in the West Indian race and least in the Mexican race cultivars. However, there is significant variability within each of the three races (Kadman and Ben-Ya'acov 1976) and even among seedlings from the same tree (Kadman 1968). Resistance to high-lime chlorosis is greatest in West Indian cultivars and there is also a considerable intra-racial variability in this trait (Ben-Ya'acov 1972). However, West Indian rootstocks perform poorly in heavy soils and under waterlogged conditions (Ben-Ya'acov et al. 1974). A major Israeli objective is to combine the West Indian tolerance to high salinity and lime with tolerance to low oxygen. Breeding for rootstock tolerance to salinity and chlorosis is also reported from Mexico (Sánchez-Colin and Barrientos-Priego 1987) and South Africa. The South African selection 'Dusa' showed in California a good measure of tolerance to saline irrigation water (Crowley and Arpaia 2002).

Pure West Indian rootstocks have not proven satisfactory in the winter-cold California soils, but hybrids especially with Mexican genes seem well adapted. A rootstock that enhances scion cold hardiness would be highly desirable in frost-prone areas, but chill-tolerant Mexican rootstocks that have been studied did not transmit their hardiness to the scion (Halma and Smoyer 1951; Ben-Ya'acov 1987, 1998).

8.4.1.3 Dwarfing

Sánchez-Colin and Barrientos-Priego (1987), reported a significant dwarfing effect by their 'Colin V-33' selection, whether used as an inter-stock or as an ordinary seedling rootstock. However, since viroid was identified in some tests of this rootstock, its use for dwarfing is doubtful. A dwarfing rootstock could be a major benefit for most avocado producers. In Israel, the West Indian cultivars of 'Nahlat' (Ben-Ya'acov et al. 1979) and 'Maoz' (Kadman and Ben-Ya'acov 1980) were found to have dwarfing effects. In other studies, trees grafted to Mexican rootstocks were smaller than comparable trees grafted to West Indian types (Ben-Ya'acov 1976).

8.4.2 Cultivars

8.4.2.1 Fruit Traits

Size – The present optimum fruit size for most markets is about 250–350 g. For the sophisticated markets of developed countries fruit outside the 170–400 g range is unacceptable. Size is the most variable trait for a given genotype, being affected by crop load, proximity to other fruit on the tree, stage of maturity, cultural practices, climatic conditions and other unknown factors (Lahav and Kalmar 1977; Whiley and Schaffer 1994). Bergh and Lahav (1996) reported that in every selfed progeny set, the average size of seedling fruit was smaller than the parent, but this phenomenon was not repeated in the Israeli breeding project.

Shape – Fruit shape segregates extensively in most self-progenies. The squat-pyriform shape of ‘Hass’, the ovate fruit of ‘Bacon’ and the thick-ovate form of ‘Gwen’ are all desirable shapes. Fruits that are too elongate are quite common in the progeny of ‘Hass’ (Bergh and Whitsell 1974). The excessive elongated shape of ‘Pinkerton’ or ‘Galil’ (selected in the Israeli breeding project) can be shortened by treatments with growth retardants (Lahav et al. 1998).

Skin thickness – The leathery easy-peeling type of ‘Fuerte’ or the thicker skin of ‘Hass’ (for spoon eating) are usually preferred. Thin-skinned fruit is prone to damage while a skin that is too thick prevents determination of ripening time.

Skin colour – The preferred colour varies with market and time. The black-skinned ‘Hass’ was down graded during the time when green skinned ‘Fuerte’ was the leading cultivar in the US. Now that ‘Hass’ is dominant, cultivars with green skins sell for less. Breeders should probably ignore such passing fashions and, in our opinion, should concentrate on quality traits. However, currently, breeding for black skins, similar to ‘Hass’ is a major objective worldwide. Fruit that have mixed green and purple skins (seen in a considerable proportion of selfed ‘Nabal’ progeny) are usually less attractive. Glossy skin surfaces as characterised by ‘Ettinger’ are commonly considered attractive. In California, the severe russeting of ‘Regina’ and the end-spotting of ‘Zutano’ are unfortunately present in a majority of their respective progeny.

Seed size – Variability in seed size is very common within the same progeny (Fig. 8.1). A small seed relative to fruit size that is tight in the pulp cavity is a superior attribute of many Guatemalan lines. ‘Irving’ (whose later-maturing fruit and slightly thicker skin indicate more Guatemalan genes) has an exceptionally low seed/flesh ratio. The West Indian ‘Ruehle’ has small seed for that race. Elongated

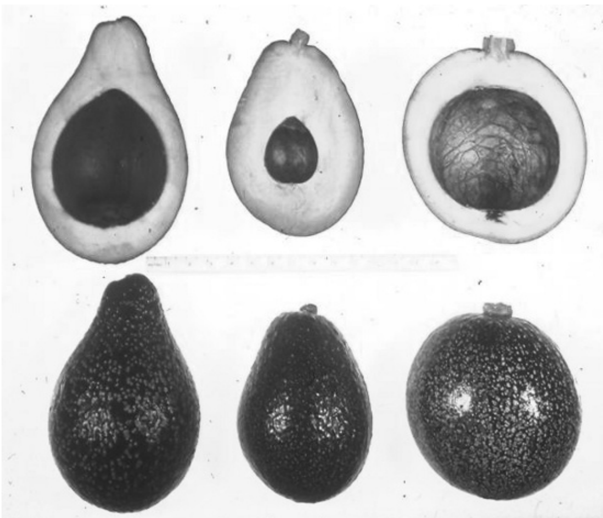

Fig. 8.1 Variation in seed size and fruit shape within seeding of the same progeny

fruits generally have a smaller seed with the long-necked ‘Pinkerton’ epitomising this trait. The semi-commercial ‘H287’ (a progeny from the Californian breeding project) has similarly a very small seed in a better shaped fruit. Progenies of both on average have smaller seeds than most other seedling groups. It is highly desirable that both seed coats remain attached to the embryo instead of to the flesh (loose seeds are frequently found in Mexican types).

Ripening – In most cultivars, the fibrovascular bundles tend to become more prominent with increasing maturity, but this undesirable trait is less marked in most Guatemalan lines. Uniform and adequate softening of fruit as it ripens is independent of race. For example, ‘Jalna’ (Mexican) and ‘Pinkerton’ (Guatemalan hybrid) are characterised with uneven ripening that may be more pronounced in some regions or in a fruit with advanced maturity (Piccone and Whiley 1986). A long period from harvest to softening is advantageous, especially when fruit is transported long distances. However, the unusually slow-ripening ‘Pinkerton’ has caused some consumer dissatisfaction because of its delayed edibility as compared with other cultivars. A longer time from softening to flesh deterioration is highly desirable. Avocado has a short shelf life compared with other fruits, although it varies between very short in ‘Anaheim’ to the better-keeping qualities of ‘Hass’, ‘Fuerte’ and others. West Indian lines have much lower oil content than those of the other two races but there is considerable intra-race variability. Guatemalans and especially Mexicans can reach well over 20% oil, but the ‘Anaheim’ and ‘Mayo’ cultivars of these two respective races may have an oil content below 8% when they begin dropping or deteriorating.

Flavour – The rich, slightly nutty taste of ‘Hass’, ‘Fuerte’ or ‘Benik’ is generally preferred over bland flavours. However, population preferences do exist and while the spicy or anise-like flavour of Mexican types such as ‘Mexicola’ or ‘Duke’ is more desirable by some consumers, the mild, sweeter taste typical of West Indian cultivars is usually preferred by other populations (such as in Central America).

Fruit diseases – In high rainfall climates, where fruit diseases are common, genetic resistance is desirable since fungicide treatments are expensive, not fully effective and are unfriendly to the environment. Ruehle (1963) lists the following relative cultivar susceptibilities: Cercospora spot or blotch (caused by *Pseudocercospora purpurea* Cooke) is much less severe on ‘Collinson’, ‘Fuchsia’ and ‘Pollock’; ‘Fuerte’ and other Mexican types are highly susceptible to anthracnose or black spot (caused by *Colletotrichum gloeosporioides* Penz.); ‘Fuchsia’, ‘Pollock’, ‘Booth 1’ and ‘Waldin’ are quite resistant while ‘Lula’ is highly susceptible to avocado scab (caused by *Sphaceloma perseae* Jenkins).

8.4.2.2 Tree Characteristics

Yield – The most important tree characteristics are precocity with consistent and high yields. Without this, excellence in the other tree characteristics is meaningless. Production consistency from year to year may be as important as overall heavy production (Bergh 1961). Because of large differences in tree size, yield is best assessed as tonnes per hectare at the respective tree spacings. A more subjective evaluation is fruit number per tree volume or "covering area" of the tree canopy (Kadman et al. 1976). The majority of fruit should reach commercial maturity about the same time and this is especially important for earlier-season cultivars that have a short life on the tree. Nothing is known about the heritability of this trait that is assessed in the second selection stage (see below).

