Estimation of the Genetic Parameters of Boron and Sorbitol Concentration in Fruit from a Pear Breeding Population, and Investigation of the Relationship between these Traits on the Occurrence of Flesh Spot Decay

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Estimation of the Genetic Parameters of Boron and Sorbitol Concentration in Fruit from a Pear Breeding Population, and Investigation of the Relationship between these Traits on the Occurrence of Flesh Spot Decay

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Pears (*Pyrus* spp.) are among the oldest crops grown worldwide. They are cultivated in all temperate regions and world production is second only to apples among deciduous tree fruits. Flesh spot decay (FSD) is one of the most important physiological disorders of Asian pears. The causes of this disorder remain unclear and no single nutrient or factor has been identified as the causal factor; however FSD has been related to environmental conditions. This study was carried out to estimate genetic parameters, in particular narrow-sense heritabilities and additive genetic correlations, of boron (B) and sorbitol contents and FSD in pear fruit. Approximately ten trees from each of twenty families of a breeding population were used in this study. Approximately ten fruit from each tree were assessed for disorder after four weeks post-harvest storage, and a composite sample from each tree was subsequently analysed for B and sugar (including sorbitol) content. Symptoms of internal browning and cavities were independent and thus not considered to be the same disorder (FSD). The heritability estimates for the prevalence of browning and cavities were low (0.18 and 0.19 respectively). B and sorbitol contents showed a positive genetic correlation (0.38) supporting the argument that the translocation of B is facilitated by the formation of complexes with sorbitol. High heritability was estimated for B content (0.72); however, that for sorbitol content was relatively low (0.18). A negative genetic correlation was estimated between both B and sorbitol and the area of the fruit affected by browning (-0.28 and -0.44 respectively). However, the opposite effect was observed in relation to the occurrence of cavities (0.51 for B and 0.60 for sorbitol). Furthermore, a negative genetic correlation was estimated between fructose and the occurrence of cavities (-0.62). Given that sorbitol-B complexes releases B when sorbitol is converted to fructose, these results suggest that there might be a link between B, these sugars and the formation of cavities in the fruit. Further work examining the B content within various parts of the fruit is required to unravel this link.

Keywords: *Pyrus*, pear, fruit disorder, flesh spot decay, boron, sugar, sorbitol, fructose, genetic correlation, heritability, empirical breeding value, browning, cavities.

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1. Introduction

Pears (*Pyrus* spp.) are among the oldest crops grown worldwide. They are cultivated in all temperate regions (Itai, 2007), and the world production of pears is second only to apples among deciduous tree fruits (Hancock & Lobos, 2008). Pears are cultivated principally for the fresh market and the canning industry (Jackson, 2003), with about 80% of the total production destined for fresh consumption (Itai, 2007).

Pear cultivars have been categorized mainly in two principal groups: European and Asian types. Barbosa *et al*. (2010) stated that the European pear (*P. communis*) has a more pyriform shape, greenish-yellow skin and melting flesh while the Asian pear has greenish-yellow or russet-brown skin and crisp flesh with a round shape predominating. Cultivars of the European pear are grown almost exclusively in Europe, North and South America, Africa and Australia while in China, Japan and Southeast Asia, the principal cultivated species are the Japanese pear *P. pyrifolia* (Hancock & Lobos, 2008) and the Chinese pear *P.* x *bretschneideri* (Brewer, 2011). Other pears cultivated widely in Asia include hybrids between *P. pyrifolia, P.ussuriensis*, and *P.* x *bretschneideri* (Rieger, 2006). Interest in Asian pears has been increasing in Western Europe, North America, New Zealand and Australia whereas the European pear has made little impact in Asia (Hancock & Lobos, 2008).

Numerous pear breeding programmes are found across the world. These programmes have been focussed on improving characteristics such fruit quality, disease resistance and climatic adaptation (Hancock & Lobos, 2008). However, little research has been carried out about the causes and inheritance of physiological disorders such as browning disorders and flesh spot decay (FSD). Browning disorders in pears can be observed in diverse forms such as spots, cavities, etc (Frank *et al.,* 2007). FSD generally appears as small brown spots in the flesh (Koto *et al*., 1972), but when fruit show severe symptoms, all parts will have spots and the necrotic cells will form cavities (Kawamata, 1982). Therefore, brown spots and cavities have been reported as symptoms of FSD in pear fruit (Kawamata, 1982; Lallu, 1989; Crisosto, 2002). However, there is no clear delineation stated in the literature which allows differentiation between the symptomatology of browning disorders and that of FSD.