It is noteworthy that there are reports describing a significant increase in yield as a result of treatments with various growth regulators (Wolstenholme et al. 1990). We believe that increasing the yield by selection of high yielders is preferable in the long run but at the same time, breeders should be aware of developments in horticultural practices and focus (in the short run) on those goals that are unachievable by agro-technical procedures.

Architecture – Tree form varies from erect as typified by 'Reed' through spreading ('Fuerte') to weeping as in 'Wurtz'. A spread about equal to tree height is considered desirable and dwarf or semi-dwarf erect trees are considered ideal. Very tall trees such as 'Bacon' or 'Ettinger' are difficult to manage and make fruit harvest expensive. Excessive vigour and fruitfulness are not fully compatible and exceptionally robust seedlings usually have little or no fruit. According to Bergh et al. (1996), tree vigour declines with inbreeding and is restored when inbred lines are crossed. Enough vigour to maintain good tree health and high productivity is essential.

Cold tolerance – Most of the major world avocado regions are subject to occasional frost damage and cold tolerance is highly advantageous for both the fruit and tree as a whole. Outstanding cold hardiness is limited to the Mexican race while above-freezing temperatures may still injure West Indian race cultivars. 'Hass' has been considered an extraordinary cold-hardy Guatemalan cultivar, but its progeny suggest that perhaps a fifth of its genes came from the Mexican race (Bergh and Whitsell 1974). Mhameed et al. (1997) and Davis et al. (1998) also provide evidence for an inter-racial origin for 'Hass'. This presumably explains both its cold tolerance and the fact that it is palatable much earlier in the season than pure Guatemalan cultivars. There are differences in tolerances within as well as between races (Fig. 8.2). For example, among cultivars believed to be pure Guatemalan, 'Nabal' and 'Reed' are unusually cold hardy and 'Anaheim' unusually sensitive to low temperatures. 'Yama' is considered one of the hardiest Mexican cultivars withstanding $-8\,^{\circ}C$ without severe injury, which would make it a favourable parent for cold hardiness breeding. Mexican seedlings appear promising in Florida for generating commercial selections with enhanced cold hardiness to withstand the freeze conditions that periodically occur there (Knight 1971).

Heat tolerance – Heat tolerance of cultivars varies significantly. However, the Mexican race has greater average heat tolerance, as exemplified by 'Mexicola', 'Mayo' and 'Indio'. Moreover, because Mexicans bloom earlier, their fruits are usually more advanced and thus less vulnerable to spring heat waves. 'Frey' and 'Hass' appear to be among the more heat-sensitive Guatemalan cultivars. 'Irving', a Mexican-Guatemalan hybrid, has shown exceptional tolerance to desert heat and low humidity in California (Bergh et al. 1996).

Fig. 8.2 Variation in cold hardiness within seedlings of the same progeny

8.5 Floral Biology

The flowers are grouped in terminal, highly compound cymes or thyrses (Scora et al. 2002) of dozens to hundreds of flowers each. Hence, a single tree may have a million flowers during one blooming period and only 100–200 fruits (Lahav and Zamet 1999). This low rate of fruit set makes hand-pollination impractical.

Knowledge of avocado floral biology is important for the generation of both selfed and crossed progeny. Avocado flowering has been reviewed by Bergh (1986), Davenport (1986) and by Gazit and Degani (2002). The avocado flower is protogynous, i.e. its pistil is receptive before pollen shedding. The flower opens twice for several hours each time and each opening is separated by at least one overnight period. The flower is functionally pistillate (female) during the first opening and staminate (male – pollen shedding) during the second opening. Under warm temperatures, avocado cultivars fall into one of two flowering groups (Lahav and Gazit 1994):

Group A – the first (female) opening starts in the morning and ends before noon. Second (male) opening occurs in the afternoon of the next day.

Group B – the reverse pattern: the female opening occurs in the afternoon and male opening the next morning.

Thus, the two groups are reciprocal, favouring cross-pollination, although self-pollination and pollination within trees of the same flowering group is quite frequent (Degani and Gazit 1984). As the weather becomes cooler, the opening and closing of the flowers are delayed. In addition, more than one night may elapse between the first and second openings (Gazit and Degani 2002).

Length of flowering varies with cultivar and climate – the cooler the temperature, the longer the flowering period. Guatemalan cultivars usually bloom later than those of the other two races. Usually, each tree flowers continuously for about two months and it is rare for the earliest to be finished before the latest begins. The breeder can manipulate flowering time to achieve overlap of desired parents. For example, flowering can be advanced as much as 2–3 months by girdling (Lahav et al. 1972) or by placing container-grown trees in a greenhouse during winter. Similarly, flowering can be delayed by holding such trees in a cool growth chamber or scions with differentiated flower buds can be stored at about 4 °C and grafted at the breeder's convenience. Using the latter technique, it is possible to carry out pollination for six months (Sedgley and Alexander 1983). Growth retardants have had limited success on controlling the flowering period (Levin 1981).

8.6 Genetics of the Juvenile Phase

The length of the juvenile phase is a major factor affecting the efficiency of fruit tree breeding programmes in general and avocado in particular. Relative to other fruit trees, this period in avocado is quite long reaching 15 years or more (Bergh et al. 1996). The efficiency of genetic improvement is inversely related to the length of the breeding cycle. Thus, it is important to shorten the juvenility phase. In breeding programmes based on controlled crosses, it may take about 18 years before a selection is released for small-scale commercial evaluation (Table 8.1). In breeding based on open-pollination and a fast release of selected progeny, the cycle may be reduced to about 11 years.

The length of the juvenile period is genetically controlled as shown in some fruits (Johnson 1940; Visser et al. 1976). The juvenile period in avocado can be significantly shortened by choice of parents (Lavi et al. 1992). 'Pinkerton' and 'Gwen' (Bergh et al. 1996) and 'Arad' (Lahav et al. 2005) produce very precocious

Table 8.1 Approximate timetable for avocado breeding

Year	Stage	Controlled Crosses	Year	Open Pollination
1	I	Preparation of parents	1	Pollination
2		Continued preparation of parents	2	Nursery
3		Breeding in cages	3	Planting in field
4		Nursery germination	4	Continued planting in field
5		Planting in field	5	Girdling
6			6	Evaluation and selection
7		Girdling, if practiced		
8		Evaluation and selections		
9		"		
10		"		
11		"		
12	II	Nursery propagation.	7	Top-working
		Top-working (may begin sooner)	8	Intense evaluation
			9	"
13		Field planting	10	"
14		Intense evaluation	11	"
15		"		
16		"		

offspring, sometimes fruiting two years from grafting. In contrast, Lavi et al. (1992) reported that the mean flowering age ranged between 4.3 years for the progeny of 'Rosh-Hanikra II' x 'Ettinger' to 8.6 years for the self-pollinating progeny of 'Nabal' (Table 8.2). In their study with 11 progeny populations, flowering age ranged between 3 and 11 years (with some seedlings up to 14 years). Fruiting age ranged between 3 and 11 years (up to 14 years in one case). No seedling flowered or fruited earlier than 3 years after planting and only 4.2% did so at the age of 3 years (Table 8.2). Note that a significant portion of each progeny population did not flower or fruit during the 10 years of experimentation. Statistical analysis showed clearly that the various progeny populations differ significantly in their flowering and fruiting ages. A difference by a factor of two between the earliest and the latest mean flowering age probably depends on genetic factors that are quantitatively controlled. Although there were some variations between different years, the above mentioned figures represent the general picture. No differences were detected between self- and cross-pollinations suggesting that there is no significant effect of heterozygosity level on the juvenile period (Lavi et al. 1992). It is noteworthy that the majority of seedlings that flowered in a certain year, fruited the same year or the following year, suggesting that the main constraint in an avocado breeding programme is time until the first flowering. It was concluded that the choice of parents might influence the length of the juvenile period and thus the efficiency of the breeding project. However, choice of parents in breeding programmes is influenced by other factors as well and thus the use of this tool is quite limited.

Table 8.2 Length of the juvenile period in 11 avocado progeny populations

		Flowering Age (Years)				Fruiting Age (Years)			
Cross[z]	Number of Progeny	*Mean*	*SD*	*Range*	*(%) flowered*	*Mean*	*SD*	*Range*	*(%) fruited*
Rosh Hanikra II × Ettinger	51	4.3[y]	0.7	3–7	90.2	5.4	1.0	3–7	62.7
Tova × Fuerte	48	5.0	1.5	3–14	91.8	5.6	2.0	4–14	37.7
Hass × Fuerte	123	5.8	1.7	3–10	86.1	6.3	1.9	4–11	70.8
Ettinger (selfed)	235	5.9	1.1	4–8	70.7	6.9	0.8	5–8	42.1
Ettinger × Tova	62	6.2	1.7	3–10	91.9	7.0	2.0	4–10	83.9
Tova × Regina	54	6.2	0.9	5–7	88.9	6.5	0.8	5–7	79.6
Hass × Ettinger	46	6.4	1.3	4–8	93.6	6.9	1.2	5–8	89.2
Anaheim (selfed)	61	6.5	0.6	5–7	68.9	6.5	0.7	5–7	32.8
Tova × Ettinger	387	7.0	2.1	3–11	77.4	7.0	1.9	3–10	37.7
Horshim × Tova	240	7.5	1.9	4–10	53.3	7.5	1.3	5–9	19.2
Nabal (selfed)	85	8.6	1.5	6–10	40.1	9.4	1.0	7–10	29.4

From Lavi et al. (1992), with permission.