FSD has a negative effect on consumer acceptance and it is one of the most important physiological disorders that affect Asian pears. This disorder is more frequent in Japanese

pear cultivars such as 'Shinseiki', 'Nijisseiki', 'Kikusui' (Crisosto, 2002) and less severe in the variety 'Hosui' (Burge *et al.,* 1991). The term FSD has not been recognized within the nomenclature of the physiological disorders that affect European pears (Brewer, 2011). However, the occurrence of cavities which is a typical symptom of this disorder has been reported in the European variety 'Conference' (Franck *et al*., 2007). The causes of this disorder remain unclear (Crisosto, 2002) and no single nutrient has been implicated as the major causal factor (Marsh, 1989), although it has been related to water stress (Behboudian & Lawes, 1994).

Boron (B) deficiency in *Pyrus* species causes different physiological disorders (Shorrocks, 1980; Dart, 2004) that affect fruit production (Shorrocks & Nicholson, 1980). It has been suggested that B applications can reduce the incidence of FSD (Satoh & Fukiwara, 1962, as cited in Raese, 1989; Khoshghalb *et al.* 2008) and browning disorders (Xuan *et al*., 2001; Wojcik and Wojcik 2003). Therefore, the prevalence of FSD might be related to the content of this micronutrient in the fruit.

B has limited phloem mobility within the plant (Oertli & Richarson, 1970, as cited in Brown *et al.*, 1999). Brown and Hu (1996) suggested that B has an apparent mobility in *Pyrus* because sorbitol is used as a primary translocated photosynthate in this species. Both Brown and Hu (1998) and Brown and Shelp (1997) found that B mobility is due to the formation of stable complexes with sorbitol. Sorbitol principally accumulates in species of the woody Rosaceae, including members of economically important genera such as *Pyrus*. These species are able to translocate this B-complexing sugar alcohol (Brown & Shelp, 1997), facilitating B mobility (Brown and Hu 1998; Brown and Shelp, 1997). Furthermore, sorbitol is unloaded into parenchyma tissue (Yamaki, 2010), this being the tissue in which FSD usually appears as small spots (Koto *et al*., 1972). In addition, Bellaloui and Brown (1999) suggested that the presence of sorbitol increase both B uptake and transport, affecting B metabolism.

Taking into consideration all the afore mentioned points, the objectives of this research were:

1) To determine the heritability of sorbitol and B content in fruit of a pear population.

2) To investigate the genetic relationship between these traits and FSD.

3) To carry out initial selection for progeny showing tolerance to FSD.

2. Literature Review

2.1 Pear Crop

Pear species belong to the family Rosaceae, subfamily Maloideae (Pomoideae), and to the genus *Pyrus* (Rieger, 2006). There are 22 broadly accepted primary species which are distributed through Europe, temperate Asia and mountainous areas of northern Africa (Bell *et al.,* 1996, as cited in Itai, 2007). It is considered that the genus *Pyrus* had its origin in the mountainous area of western and south-western China (Itai, 2007). Vavilov (1951, as cited in Itai, 2007) established two sub-centres (Central Asia and Eastern China) of diversity for this genus. According to the geographic origin, wild species such as *P. pyraster* L., *P elaeagrifolia* Pallas, *P. spinosa* Forssk., *P. syriaca* Boris could be considered to be the ancestors of the cultivated pear (Fischer, 2008). Wild pears can be found in the entire Eurasian zone (Yamamoto & Chevreau, 2009).

The majority of wild pears are diploid and crossable with cultivated pears and amongst themselves, which can explain the relatively high variability of the genus *Pyrus* (Moore and Ballington 1992; Fischer & Weber 2005, both as cited in Fischer, 2008). Although all pear species are self-infertile with gametophytic self-incompatibility system (Crane & Lewis, 1942; Westwood & Bjornstad, 1971, both as cited in Hancock & Lobos, 2008), incompatibility barriers to interspecific hybridization are not a major problem (Itai, 2007). Nevertheless, male sterility and the action of what could be a lethal gene that appears to prevent plant growth have been observed in New Zealand's pear breeding programme (Brewer, 2011).