[y] Order is from low to high mean of flowering age, based only on seedling that flowered and yielded during the experiment.

[z] Data refer only to the seedlings that flowered and fruited during the experiment.

8.7 Breeding Techniques

8.7.1 Shortening the Juvenile Period

Several horticultural practices have been applied to shorten the juvenile period. Top working has been used on a limited scale in Australia (Sedgley and Alexander 1983), Mexico (Sánchez-Colin and Barrientos-Priego 1987), California and Israel. Alternative approaches are to cut off a tree or discarded seedling and graft one new seedling on each or graft up to 50 scions of different lines into branches of a large tree. This can be efficiently done on whole rows of trees cut down a year ahead of time to produce numerous upright shoots. The multi-graft approach reduces space but requires much more labour, including ongoing pruning to protect less-vigorous shoots and has the risk of confused identities. Both approaches, but in particular the multi-grafting technique, have the added risk of contaminating new seedlings with viroid from the recipient mother tree. Grafting on West Indian rootstocks is always recommended under saline conditions. In our experience top working did not affect the length of the juvenile period.

Attempts have been made in California and Israel to induce earlier fruiting by bending seedling branches into a horizontal position, but no appreciable gain was achieved. A shorter juvenile period is reported in Mexico by training the seedlings as a single stem then allowing them to branch when 2 m high (Barrientos-Priego et al. 1991). Breeders in Israel and Mexico have noticed that larger planting distances shorten the juvenile period, probably due to better illumination.

Growth retardants offer theoretical advantages for enhanced fruitfulness by shifting vegetative/reproductive competition to favour flowering/fruit set but may introduce undesirable distortions. In Israel, no effect on the juvenile period was observed after application of various growth retardants (unpublished data).

Girdling is probably the best way to shorten the juvenile period (Lahav et al. 1986). The earliest autumn girdling increased the proportion of seedlings that flowered from 47% to about 100% that nearly tripled flowering intensity (from rating 1.0–2.7) and significantly increased the proportion of seedlings setting fruit (14.9% vs. 65.4%). Most importantly, this resulted in a 7-fold increase in the number of fruits per tree (1.3 vs. 9.3) (Table 8.3). Note that in breeding programmes, nine fruits per tree permit a reliable evaluation of the seedling. In conclusion, the recommended practice to shorten the juvenile period available to the breeder are choice of parents (without reducing genetic variation) and girdling.

8.7.2 Pollination

Commercial avocado production requires pollen transfer by large flying insects, primarily honeybees in subtropical regions (Vithanage 1990). Pollen requires about 2 h to grow from the stigma to the ovary, depending on the prevailing temperature and about 48 h for sperm-egg union (Sedgley 1979). Pollen tube progress can easily be

Table 8.3 Effect of girdling date on flowering and fruit set of avocado seedlings[z]

Date of Girdling	Number of Seedlings	(%) Flowered	Flowering Intensity[y]	(%) Fruited	Average Number of Fruit per Seedling
12 Sept. 1983	73	99.2[a]	2.7[a]	65.4[a]	9.3[a]
18 Oct. 1983	64	93.7[a]	2.3[b]	54.8[ab]	6.3[ab]
22 Nov. 1983	43	90.9[ab]	2.0[b]	42.6[ab]	4.7[bc]
5 Jan. 1984	27	61.4[b]	1.0[c]	27.4[bc]	1.7[bc]
Ungirdled control	74	47.3[b]	1.0[c]	14.9[c]	1.3[c]

From Lahav et al. (1986), with permission.
Values within columns followed by different letters are significantly different.
Means were separated by Tukey-Kramer test ($P = 0.05$).

[z] All values are least-square means.
[y] Flowering intensity ranked from 1 = very little to 5 = profuse.

followed by fluorescence microscopy after staining with aniline blue (Tomer and Gottreich 1975).

Hand-pollination is feasible for producing a few fruits but cannot be applied for breeding projects where large amounts of seedlings are needed. Hand-pollination is carried out by picking male-stage flowers with dehisced anthers and daubing stigmas of female-stage flowers. For controlled self-pollination, male-stage flowers can be harvested, placed in agar and stored at 4 °C until female-stage flowers open. The pollen from each sac usually sticks in a clump to the opened valve until it is removed by insects or drops with the flower. Methods of pollen collection, such as vacuum devices, have not worked well with avocado. Avocado pollen has remained viable for up to 6 days under field conditions averaging about 27 °C and 60% relative humidity (Papademetriou 1974). Storage viability can be extended by reducing both temperature and humidity. Thus, Sedgley (1981) successfully stored pollen for 1 month at 4 °C and 1–23% relative humidity and for one year at −196 °C and 0% relative humidity in liquid nitrogen. This procedure permits crosses between a wide range of avocado cultivars with various flowering times. However, due to the gradual loss of pollen viability over time, it is advisable to use fresh pollen where feasible. Net caged unpollinated trees have set only few fruits in California (Peterson 1955) or Israel (Gazit 1977).

8.7.2.1 Controlled Pollination

The advantage of controlled pollination is the knowledge of parents' identity while the cost per seedling is much higher. Self-pollinated seedlings can be obtained by three basic procedures:

1. Fruit can be harvested from a source at a sufficient distance from any other avocado trees. Note that some cross-pollination by bees (or other insects) can occur over long distances (Torres and Bergh 1978; Vrecenar-Gadus and Ellstrand 1985; Degani et al. 1989). The further away from contaminating pollen, the greater the likelihood of selfing.

Fig. 8.3 Breeding cages with two cultivars of complimentary flowering groups enclosed in each age

2. The tree can be caged within a bee-proof net in the presence of a hive (Fig. 8.3). Sometimes high levels of cross-pollination have been detected probably due to wind-pollination (Degani et al. 2003).
3. Seedlings can be pollinated by hand within a cage or bee-proof sleeves that enclose part of a branch. This is impractical for breeding purposes and can only be used to generate a small number of fruits.

Cross-pollinated seedlings are obtained under similar conditions:

1. Two different cultivars in close proximity but at a distance from other avocado trees can be allowed to cross-pollinate. The closer the two cultivars are situated, the greater the likelihood of crossing. However, there are differences in the effectiveness of cross-pollination between different cultivars (Degani et al. 1990) and progeny have to be identified by molecular markers (see below).
2. Flowering branches or trees of the pollinator can be enclosed in a cage with bees. This method will produce a variable mixture of crosses and selfers (Degani and Gazit 1984; see 1 above).
3. Seedlings can be cross-pollinated by hand inside cages or sleeves. This technique should produce only crosses, but conceivably both selfing and out-crossing could occur. This method (successful only under specific conditions) will produce a very limited number of progeny, definitely insufficient for breeding purposes.

None of the above methods can ensure the identity of the progeny which would mostly be mixtures of selfing and crossing. Whenever important to know the seedling identity, molecular markers have to be applied. For isozymes, see Degani et al. (1990); Torres et al. (1978); Goldring et al. (1985). For DNA markers, see Lavi et al. (1991a). The only exception is truly isolated trees (or a commercial block of a single cultivar), which would give rise only to selfers.

Based on our experience, we do not recommend controlled pollinations or identification of progeny by molecular markers for breeding purposes since the breeding objectives of avocado are "general" (see Section 8.4). We have to ensure the maximum genetic variation of the seedling population and this could be achieved by open-pollination. On the other hand, progeny identification is required for genetic studies.

8.7.2.2 Open-Pollination

Seeds are collected from the selected tree(s) and depending on the degree of isolation and the cultivar, various proportions of out-crossed progeny can be produced. The advantage with this approach is its low cost permitting the rapid evaluation of many seedlings. The disadvantage is that little is learned of the inheritance of commercial traits.

8.7.3 Increasing Seed Set

Hand-pollination is impractical for breeding programmes due to the high cost and the low fruit set. Schroeder (1958) cross-pollinated over 10,000 'Fuerte' flowers and only 4 mature fruits were obtained. Eisenstein and Gazit (1989) reported a yield of 1.3–27.2% fruitlets in hand-pollination of various crosses.

A number of techniques can increase fruit set from hand-pollination:

1. Select trees growing in an optimal location and provide a high level of management (Bergh 1967) (e.g. irrigation, nutrition, disease and insect control and wind protection).
2. Avoid excessive shading by using a screen material that transmits as much light as possible and remove limbs shading the breeding site (Lahav 1970).
3. Hybridise or self-pollinate in the productive ('on') year for that tree, since all avocado trees are alternate bearing to some degree.
4. Girdling maximises fruit yield and especially fruit number. Girdling after fruit set may increase its chances of fruitlets' survival (Lahav et al. 1971, 1972).
5. Select a heavy-setting cultivar as the seed (maternal) parent.
6. Use a potent pollen parent (Gafni 1984).
7. Cross-pollinate cultivars belonging to complementary flowering groups (Lahav and Gazit 1994) that flower at the same time.
8. Pollinate during optimal weather conditions.

8.7.4 Growing the Seedlings

An avocado seed left at ordinary room temperature and humidity remains viable for only a few days after its removal from the fruit as the embryo has no protection against desiccation (Storey et al. 1986). However, avocado seeds remain viable for up to 15 months when stored at 5 °C at high humidity (Halma and Frolich 1949). Humidity can be easily maintained in storage by placing seeds in sealed

Table 8.4 Seedling productivity as related to their grafted duplicates (±SE)

Number of Seedling Evaluated	Evaluation of Seedling Productivity	Evaluation of Graft Productivity
5	Low 1	2.3 ± 0.5
56	Low – Medium 2	2.6 ± 0.3
76	Medium 3	3.0 ± 0.3
59	Medium – High 4	3.5 ± 0.3
33	High 5	4.4 ± 0.6

After Lahav et al. (1995), with permission.
Productivity was evaluated from 1 (low) to 5 (high).
$R = 0.55^{***}$ (The three asterisks indicate level of significance $P = 0.001$)

polyethylene bags. Details on seed treatment, storage and germination procedures were summarized by Bender and Whiley (2002).

Under optimal growth conditions (night-day temperature range of about 23–25 °C), most viable seeds will germinate within a month (Alexander 1977). Vigorous seedlings will attain a height of about 1 m in three months, while less vigorous seedlings may require six months to reach a suitable size for field planting. Cooler temperatures greatly retard seedling growth.

Growing seedling progeny on their own roots is cheaper, but under some conditions (such as saline water or root rot) it may be necessary to graft onto salt resistant or Phytophthora tolerant rootstocks. About half the Israeli progeny from breeding programmes are grafted due to salinity conditions. Avocado seedlings and their grafted duplicates showed no significant performance differences (Lahav et al. 1995) and have a high and significant similarity in productivity (Table 8.4). Horticultural approaches aimed at economising field space have been discussed above under 'Length of the juvenile phase'. Detailed instructions with illustrations on avocado propagation were reviewed by Whitsell et al. (1989) and Bender and Whiley (2002).

The most efficient seedling spacing is the closest that permits adequate fruitfulness. This will vary with the average vigour of each seedling group. Suitable planting distances may be 1–2 m in the row and 4–6 m between rows (Fig. 8.4). Generally, the close planting distances increase the juvenile period and should therefore be avoided. At the closer spacing some of the smaller trees may have their fruiting delayed by shading, but this can be relieved through gradual reduction in tree density by removing inferior seedlings. The breeding block is replaced with new seedlings after 6–8 years, which is ample time for individual seedlings to exhibit the major breeding objective of precocity, but allowing a safety margin to cover delayed fruiting due to genetic or environmental factors. Recommended management techniques for nursery and field planted seedlings are summarized by Bergh et al. (1996).

8.7.5 Assessment of the Seedlings

A detailed list of avocado descriptors was prepared by the International Plant Genetic Resources Institute (1995). In the California breeding programme, recorded

Fig. 8.4 Avocado seedlings planted on ridges (two rows on each for drainage). Each seedling is protected against sunburn and frost by a mesh cylinder (from Bergh and Lahav (1996), with permission)

evaluations are usually made only on the seedlings selected for further testing, scoring only the important commercial traits. Trees are judged by size, shape, productivity and flowering. Fruit is evaluated by size, shape, colour, russet, attractiveness, time of maturity, skin thickness and roughness, seed size and tightness in the cavity, flesh attractiveness and fibre in the flesh (see Section 8.4.2.1). The ripened fruit is evaluated again in the laboratory focussing on flavour (nuttiness, sweetness, bitterness, other defects or comments), peeling ability and the time from harvest to softening (eating-ripe).

The Israeli breeding programme evaluated every seedling produced and records numerous parameters for genetic study, including nearly all of those listed for California plus the following:

1. *Tree*: The distance between buds, leaf and flush colour, flush lenticels and leaf size, shape, habit, margin waviness and anise scent.
2. *Flowering*: Time of year, intensity and flowering group.
3. *Fruit*: Length of inflorescence stalk (peduncle) and fruit stalk (pedicel), thickness of fruit stalk, its attachment position, suitability for snap picking (instead of having to cut the stem), skin gloss, seed surface, flesh texture, oxidation of cut fruit and shelf life.

Some traits are evaluated quantitatively by measurements but most are visually estimated. Evaluation of economic traits is summarized in Table 8.5 (Lahav et al. 1995). For genetic studies many traits can be assessed but for selection of new cultivars the assessment is usually limited to few economic traits.

It is currently recommended that two selection stages be performed. The first stage is aimed only at fruit assessment and is carried out in the first year of cropping. In the second stage, selected seedlings are grafted onto mature trees and are subsequently assessed for all fruit and tree characteristics under commercial conditions. This two-stage selection process ensures faster and efficient breeding outcomes (see Table 8.2).

Table 8.5 Evaluation of economic avocado traits

Traits	Evaluation Criteria
Tree size	Very big (1); Big (2); Medium (3); Small (4); Dwarf (5)
Flowering Intensity	Profuse (1); High (2); Medium (3); Light (4); Very light (5); None (6)
Flowering Time	Precocious (1); Early (2); Early mid-season (3); Late mid-season (4); Late (5); Very late (6)
Fruit Weight	In grams
Fruit Size Uniformity	High (1); Medium (2); None (3)
Fruit Shape Uniformity	High (1); Medium (2); Slight (3)
Fruit Density on the Tree	Dense (1); Medium (2); Light (3); Very light (4)
Damage by Snap-Picking	Minimal (1); Slight (2); Medium-severe (3)
Skin Thickness	Mexican type (1); Like 'Fuerte' (2); Like 'Tova' (3); Like 'Hass' (4); Like 'Nabal' (5); West Indian type (6)
Ease of Peeling	Excellent (1); Good (2); Medium (3); Difficult (4); Impossible (5)
Separation of Seed from Flesh	Easily (1); With some difficulty (2); Impossible (3)
Seed Weight (% of Fruit Weight)	< 6% (1); 6–10% (2); 11–15% (3); 16–20% (4); 21–25% (5); 26–30% (6);> 30% (7)
Taste Evaluation	Excellent (1); Very good (2); Good (3); Poor (4); Bad (5)
Darkening of Cut Surface (6 h After Cutting)	None (1); Slight (2); Severe (3)
Harvest to Softening Time (Room Temperature at About 20°C)	<6 days (1); 6–10 days (2); 11–15 days (3); 16–20 days (4); 21–25 days (5); 26–30 days (6);>30 days (7)
Shelf Life	In days

From Lahav et al. (1995), with permission.

8.8 The Genetic Basis of Fruit Abscission

Fruit abscission is a major problem that significantly affects yield. Moreover, it is the major reason for the low success rate of hand-pollination. Over the years, many explanations have been suggested for the occurrence of fruitlet drop by plant physiologists. In most cases, no horticultural practice has been successful in significantly reducing fruit drop, although shoot tipping in spring (Biran 1979) and the mid-anthesis foliar application of growth retardants (Wolstenholme et al. 1990) partly reduced it. Later it has been shown in Israel (unpublished data) that growth retardants reduced significantly fruit drop. Genetic selection was found to be an important factor in avocado fruitlet abscission. Thus, it was shown that the abscised fruitlets had different genotypes compared with the fruitlets that remain on the tree (Degani et al. 1986). This result suggests that abscission does not occur at random but depends on the seed genotype – selective drop.

Seedlings originated from self-pollinated 'Ettinger' trees that were caged under a net in the presence of a beehive were genotyped with leucine aminopeptidase (LAP), malate dehydrogenase (MDH), acid phosphatase (AP), glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM) and triosephosphate isomerase (TPI). Selfing was proved by MDH, AP and GOT. Segregation was analysed by PGM and TPI and resulted in the expected Mendelian ratio. On the other hand, analysis of this population in the LAP-2 locus resulted in a significant deviation from the expected ratio. In another experiment, three 'Ettinger' trees were individually caged and fruitlets were sampled both from trees and after dropping at various stages of fruit maturity. Deviation from the expected Mendelian ratio was increased significantly during the late stages of fruit maturity. This experiment was repeated with similar results in the consecutive year. Among 48 mature fruits that were picked from an 'Ettinger' tree, 45 were FS, 3 were FF and none were SS (Degani et al. 1986).