Most of the cultivated pears are functional diploids ($2n = 34$). However, there are some polyploidy (triploids and tetraploids) cultivars of *P. communis* and *P.* x *brestschneideri* (Itai, 2007).

Kikuchi (1946, as cited in Itai, 2007) categorised *Pyrus* species into three groups. The first group is known as Asian pea pears. They are small fruited species with two carpels and are utilised for ornamental purposes or rootstocks. The second group includes large fruited species with five carpels. In this group, there are three main species: *P. communis* L. (European pear), *P. brestschneideri* Rehd. or *P. ussuriensis* Maxim. (Chinese pear) and *P. pyrifolia* Nakai (Japanese pear). The third group involves hybrids with 3 to 4 carpels.

However, fruit with more than 4 carpels (5-8) have been found in particular genotypes (Brewer, 2011) and thus, they do not fit into categorisation made by Kikuchi.

2.1.1 Pear Cultivars

Pear cultivars grown worldwide include European (*P. communis*) and Asian (*P.* x *brestschneideri, P. pyrifolia* and *P. ussuriensis*) types (Rieger, 2006). European pears are thought to have been in Europe since as early as 1000 BC (Itai, 2007) whereas Asian pears are thought to have been domesticated in prehistoric times and to have been grown in China for at least 3000 years (Lombard & Westwood, 1987, as cited in Itai, 2007). There are records of the cultivation of Japanese pears since the year 693 (Kajiura 1994, as cited in Itai, 2007). Currently, more than 1000 varieties of pears are known and there are also as many landraces (Fischer, 2008). The pear tree as currently grown commercially is a compound tree formed by a fruiting scion grafted on to a rootstock (Jackson, 2003), and often with an inter stock ('Buerre Hardy') if Quince rootstock is used to control vigour (Brewer, 2011).

Pear cultivars cultivated around the world are principally originated from *P. communis, P.* x *brestschneideri, P. pyrifolia* and *P. ussuriensis* (Yu-Lin, 1996). However, the first three are the major species commercially cultivated (Rieger, 2006). About 20-25 European and 10-12 Asian cultivars represent virtually all of the commercially grown pears (Rieger, 2006).

P. communis was developed in Europe and is the principal commercial species in Australia, Africa, South America, North America and Europe (Rieger, 2006; Itai, 2007) while *P.* x *brestschneideri* is the main species in northern and central China. In addition, *P. pyrifolia* is the principal species in Korea, southern and central China and Japan (Itai, 2007).

2.1.2.1 European Pears

The dominant cultivars of European pears (*P. communis*) were selected in the eighteenth and nineteenth centuries (Jackson, 2003). This species does not occur in nature and probably derives from *P. caucasia* and *P. nivalis* (snow pear) (Rieger, 2006). European pears vary in shape, juiciness, skin colour and texture (University of Kentucky, 2010). This

type of pear combines a buttery juicy texture with good flavour and aroma (Itai, 2007). There are some cultivars which show resistance to diseases and physiological disorders. Fruit are harvested before they are completely ripe (University of Kentucky, 2010) because they are subject to core breakdown if allowed to ripen fully on the trees (Rieger, 2006). Moreover, fruit can be stored for 2 to 4 months (even longer for some cultivars) at the proper temperature and relative humidity, depending on the cultivar (University of Kentucky, 2010). A chilling period is required before fruit are able to ripen, and the duration of this period differs with the cultivar. Summer pears require only a very short chilling induction, whereas winter pears typically require a longer period of chilling. The fruit then requires a ripening period at room temperature before being ready for consumption (University of Kentucky, 2010). European pears are generally served when soft and juicy, which takes about a week to occur after removal from cold storage (Beautel, 1990).

2.1.2.2 Asian Pears

Asian pears have a range of common names such as Oriental pears, Chinese pears, Japanese pears, water pear, apple-pear, sand pear, salad pear (Johnson, 1983) or nashi (Rieger, 2006). Cultivars of Asian pears have been developed from the species *P*. *pyrifolia*, *P*. *ussuriensis* and probably other *Pyrus* species native to northern Asia. However, several commercial Japanese cultivars have resulted from breeding within *P*. *pyrifolia* (Johnson, 1983).