Genetic selection, expressed in the high frequency of FS genotypes, the low frequency of the FF genotypes and the absence of the SS genotypes, is the most probable explanation for fruitlet abscission (Degani et al. 1986). This conclusion is supported by the fact that the SS genotype was found at the early stage of fruit development and never among mature fruits. It is believed that the LAP-2 locus serves as a genetic marker linked to some other locus having selective adaptation. One can assume that similar genetic selection might operate on other loci, thus explaining the massive fruitlet abscission in avocado. If so, this phenomenon has a major evolutionary impact allowing a 10^4-fold selection rate, i.e. 100–200 mature fruits from a potential of about 1,000,000 flowers per tree at each generation (Lahav and Zamet 1999), thus providing the avocado a large evolutionary flexibility by 'choosing' the most adaptable seeds.

8.9 Classical Breeding Systems

8.9.1 Selection of Naturally Occurring Superior Variants

Before the onset of avocado breeding programmes, every avocado cultivar being grown on a large scale throughout the world originated as a random seedling. 'Fuerte', which for decades was the leading cultivar in the Mediterranean/subtropical regions worldwide, is a good example. Introduced into California in 1911 as budwood from a seedling tree in Atlixco (Mexico), it gradually gained prominence. Later it was exported from California to Israel, South Africa, Chile, Australia, Mexico and other subtropical countries where it became a leading cultivar.

Many early California cultivars originated in Mexico or Central America, either from asexual propagation of selections made there or from imported seeds (Bergh 1957). The Guatemalan cultivars 'Benik', Itzamna' and 'Nabal' were introduced as budwood while 'Dickinson' came from imported seed. Later, introductions to California have been almost entirely limited to those collected in a search for resistance to Phytophthora root rot (Coffey 1987). These introductions have also been mainly from Central America and neighbouring countries where *Persea* species abound.

Seeds imported from California, Mexico and Central America formed the basis for the Florida industry (Ruehle 1963), which later became a source of germplasm for tropical areas in the same way as California for the subtropics. Florida cultivars, some entirely of the West Indian race, others Guatemalan/West Indian hybrids, have been successful in various countries of Central America, South America and the West Indies, in coastal Mexico and in the tropical regions on other continents.

Several hundred random seedlings selected in California have been named and five (‘Hass’, ‘Pinkerton’, ‘Reed’, ‘Bacon’ and ‘Zutano’) are currently grown on a commercial scale. Similar local randomly selected seedlings have achieved some commercial success in other countries, notably ‘Ettinger’, and to a lesser degree ‘Horshim’ in Israel and ‘Sharwil’ in Australia and Hawaii.

In nearly every region where avocado is grown, local seedlings have been selected and named. Note that numerous mediocre-quality cultivars aggravate marketing problems. Since modern avocado industry is based on grafted trees and no superior somatic mutation had been reordered in avocado, we consider the chance to detect naturally occurring superior cultivars to be quite small.

8.9.2 Inter-specific Crosses

Attempts have been made to generate resistant rootstocks by inter-specific hybrids of avocado with *Persea* species immune to the disease. Up to the present time, all such crosses have been unsuccessful. Both graft and cross incompatibility appear to be complete between the sub-genus *Persea*, which includes the avocado, and sub-genus *Eriodaphne*, which includes all the known immune species.

Within the sub-genus *Persea*, ‘Martin Grande’ (G755) appears to be a natural hybrid between *P. americana* var. *guatemalensis* and *P. schiedeana* (Ellstrand et al. 1986; Furnier et al. 1990). ‘Martin Grande’ has about as much resistance to *Phytophthora cinnamomi* as any compatible line known. However, production from trees grafted to this rootstock has been poor in several countries where it has been evaluated (Whiley et al. 1990).

P. floccosa Mez has the valuable trait of setting much larger numbers of fruits than do other taxa in the *Persea* sub-genus. However, its fruits are very small and the seed relatively large. It has been hybridised with several large-fruited, small-seeded cultivars and a few of the better F_1’s have been selfed or “backed crossed” to commercial cultivars. The results have not been promising. Heavy setting ability has been lost at least as rapidly as commercial quality has been approached and flavour has been mediocre at best.

Inter-species hybridisation by somatic hybridisation and genetic engineering is discussed by Pliego-Alfaro et al. (2002).

8.9.3 Mutations and Polyploidy

Occasional spontaneous mutations have long been recognised in the avocado. Tree shape, leaf size, shape and colour, fruit size and shape or skin surface and thickness have been clearly different on certain ‘sported’ limbs. While trees of a number of cultivars have been affected, ‘Fuerte’ appears to be the most unstable with a pronounced tendency for somatic mutation. Several such mutations as ‘Weisel’, ‘Newman’ and ‘de Bard’ (Hodgson 1945) have been selected for commercial production. In contrast, ‘Hass’ seems to be comparatively stable. No mutation has yet proven horticulturally beneficial.

Somatic mutations affecting a quantitative trait like yield are much more difficult to detect, especially because of the highly erratic nature of cropping. Good evidence for genetically determined yield differences was first obtained for ‘Fuerte’ (Hodgson 1945). In addition, large-scale studies in Israel raise the possibility that there are ‘Hass’ variants differing in their yield (Ben-Ya’acov 1973). The sound nursery practice of taking buds from limbs of demonstrated high-yielding ability guards against detrimental mutations.

In the hope of enhancing its moderate root rot resistance, ‘Duke’ scions were irradiated with fast neutrons in the California programme. One resulting selection ‘D9’ was tested as a commercial stock because of its considerable root rot resistance and somewhat dwarfing impact. ‘D9’ was found more productive than ‘Martin Grande’ but less than ‘Borchard’ and ‘Duke 7’ (Arpaia et al. 1992). A radio-induced mutation-breeding programme with ^{60}Co gamma rays was conducted in Mexico by De la Cruz et al. (1999). They reported higher number of fruits/tree in some of the irradiated trees. Bringhurst (1956) generated tetraploidy by colchicine in ‘Fuerte’ and ‘Mexicola’. He reported some gigas characteristics in the vegetative organs but fruit set was reduced to almost nil.

8.10 Genetic Analysis

8.10.1 Qualitative Traits

The genetics of fruit skin colour, flowering group and anise scent was studied in breeding populations of 1,699 seedlings (Lavi et al. 1993a). The three traits were recorded over a two year period. Parent cultivars included: ‘Anaheim’, ‘Ettinger’, ‘Fuerte’, ‘Hass’, ‘Horshim’, ‘Irving’, ‘Nabal’, ‘Pinkerton’, ‘Reed’, ‘Regina’, ‘Rincon’, ‘Rosh-Hanikra II’, ‘Tova’ and ‘Wurtz’. Isozyme analysis was used to distinguish between hybrids and self-pollinated seedlings (Degani and Gazit 1984). However, the possibility that some seedlings were wrongly classified cannot be ruled out.

In all types of crosses the average X/Y ratio (X and Y being green or purple skin colour, A or B flowering group and (+) presence or (–) absence of anise among the progeny was one or higher with a wide variation in the ratio between crosses (Table 8.6). It is interesting that selfing in each trait (fruit skin colour: green × green or purple × purple; flowering group: B × B or A × A; and anise scent: (−) × (−) or (+) × (+) resulted in more progeny of the first phenotype (green skin, flowering group B and no anise scent) (Lavi et al. 1993a). These results rule out the

Table 8.6 Progeny distribution in three avocado traits

Traits	Crosses	Progeny Phenotype		Ratio
Fruit Skin Colour		Green	Purple	Green/Purple
	Green × green (selfings)	121	14	8.6
	Green × green (crosses)	273	20	13.6
	Green × green (total)	394	34	11.6*
	Green × purple	10	4	2.5
	Purple × green	71	48	1.5
	Purple × purple (selfings)	5	4	1.2
	Total and weighted mean	480	90	5.3*
Flowering Group		B	A	B/A
	A × A (selfings)	11	9	1.2
	A × B	148	111	1.3
	B × A	32	17	1.9
	B × B (selfings)	35	13	2.7
	Total and weighted mean	226	150	1.5*
Leaf Anise Scent		No anise	Anise	No anise/Anise
	Anise × anise (selfings)	225	29	7.8
	Anise × anise (crosses)	57	24	2.4
	Anise × anise (total)	282	53	5.3*
	Anise × no anise	59	11	5.4
	No anise × anise	555	119	4.7
	No anise × no anise (selfings)	257	17	15.1
	No anise × no anise (crosses)	270	65	4.2
	No anise × no anise (Total)	527	82	6.3*
	Total and weighted mean	1423	265	5.4*

From Lavi et al. (1993a), with permission.

*Weighted mean

possibility of a single gene coding for these traits. The results could be explained by the assumption that the traits are coded by several loci with several alleles in each while the various phenotypes may result from various heterozygous combinations. Furthermore, it was suggested that the inheritance of these traits is based on a threshold value beyond which the phenotype shifts from one phase to another as suggested by Carter (1969).

8.10.2 Quantitative Traits

Hybridisation is the only way to combine complementary desirable features of different cultivars and the most efficient way to obtain a desirable intermediate trait when the available breeding materials have extreme phenotypes. For example, the commercially important 'Booth' numbered selections in Florida are evidently natural hybrids of the Guatemalan and West Indian races and are intermediate in the harvesting season as well as other useful traits. Parallel inter-racial hybridisations between Guatemalan and West Indian types have given rise to important intermediate cultivars in Hawaii. The assumption behind hybridisation is that most of the genetic variance is additive and therefore combining alleles from two parents will result in offspring having the desired performance (Hansche 1983). An analysis of several quantitative traits in avocado was conducted in order to estimate the variance of components and their heritability. It was shown (Lavi et al. 1993b) that genetic variance (both additive and non-additive) is large for most avocado traits. In four of the nine traits (tree size, flowering intensity, fruit density and inflorescence length), relatively large non-additive variances in components were detected. In the second more detailed stage of this study, 14 cultivars were used to carry out 12 crosses and 12 selfings. The number of progeny varied between 2 and 431 seedlings per each self or cross and the total population consisted of 1,938 seedlings. The parent cultivars represented much of the variation that exists within avocado. Parentage verification was based on isozyme analyses. Only seedlings of known parentage were used in this study and the traits were assessed by measurements or by visual scoring with results averaged for 2–5 years.

The value of the non-additive genetic variance was significantly higher than zero for all traits (anise scent, fruit density, flowering intensity, fruit weight, harvest duration, inflorescence length, seed size, softening time and tree size). These values ranged from 36.3% in time to softening to 49.3% in harvest duration. In contrast, the additive genetic variance was non-significant in all traits. However, significant environmental variance was present and except for flowering intensity the estimate accounts for 33–35% of the total phenotypic variance. The values of the narrow sense heritability (h^2n) ranged from 0 in fruit density and flowering intensity to 0.5 in seed size and 0.48 in softening time (Lavi et al. 1993b).

These estimates indicate that non-additive genetic variance is a major component of the total genetic variance and is significantly greater than zero in all traits. The high level of heterozygosity known to exist in avocado (Lavi et al. 1991b) could explain the prevalence of a large non-additive (dominant) genetic variance (Fisher 1930). Thus, these results explain the common frustration after crossing two cultivars and obtaining a wide range of phenotypes among the progeny (rather than the naive expectation of combining traits from both parents). It must be emphasized that these conclusions are limited to the set of cultivars used in this study. The importance of the broad sense heritability (h^2b) originates from the fact that best performing seedlings in the selection plots could result from either environmental or genetic factors. The higher the value of the h^2b, the greater the confidence that genetic factors are responsible for performance. In such cases, a few grafted trees must be generated from each selected seedling for the next phase of evaluation. The choice of parents for breeding depends, first of all, on the breeding objective. In the case of the avocado, the main objective is to obtain new cultivars better than those currently available (see Section 8.4). Since the major variance components in most traits important to breeders are non-additive, parents should be chosen in order to maximise the genetic variance in the progeny. Thus, the chance to obtain the desired combination of genes and alleles is increased. This should be achieved by choosing a broad spectrum of parent cultivars and may include some with inferior performance.

8.11 Genetic Markers

8.11.1 Isozymes

Isozymes have been used mainly to assess the level of cross-pollination. For additional information on this topic, see Gazit and Degani (2002).

8.11.2 DNA Markers

Development of DNA markers (Botstein et al. 1980) paved the way towards new applications of this tool mainly due to the high level of polymorphism and the abundance of these markers. Several classes of DNA markers were developed to show existing polymorphism between individuals of the same species as well as between species. Several classes of these DNA markers were applied to avocado including RFLPs, Rapid Amplified Polymorphic DNA (RAPD) and Variable Number Tandem Repeats (VNTR) (DFP and SSRs). These markers have been applied to obtain various goals (Furnier et al. 1990; Pliego-Alfaro et al. 2002; Scora et al. 2002).

8.11.3 Level of Heterozygosity

Two types of VNTR markers were used for estimating the level of heterozygosity (Lavi et al. 1994b). Multilocus DNA markers were used to analyse avocado progeny resulting from either crossing or selfing cultivars. In five crosses, the heterozygosity level was found to be 100%, while in two self-pollinated families, heterozygosity was 90% and 94%. Typing of 59 loci with SSR markers in 5 avocado cultivars revealed an average heterozygosity (AH) of 0.58 ranging between 0.50–0.66 (based on Nei and Roychoudhury 1974); $AH = 1 - \sum pi^2$, where pi is the frequency of the ith allele. Gene Diversity (GD) varied between 0.42 and 0.66 calculated according to Rongwen et al. (1995); $GD = 1 - \sum pi^2$, where pi is the frequency of the ith pattern. Percentage heterozygosity calculated as the proportion of heterozygous genotypes from all those tested, varied between 38 and 70%. The percentage of fragments that exhibited Mendelian inheritance was 62.5–85% ($P < 0.05$) for DFP fragments and 85% for the SSR alleles.

Both RFLP and isozyme markers show low levels of heterozygosity compared to the above-mentioned results. This is due to the nature of the VNTR markers (Lavi et al. 1994a). A low level of self-pollination is probably the cause for the high level of heterozygosity in avocado (Lavi et al. 1993b; Mhameed et al. 1996).

The (AG)n markers were found to be the most efficient among the SSR markers analysed in avocado having heterozygosity levels of 0.58–0.70. Analysis of 11 cultivars with 17 SSR markers revealed on average 6.1 alleles per marker, an AH level of 0.79 and an average GD level of 0.78.

8.12 Genetic Linkage Map

SSR markers were generated by screening a genomic library of avocado having short DNA inserts (about 500 bp). The library was screened with all the dinucleotides and some of the tri- and the tetra-nucleotides. (A)n and (AG)n were the most frequent to occur. The total number of micro-satellites in the avocado genome was estimated to be about 45,000. From this library, 238 positive colonies were isolated and their sequence was determined. In 113 colonies a sequence defined as SSR was found and primers to the flanking regions were synthesized in 62 of them (Sharon et al. 1997). These 62 markers, together with 30 other markers synthesized earlier (Lavi et al. 1994a) and an additional SSR marker synthesized on the basis of a GeneBank sequence to the avocado cellulase gene were applied to several analyses.

Fifty offspring of the cross ‘Ettinger’ × ‘Pinkerton’ were genotyped with 93 SSR markers (Sharon et al. 1997) of which 51 were found to be polymorphic and reliable. Ten markers were inherited in a non-Mendelian fashion (4 with a significance level of $P \leq 0.01$). These markers together with 17 polymorphic RAPD markers (identified by screening 100 RAPD markers) and 23 DFP markers were used to draw a genetic linkage map of avocado. Two-point analysis resulted in 29 linked marker loci (LOD score $\geq$ 3). The map consists of 12 linkage groups having 2–5 markers per group (a total of 35 markers) covering 357.2 cM. Comparison of the parental with the maternal map shows that the crossing-over frequency was on the average 21% higher in the maternal parent (‘Pinkerton’). No linkage was detected between SSR and DFP markers and within RAPD markers. Based on the Poisson distribution, the markers were found to be randomly allocated to the linkage groups.

Note in proof. Michael Clegg of the University of California, Riverside, has recently started a project aiming at generation of additional SSR markers and identification of linkage between these markers and genes controlling traits of interest to the avocado industry (Clegg 2005). This project contains several aspects including phenotyping of important traits in experimental trees, estimation of heritabilities and genotyping of out-crossed avocado progeny by seven SSR markers. More results are expected in the next two years of this project.

8.13 Modern Breeding Methodologies

8.13.1 Cryopreservation

Efendi and Litz (2003) have developed two cryopreservation procedures for avocado: (1) Slow cooling at −1 °C/min from 25 °C to −80 °C followed by rapid cooling to −196 °C; (2) Rapid cooling from 25 °C to −196 °C. All tested embryonic cultures that recovered from the cryogenic storage grew normally. Cryopreservation is an alternative method for long-term conservation of avocado genetic resources which have so far been maintained ex situ in field repositories at high cost and under continuous various environmental threats. Cryopreservation is expected to improve and efficiate avocado breeding programmes.

8.13.2 Generation of Rootstocks by Somatic Hybridisation

Resistance to *Phytophtora cinnamomi* was identified in several *Persea* spp. within the sub-genus *Eriodaphne* (Bergh et al. 1996). These species include *P. borbonia*, *P. cinerascens* and *P. pachypoda*, which are all sexually and graft incompatible with avocado (*P. americana*). Thus Litz (2005) suggested overcoming this incompatibility by the fusion of avocado (*P. americana*), protoplasts with protoplasts of resistant *Persea* spp. The same procedure could obviously be applied to generation of rootstocks harbouring other horticultural important traits.