Asian pears are characterised by a crisp texture and unique flavour (Itai, 2007). They are ready to be consumed as soon as harvested when mature (Beautel, 1990) as they ripen on the tree (Rieger, 2006). This ready-to-eat feature may make them more acceptable to some people than European pears. Moreover, Asian pears do not change texture after harvest or storage as do European pears (Beautel, 1990). Fruit of some cultivars can be stored at 0^{o}C for 1 to 3 months without problems; nevertheless, varieties such as ‘Hosui’ and ‘Shinko’ had shown internal breakdown in the core area of the fruit after 4 months of storage (Beautel, 1990).

Fruit from cultivars of *P.* x *brestschneideri* are large, round or elongate-round in shape with a long stem. They have white flesh which is crisp, juicy and sweet with few grit cells

and also have a deciduous calyx. These fruit are suitable for eating when matured on the tree. Fruit of this group have a long shelf-life. They can keep their eating quality for 2 to 3 months under ambient temperature (21^{o}C). However, the fruit flavour is not as rich as that of cultivars of *P. ussuriensis* (Yu-Lin, 1996).

The cultivars of *P. pyrifolia* produce fruit which are usually oblate, elongate-round or more rarely pyriform, have brown or yellow-green skin, with the calyx either deciduous or persistent. Flesh quality varies, some with fine texture and low flesh grit cells while others have a coarse texture with many grit cells, crisp, juicy, sweet and frequently with low aroma. The fruit is able to be eaten after harvesting. However, the storage ability is not as long as that of cultivars of *P.* x *brestschneideri* (Yu-Lin, 1996).

Fruit from cultivars of *P. ussuriensis* have small size, round or oblate shape and persistent calyx. These fruit are suitable for eating only when they soften. Therefore, like fruit from *P. communis* cultivars, they are not ready for eating at harvest. Their flavour is very rich and they frequently show a strong aroma. However, the flesh usually contains many grit cells (Yu-Lin, 1996).

2.2 Pear Breeding

Pear breeding programmes are found worldwide, principally in North America (Canada and USA), France, Italy, Norway, Sweden, Romania, England, China, Korea, Taiwan, South Africa, Brazil and New Zealand. Improvement of fruit quality is usually the main objective in pear breeding programmes, although what constitutes fruit quality varies by pear species (Itai, 2007) and locations (Hancock & Lobos, 2008). The principal characteristics which are studied to improve fruit quality are: fruit weight, flesh firmness, soluble solid content, organic acid content, flavour, ripening time and storage potential. Other important commercial traits of pear breeding are: fruit appearance, disease resistance, resistance to insects, growth habit and self-compatibility.

Information on the inheritance of fruit characteristics in pears is important for increasing breeding efficiency and clarifying the way in which the main traits influence fruit quality. However, genetic studies in pears have been scarce and there is limited information about genetic linkage maps and molecular markers in this crop. Breeding pears is complicated by their long juvenile phase and complex genetic structure. A high level of

heterozygosity occurs in pears, thus segregation must be taken into consideration for breeding populations (Itai, 2007).

In New Zealand, the pear breeding programme has been intercrossing cultivars of Japanese, Chinese and European species. Initially, selection has been carried out for the traditional pyriform style of pears; however, innovations in fruit texture, flavour and skin colour have been made from interspecific hybridisation. In populations derived from a wide range of interspecific crosses, moderate to high heritability has been shown in most of the fruit traits of interest to a breeder (White *et al.,* 2000b). Therefore, the strategy of inter- and intra- species crossing has created diversity from which to select new cultivars that represent European and Asian groups as well as combinations of the characters of both (White & Brewer, 2002), making the selection of new genotypes easy using the conventional breeding methods (Fischer, 2008).

A range of novel pears have been generated by interspecific hybridisation among European, Japanese and Chinese types (Brewer *et al.,* 2008). However, combination breeding (controlled crossing) is a procedure which requires much time (Fischer, 2008).

In recent times, there has been increasing interest in the genetics and physiology of fruit crops (Fischer, 2008). However, very little research has been done about inheritance of other traits in pears, especially in non-traditional quality characteristics such as mineral and sugar content in fruit.

2.2.1 Selection Procedure in Pear Breeding Programmes

Fruit tree breeding methods usually include three principal steps: generation of genetic variation, selection of elite genotypes, and extensive experimentation on these promising genotypes before market release (Schmidt & van de Weg, 2005, as cited in Yamamoto & Chevreau, 2009).