Putative somatic hybrids have been recovered by this approach although at a very low frequency of less than 0.001% (Witjaksono 1997; Witjaksono and Litz 1998, 1999). A newer approach for somatic fusion was developed by Litz (2005). This procedure is based on callus cultures that have been initiated from stem segments of micro-propagated plantlets generated from various resistant *Persea* species.

Somatic embryos from inter-specific hybridisations of *P. americana* (avocado) and *P. cinerascens* were recovered several years ago based on morphological markers. Some of these hybrids began to germinate in vitro. Further data regarding these hybridisations are not available at this time.

8.13.3 Propagation of Avocado via Tissue Culture

Success with in vitro propagation of avocado has been achieved through saving aborted embryos (Sedgley and Alexander 1983). The embryo culture medium stimulated the production of shoots, which were then micro-grafted to rootstocks. This method may be useful for rescuing especially valuable hybrids or selfs, but no report of its application is available.

8.13.4 Tissue Culture and Transgenic Plants

Due to the difficulties in classical avocado breeding, a generation of transgenic avocado trees having the desired traits is obviously a major goal that will make avocado breeding of both cultivars and rootstocks much more efficient. In order to achieve this goal there is a need to overcome two main obstacles:

1. Development of a regeneration protocol that will allow gene transfer to avocado.
2. Availability of the genes, which control the important traits.

For a list of avocado genes, which have been isolated and the gene transfer methodology for avocado, see Pliego-Alfaro et al. (2002). The following is a short summary of this subject:

A transformation system for the generation of new cultivars has to be based on the ability to transform existing cultivars. In other words, there is a need for a regeneration system from explants taken from mature trees. Available regeneration systems, which are based on either juvenile material or embryo cultures, do not serve this purpose. Only regeneration from mature material would serve breeding purposes, either for large-scale propagation of rootstocks with resistance to Phytophthora root rot and other desired traits or for the production of new cultivars (Pliego-Alfaro and Bergh 1992).

An embryonic avocado culture (derived from immature zygotic embryos of 'Thomas') was transformed using *Agrobacterium tumefaciens*. The reporter genes were GUS and nptII. The maturation of the transgenic embryos was achieved but regeneration to mature plants was not successful (Cruz-Hernandez et al. 1998).

Rahajo et al. (2003) reported the transformation of avocado with various genes (see below). Embryonic cultures were induced on semi-solid medium and transferred into liquid medium. The embryonic suspension cultures were transformed with *Agrobacterium tumefaciens* using the vector pGPTV containing glyphosate resistance and the CaMV 35S promoter. The transformation experiments included: 'Gwen' with the genes Chalcone synthase and nptII (as a selection marker) and 'Hass' with the antifungal protein (AFP) and the glyphosate resistance. Transformed plants with the genes: AFP; AFP+Chalcone synthase and SAMases (a bacterial gene mediating the breakdown of S-adenosylmethionine – SAM (which is precursor of ethylene and thus can delay ripening) and ACC deaminase (degrading ACC – a precursor of ethylene) have been regenerated by micro-grafting. Plants transformed with the AFP are reported to be assessed in greenhouse. No further information is available at the time of writing this chapter.

Although we consider the production of transgenic plants as a significant breakthrough in avocado breeding, we are aware of the major difficulties currently preventing the achievement of this goal. At present, the use of classical breeding techniques is the only available way to generate new cultivars. We believe that there is a simultaneous need to improve the efficiency of classical breeding and to develop modern breeding technologies.

8.14 Linkage Between DNA Markers and Loci Controlling Important Traits

Genetic linkage between DNA markers and genes controlling important traits could be applied to improve breeding projects. This is achieved through Marker Assisted Selection by selecting for the marker rather than for the trait and thus making breeding more efficient by saving time and space. In the long term, this linkage can be used for isolation of the genes responsible for these traits (Tanksley et al. 1995).

Only a few reports of such linkage are available for avocado that is lagging behind as compared with other species. Association between DFP fragments and 16 avocado trait loci was tested in two families by one-way analysis of variance and multiple regression (Sharon et al. 1998). The DFP fragments P4, P8, E2 and E5 in 'Pinkerton' × 'Ettinger' progeny were found to be associated with harvest duration, skin colour, skin thickness and skin surface respectively. The fragments P1, P8, B1 and B4 in the 'Pinkerton' × 'Bacon' progeny (half sibs of the first population) were found to be associated with fruit weight, skin colour, seed size and peeling respectively. Based on the two populations, the fragment P8 was found to be associated with the black-purple fruit skin colour. The intensity of this fragment in the DNA pools of progeny having green skin colour compared to those having black-purple skin colour supported this association. These results are interpreted as an association and maybe genetic linkage between the DNA fingerprint fragment P8 and locus (i) regulating avocado fruit skin colour.

The multi-locus markers are very useful for identification purposes but less so for linkage analysis. For this purpose the single locus VNTR markers are better suited. These markers are based on micro-satellite sequences flanked with conserved sequences. Primers based on these flanking sequences allow the use of Polymerase Chain Reaction (PCR) for their genotyping. These SSR markers are very polymorphic, very abundant and very reliable and thus served as the marker of choice for the human genome. SSR markers have been successfully used in several plants (Akkaya et al. 1992) including the avocado.

Sixty progeny of the ‘Pinkerton’ × ‘Ettinger’ cross were analysed to identify linkages with loci coding for agriculturally important traits (Sharon et al. 1998). One-way analysis of variance resulted in the identification of linkages with seven of the nine analysed traits. High levels of significance ($P \leq 0.01$) were detected in the traits. For example, skin gloss was linked to two SSR markers ($P = 0.0014$); seed size was linked to one SSR marker ($P = 0.0006$); and the amount of fibre in the flesh linked to SSR markers on linkage group 3 (especially with the marker AVAO4) ($P = 0.00001$). The application of Interval Mapping to allocate loci coding for these traits resulted in three cases where the LOD score value was ≥ 2 (skin gloss in linkage group 6; skin surface in linkage group 9; and fibres in linkage group 3). Further analysis revealed allelic interaction in the locus (i) controlling fibres in the flesh. The level of significance was found to be very high in the marker locus AVAO4 in certain genotypes (Sharon et al. 1998). These results point towards the potential of using SSR markers in genetic studies and the benefits of using this technology in breeding fruit trees such as avocado.

We are not aware of application of SNPs – which lately became the marker of choice in human and other species – to avocado.

8.15 Achievements

Many new avocado cultivars are currently available. However, we will limit this discussion to those cultivars from breeding programmes that have attained (or show clear promise of attaining) commercial significance. At the moment, the Californian and Israeli avocado breeding projects are in their final stage of evaluation of new and better performing progenies from already grown seedlings. The major objectives in both programmes are to find better than ‘Hass’, black skin cultivars and cultivars from the B flowering group that might increase productivity of ‘Hass’. Selections of outstanding rootstocks and cultivars exist in South Africa and Australia, and a small scale breeding project in Mexico.

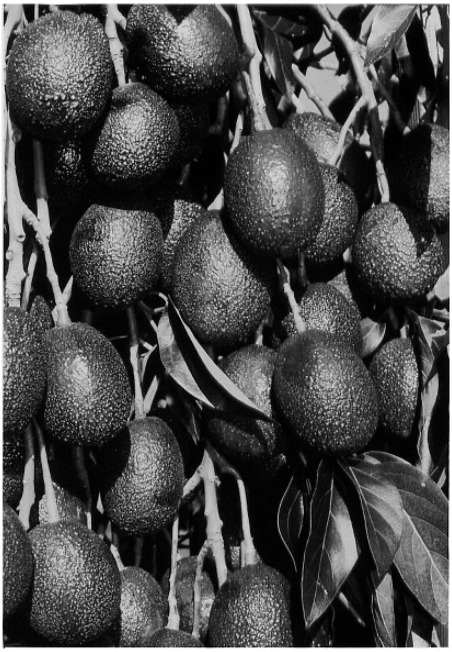

Fig. 8.5 Heavy set on 'Gwen' trees, 19 months after top-working

'Gwen' is a selection from the University of California avocado breeding programme (Bergh and Martin 1988; Martin and Bergh 1988, 1989). It is a seedling of 'Thille', which is in turn a seedling of 'Hass'. Propagated as either nursery trees or top-worked in the field, it may out-crop 'Hass' several fold in the early years of fruiting (Fig. 8.5) and about twofold indefinitely. However, it has not shown this level of production everywhere. Its smaller tree size makes picking cheaper. The length of its season is similar to 'Hass', but starts about a month later. In California, its flavour is superior to 'Hass' over most of its season.

'Lamb Hass' (BL 122) is another product of the California avocado breeding programme. It is a 'Hass'-like cultivar that produces 50% more fruits as compared to 'Hass' trees of similar age and growing conditions (Martin 1993). 'Lamb Hass' is a precocious, consistent bearer, which holds fruits very late in the season even in fierce wind conditions.

'Sir Prize' (4-18-15) is a selection from the University of California avocado breeding programme that is an early season cultivar with a green skin. Skin thickness is similar to that of 'Fuerte' although somewhat pebbly. Peeling is good and fruit quality excellent (Martin 1993). 'Sir Prize' is a good producer but has an extreme alternate bearing habit. It belongs to the B flowering group cultivars and shows promising results as a pollinator to 'Hass' (Arpaia 2004).

Some of the newer promising University of California releases are 'Harvest' (Fig. 8.6), a very heavy 'Hass'-like producer with excellent shelf life and 'Gem'

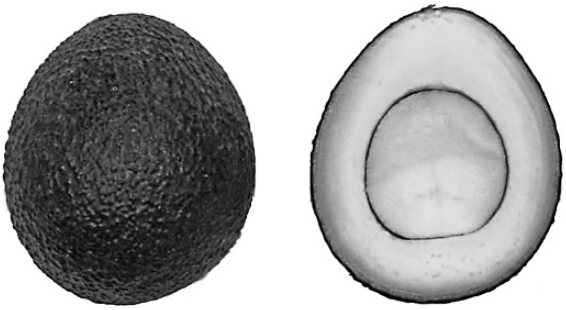

Fig. 8.6 “Harvest”, a ‘Hass’-like heavy producer, new release from the California breeding programme (from M.L. Arpaia, with permission) (*See* Color Insert)

(3-29-5) (Fig. 8.7) a good producer that shows less tendency to alternate bearing than most of the other selections (Witney and Martin 1998; Arpaia 2004).

‘Ardith’ (OO-28) was selected by the California breeding programme, but has only been commercialised in Israel. The tree is medium-sized and spreading. It is an oval, green skinned fruit of 200–300 g with a small seed. The fruit is late maturing, achieving excellent flavour only at the end of the ‘Hass’ season (Blumenfeld and Elimeleh 1986).

Two other Californian selections that have only been commercialised in Israel are the green, highly productive, mid-season ‘Fino’ (TX 531) and the ‘Hass’-like but much larger in fruit size ‘Ace’ (T 142).

‘Iriet’ is the first cultivar generated by the Israeli avocado breeding programme (Lahav et al. 1989). It is a progeny of ‘Hass’ and an unknown pollen donor producing a small-medium tree. The fruit is pyriform, 300–500 g and glossy black (Fig. 8.8), with a very small seed and an excellent nut-like flavour. The season of maturity is late.

Fig. 8.7 ‘Gem’, a new cultivar with reduced alternate bearing habit, released from the Califronia breeding programme (from M.L. Arpaia, with permission) (*See* Color Insert)

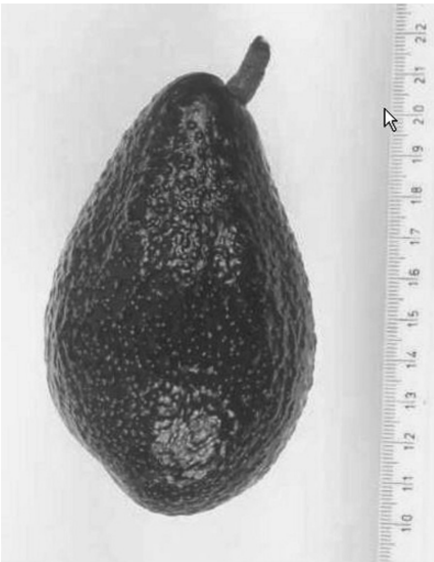

Fig. 8.8 'Iriet', a cultivar excellent in taste, from the Israeli breeding programme (*See* Color Insert)

'Eden' (Lavi et al. 1997) resulted from a cross in Israel between 'Pinkerton' and 'N-151-2' (a UC progeny). This was verified by analysis of mini-satellites and SSRs. It is a precocious producer with uniform dark green ovate fruit of 250–400 g.

'Galil' selected in Israel, is a green-skinned, Mexican type with a long neck (Fig. 8.9) and is an open-pollination seedling of 'Oshri' (a local selection). It is the earliest maturing summer cultivar harvested before 'Ettinger' in a season of great demand for avocado (Lahav et al. 1998).

'Arad' is an Israeli seedling of 'XX 102' that was caged with 'N-151-2' (both UC progenies). The 300 g fruit is green and harvested in mid-season. 'Arad' excels in precocity carrying many fruits already in the second year after grafting (Lahav et al. 2005).

'Lavi' is a seedling originated in Israel from a 'Hass' tree pollinated by an unknown donor (Regev et al. 2005). The tree is smaller than 'Lamb Hass' and its productivity is good. The fruit is more ovate and the peel thicker than 'Hass'. 'Lavi' was found to be especially interesting since its fruit is larger than 'Hass'.

'Naor' is an Israeli seedling of self-pollination of 'Horshim'. It is one of the most interesting selections since the fruit is very similar to 'Hass' but significantly larger in size (260–360 g). The harvest season is somewhat shorter than 'Hass' (Regev et al. 2008a).

Some interesting seedlings are currently under evaluation in Israel. One of them is 'Moti' (137–18), a seedling of 'R27T27' (originated from Hawaii) pollinated by unknown donor. The fruit is green, relatively large sized (350–400 g) and harvested after mid-season (Regev et al. 2008b). It excels in productivity but has tendency to alternate bearing. Another interesting seedling is 'Bar' (30–10) which is

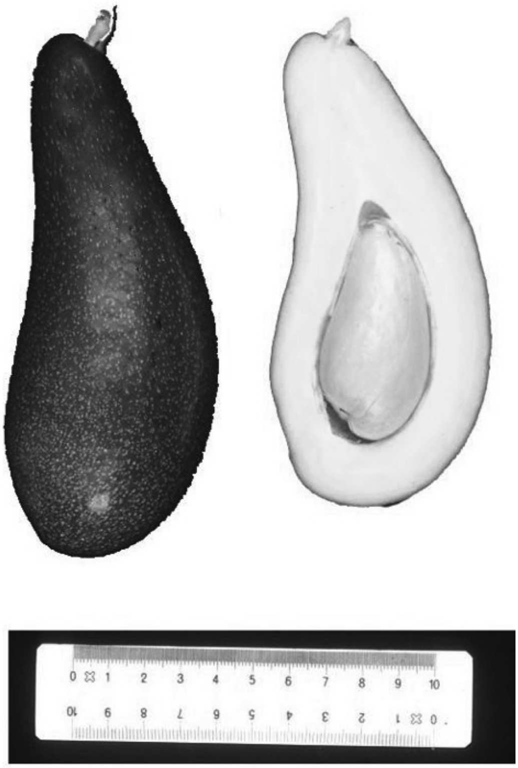

Fig. 8.9 'Galil', a very early maturing cultivar from the Israeli breeding programme (*See* Color Insert)

a 'Hass'-like cultivar but belongs to the B flowering group and has the potential to pollinate 'Hass' (Regev et al. 2008c).

8.16 Conclusions

Prospects for a significant increase in avocado consumption are bright since the fruit is exceptionally nutritious and as yet is quite unknown to many potential consumers worldwide. The future of avocado breeding is assured since past breeding programmes have so far barely scratched the surface of accessible genetic variation. This is even truer in most tropical regions where inferior local seedlings predominate, thus providing immense scope for improvement.

Based on our own experience, we offer the following recommendations for breeding avocado:

1. Unless a specific objective is required, open-pollination is preferred to controlled-pollination because the latter is more expensive and has no advantage.

2. In order to achieve an efficient breeding process we propose two selection stages. The first stage is carried out as soon as possible (even after one year of fruiting) on seedling populations (from either controlled crosses or open-pollination). This stage is aimed at identifying the seedlings' performance regarding fruit traits only. At this stage about 1% of seedlings are selected. The first stage lasts for about 6 years for progeny from open-pollination or about 11 years for controlled crosses. In the second stage, selected seedlings from stage one are grafted onto two or more mature trees in several locations (depending on the breeding goals) and managed under commercial orchard practices. At this second stage, the selected seedlings are assessed for yield, shelf-life and suitability for various climatic and soil conditions. At this second stage, selected seedlings are assessed by a wide team of growers, extension officers and breeders. Grafted trees of the most promising selections are prepared for the next stage of semi-commercial plots. The second stage is 4–5 years long.
3. High density planting should be avoided especially in the breeding orchard in order to shorten the long juvenile period.

The rapid advances in molecular biology, somatic hybridisation and other aspects of biotechnology have opened up new approaches in avocado breeding. The recent development of genetic markers for avocado and their applications to the classical breeding offers tremendous potential for avocado improvement. The introduction of specific genes for disease resistance from wild species into popular cultivars should be a reality in the foreseeable future. All these new technologies open new horizons to avocado breeding.

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