Strong directional selection may be carried out for the traits which are inherited independently because this allows further development of new combinations (Brewer *et al.,* 2008). In practice breeders usually select with regard to several traits. Correlated responses to selection with regard to other traits can occur even when the breeder is selecting just for one trait. Understanding these correlations and using multiple selection

is commonly applied to breeding populations. These may involve qualitative as well as quantitative variation (Bos & Caligari, 2008).

Selection pressure on different traits is most common in the first few generations when the number of individuals is very high rather than later generations (Bos & Caligari, 2008). Selections from the first combinations have to be evaluated and used as parents for the next populations. Thus, seedlings from further generations will also continue being evaluated in order to know if an increment in the genetic gains was obtained in the progeny. The best individuals (genotypes) must be extensively assessed in pre-commercial trials before being released for commercial production (White & Brewer, 2002).

A promising tool for increasing selection efficiency is marker-assisted selection (MAS) because of its ability to make assessments at the seedling stage and thus to eliminate undesirable genotypes at an early stage (Itai, 2007). However, no molecular markers have been developed for diagnosis of physiological disorders such as FSD in pears, limiting the use of this technique.

2.3 Boron

Plants show different needs for certain micronutrients; however, the elements that are essential for all higher plants are: boron, chlorine, copper, iron, manganese, molybdenum, nickel and zinc (Hansch & Mendel, 2009). B is an essential micronutrient element required for growth and development in plants (Marschner, 1986).

2.3.1 Functions of Boron

B is required in important processes such as protein synthesis, transport of sugars, cell expansion, respiration and the metabolism of RNA carbohydrates and plant hormones (Hansch & Mendel, 2009). It is required principally by growing tissue because it plays a main role in the formation of plant cell walls (Hu & Brown, 1994; Brown *et al.*, 1999). B promotes the structural integrity of biomembranes (Marschner, 1986) and thus it is important in lignification and cell wall synthesis and structure.

B deficiency inhibits plant growth (Hu & Brown, 1994), affects sugar synthetic functions (Yokomizo, 1977) and produces physiological disorders in the fruit (Shorrocks &

Nicholson, 1980; Dart, 2004). Therefore, the most important effect of the deficiency of this micronutrient is on fruit set and quality (Brown and Shelp, 1997; Jackson, 2003). Moreover, in terms of cell structure, cell wall plasticity extensibility is significantly decreased under conditions of B deficiency (Hu & Brown, 1994).

Rease (1989) and Satoh and Fujiwara (1962, both as cited in Wojcik & Wojcik, 2003) reported that pear fruit affected by B-deficiency was cracked and corked and the yield per tree was strongly reduced. On the other hand, foliar B sprays have been demonstrated to be an effective means of improving bud and flower concentrations, resulting in increased fruit set and yield in *Pyrus* species (Batjer *et al*., 1978; Johnson, 1995, both as cited in Brown & Shelp, 1997).

2.3.2 Localisation and Mobility of Boron

B is chemically localized and structurally essential in the cell wall of plants, constituting a critical factor for normal cell wall expansion (Hu & Brown, 1994). More than 90% of B in plants is found in cell walls (Marschner, 1986; Hu & Brown, 1994) and the majority of cellular B is associated with pectins within the cell wall (Hu & Brown, 1994). In addition, B may accumulate in the meristematic zones of fruits (Sotomayor *et al.,* 2010).

The mobility of B in phloem shows contradictions, demonstrating the limitations of this concept. For instance, B deficiency occurs quickly in growing tissues when B is limiting while it is accumulated in source tissues when this nutrient is in sufficient or excessive amounts. Therefore, the distribution of B between plant organs and the symptoms of B deficiency and toxicity have demonstrated that B shows a restricted mobility (Brown & Shelp, 1997).

B present in phloem may be derived indirectly from developed leaves or directly from the xylem. Phloem rather than xylem is the predominant transport route of B for developing sink tissues. (Brown & Shelp, 1997). B has been regarded as showing only limited phloem mobility (Oertli & Richarson, 1970, as cited in Brown *et al.*, 1999).

The main factor that enables phloem B mobility in a plant species is the synthesis of sugar alcohols (sorbitol, manitol or dulcitol) and the subsequent formation of stable complexes with sorbitol (sorbitol-B-sorbitol complex) (Brown and Hu 1996; Hu *et al*., 1997; Brown and Shelp, 1997).

Thus, it has been reported that B is highly mobile in *Malus*, *Prunus* and *Pyrus* species (van Goor & van Lune, 1980; Hanson, 1991, Picchioni *et al.*, 1995, Hu, 1996, all as cited in Hu *et al*., 1997) because they use sorbitol as a primary translocated photosynthate (Makkee *et al.*, 1985, as cited in Brown & Shelp, 1997; Brown and Hu, 1996; Brown *et al.,* 1999).

Overall, the uptake, transport and function of B in plants appear to be dependent on the formation of B complexes (Hu *et al.*, 1997). Moreover, the B distribution within organs, which is observed in sorbitol rich species, suggests that B acquired from the soil is phloem mobile (Brown & Shelp, 1997).

2.3.3 Boron Related to Pear Disorders

B deficiency can produce internal and external disorders in the fruit such as cork formation, small deformed fruit and browning near the core (Shorrocks & Nicholson, 1980; Dart, 2004). Khoshghalb *et al.* (2008) suggested that a shortage of this mineral can produce a physiological disequilibrium and destabilization of cell walls which might cause browning disorders and cavities formation. On the other hand, Xuan *et al*. (2001) suggested that B foliar sprays helps to maintain membrane integrity, decreasing the need for protection by anti-oxidants and thus diminishing the occurrence of browning.

When membrane damage occurs, the normal cellular compartmentalisation is lost and phenolic substrates may be enzymatically oxidized, producing browning symptoms (Franck *et al.,* 2007). Thus, B could be related to the occurrence of browning because this mineral plays a main role in the membrane structure (Xuan *et al*. 2001).

Necrotic spots, blossom end rot and hard fruit have been observed in fruits of 'Chojuro' variety without foliar B supply whereas B-treated pears have been shown to contain higher amounts of ascorbate (antioxidant) or vitamin C at harvest and during the whole period of storage (Xuan *et al*., 2001). The same authors found that brownheart symptoms appeared in the variety 'Conference' after two months of storage whereas fruit treated with B (foliar applications before harvest) did not show any disorder. Therefore, B seems to enhance the ability of fruit tissue to prevent browning disorders. In addition, Satoh and Fujiwana (1962, as cited in Kawamata, 1982) thought that FSD was produced by B deficiency; however, Koto *et al*. (1972) reported that applications of B did not reduce the incidence of this disorder.

2.4 Sugar Content in Pears

Sugars, organic acids, amino acids and aromatic compounds all influence the taste of fruit. Of these components, one of the most important compounds of fruit flavour and quality is the sugars (Itai *et al*., 2010). Sugars are distributed by a system of sieve elements (phloem) to sink tissues such as developing fruit, seeds and leaves (Teo *et al*., 2006). Their composition (i. e. sucrose, glucose, fructose and sorbitol) and amount accumulated in fruit directly influence the perceived sweetness (Itai *et al*., 2010).

Fruit sugar content does not vary significantly in the radial axis of the fruit, but there are significant variations along the stem-to-calyx axis (Wang & Sheng, 2005). The same authors stated that greatest sugar content was found in the top or proximal, stem-end of the fruit while the lowest content was observed in the distal or calyx end. However, the sugar content was more stable in the equatorial area (e.g., the middle of the stem-to-calyx axis) (Wang & Sheng, 2005). Moreover, considerable differences in sugar content have been observed between unripe and ripe pears (Choi *et al.*, 2009).

The principal photosynthetic products of pears, and Rosaceae in general, are sorbitol and sucrose (Choi *et al.*, 2009). Differences in sugar composition have been reported between cultivars of Japanese (Moriguchi *et al*., 1992; Shi, 2007; Choi *et al.*, 2009; Itai *et al*., 2010), Chinese (Moriguchi *et al*., 1992) and European pears (Hudina & Stampar, 2000b). In terms of species, a fluctuation in the sorbitol content in *P. ussuriensis* Maxim has been reported by Yu *et al.* (2010), showing a downtrend of this sugar during fruit development. The same trend was found by Hudina & Stampar (2000a) in a study carried out in *P. communis*.

2.4.1 Sorbitol

Sorbitol, which is also called glucitol, is a sugar alcohol present in amounts which vary between fruit species (Brown & Hu, 1996). It is a primary photosynthate and a major soluble carbohydrate in the phloem of rosaceous fruit trees (Makkee *et al.*, 1985, as cited in Brown & Shelp, 1997; Brown and Hu, 1996; Moing *et al.*, 1997, as cited in Kanayama 2009; Brown *et al.,* 1999). It regulates coenzyme activity, plays a function as a reducing agent and takes part as a compatible solvent in the osmoregulation of water stress resistance (Hudina & Stampar, 2000a). Sorbitol synthesis follows a different metabolic

path to sucrose synthesis, being obtained by reduction of glucose, changing the aldehyde group to an additional hydroxyl group (Madore, 1994, as cited in Hudina & Stampar, 2000a).

The content and composition of sorbitol are influenced by various environmental factors such as stress (water stress and defoliation), foliar fertilization and regular water supply (Hudina & Stampar, 2000a).

Sorbitol is the main sugar in leaves of the Rosaceae family and accounts for about 80% of the total sugars (Choi *et al.*, 2009). Along with sucrose, it serves as the main sugar translocated from mature leaves to sink organs such as fruits and young leaves (Yamaki, 2010). Although, sugars are principally accumulated in vacuoles in cells (Yamaki, 2010), sorbitol is more common in intercellular areas (Hudina & Stampar, 2000a).

Sorbitol is the main translocational substance of *Pyrus* spp., representing 60-90% of all carbohydrates transported from leaves to other parts of the plant (Loescher, 1987, as cited in Hudina & Stampar, 2000a). Moreover, it has also been reported as the main translocated sugar (65-70%) in apples (Klages *et al*., 2001).

Yamaki and Moriguchi (1989) and Moriguchi (1989, as cited in Moriguchi *et al*., 1992) reported that sorbitol is actively transformed into glucose or fructose after it is unloaded in the fruit of Rosaceae. This transformation is effected by the action of the enzyme sorbitol dehydrogenase (Yamaki, 2010).

Differences in sorbitol contents have been reported in fruit crops. For instance, Yamada *et al.* (1998, as cited in Choi *et al*, 2009) alleged that sorbitol only represents 3-8% of soluble sugar content in apple fruit during the growing season because it would appear to be degraded once it has been transported to the fruit.

Choi (2009) found sorbitol content of around 10% during fruit development in the pear cultivars ‘Niitaka’ and ‘Whangkeumbae’. However, Moriguchi *et al*. (1992) reported that sorbitol was the main sugar during early stages of fruit development in the cultivar ‘Chojuro’ (Japanese pear), representing 80% of the total sugars. Moreover, the same author found that sorbitol was also the major sugar in young fructification and continued at high levels throughout the season in the cultivar ‘Yali’ (Chinese pear). In addition, Moriguchi *et al*. (1992) and Hudina and Stampar (2000) found sorbitol content of around 20% in mature fruit from different European and Asian pears.

2.5 Principal Fruit Physiological Disorders in Pears

Among the most important physiological disorders in pears are browning disorders and FSD. Browning disorders, which can occur in European pear cultivars such as 'Conference' and 'Williams Bon Chrétien' (also known as 'Barlett') develop during long-term storage and result in economic losses. Damaged fruit cannot be distinguished externally from sound fruit (Franck *et al.,* 2007). On the other hand, FSD is a physiological disorder principally of Japanese pear (Koto *et al*., 1972), limiting their postharvest life (Joublan *et al.,* 1998). It has been reported in cultivars such as 'Kikusui', 'Shinseiki', 'Nijisseiki', 'Kosui' and 'Hosui', especially in overmature fruit (Lallu, 1989; Crisosto, 2002).

Brown regions and cavities are symptoms associated with both browning disorders and FSD (Kawamata, 1982; Lallu, 1989; Crisosto, 2002; Franck *et al.,* 2007), and hence the distinction between the two is unclear.

2.5.1 Browning Disorders

Browning disorders can be observed in diverse forms such as radial, asymmetrical, brown and/or dry spots, cavities and brown core. Spots are usually located in the extension of the five carpels while cavities can be found in different locations such as small spots in a star pattern in between the five carpels, randomly localised dried lesions or randomly localised cavities, commonly of a large size (Franck *et al.,* 2007).

There is no standardisation in nomenclature of browning disorders in pears, thus, different authors categorise the symptoms in different ways, suggesting that the classification of the observed symptoms remains subjective. However, they can fit into the general term "browning disorder", even though there is evidence that different mechanisms might be involved (Franck *et al.,* 2007). The same authors suggested dividing the symptoms into three groups: flesh browning, cavities, and both browning and cavities. Giraud *et al.* (2001) made a distinction between injuries related to senescence (categorising them as core breakdown) and CO_2-injuries (categorising them as brown heart). Larrigaudiere *et al.* (2004) found different metabolic behaviour between core browning and brown heart (both browning disorders), concluding that core browning was principally due to senescence and that storage at high CO_2 conditions only accelerated

the symptom expression. Furthermore, the description of core breakdown of Giraud *et al.* (2001) corresponds well with that of core browning made by Larrigaudiere *et al.* (2004).

Lammertyn *et al*. (2003a, 2003b) used magnetic resonance imaging to study the development of internal browning and cavities in 'Conference' (European pear), and found that cavities arise from brown tissue. The same authors found that the browning patterns did not grow over time but became more severe (e.g., intense colouration). This may be related to the different pear tissue structures which occur from the centre of the fruit towards the boundary. These authors categorized the symptoms found in the cultivar 'Conference' as core breakdown.

In core browning, flesh breakdown may extend to the rest of the fruit, with a zone of about 1 cm below the skin which remains unaffected whereas in brown heart, lesions usually dry out to form large cavities in the flesh. However, it is not clear if both disorders are the product of one single disorder or two different ones because it is common to see pears with only one kind of symptoms (Larrigaudiere *et al.,* 2004).

The principal pear features affected by preharvest factors and known to affect browning susceptibility are fruit size, vitamin C, phenolic contents and gas transport properties (Lentheric *et al.,* 1999; Lammertyn *et al.,* 2000; Hamauzu & Hanakawa, 2003, all as cited in Franck *et al.,* 2007). Moreover, the development of this disorder also depends on preharvest conditions such as climate conditions and crop load (Franck *et al.,* 2007). Blanpied (1975) suggested that heavy cropping on the tree reduced browning incidence in the cultivar 'Barlett'. Less productive trees from 'Passe Crasane' cultivar (Eccher Zerbini *et al.,* 1977) and fruit from the top of the tree of the 'Conference' cultivar (Franck *et al.,* 2003) have been shown to be more susceptible to browning.

Postharvest storage conditions, in particular a combination of low temperature, excessive CO_2 concentrations and long duration, can also increase the occurrence of browning disorders (Blanpied, 1975; Lammertyn *et al.,* 2000; Larrigaudiere *et al.* 2004).

Franck *et al.* (2007) suggested that late picking usually gives large fruit and this may increase browning susceptibility. Moreover, the same author stated that fruit with small internal air spaces are likely to be more susceptible to browning, especially in combination with a high respiratory activity.

Franck *et al.* (2007) postulated that browning disorders developing during postharvest ripening and storage are caused by an imbalance between oxidative and reductive

processes because of metabolic gas gradients inside the fruit, producing an accumulation of oxidative stress. This may induce loss of the membrane integrity which becomes macroscopically visible through the enzymatic oxidization of phenolic compounds to brown coloured polymers. Furthermore, Mayer (1987, as cited in Franck *et al.,* 2007) found that the occurrence of browning is caused by the enzymatic oxidation of phenolic compounds by polyphenoloxidase to *o*-quinones, which are very reactive and form brown coloured polymers. Amoit *et al.* (1992) and Nicolas *et al.* (1994, both as cited in Franck *et al.,* 2007) stated that the main factors involved in enzymatic browning are: phenolic concentration, polyphenoloxidase activity, ascorbic acid and peroxidases. Eccher Zerbini *et al.* (2002) suggested that browning in pears is initiated when ascorbic acid concentrations drop below a threshold level. Moreover, Larrigaudiere *et al.* (2004) found that core browning was mainly correlated with the fermentative enzyme alcohol dehydrogenase while brown heart was correlated with the antioxidant enzymes ascorbate peroxidase and catalase, suggesting that different metabolic pathways are involved in these physiological disorders.

2.5.2 Flesh Spot Decay

In general, FSD occurs at the point close to the peduncle and develops towards the equator of the fruit (Koto *et al*., 1972). Thus, it is more pronounced above the equator of the fruit, towards the stem end (Marsh, 1989). However, it can also be observed all the way down to the calyx (Crisosto, 2002). This disorder can occur in fruit while still on the tree; but it often worsens after 6 to 10 weeks of cold storage (Lallu, 1989; Crisosto, 2002). FSD typically appears as small brown spots in intracellular areas of the parenchyma near the end of the vascular bundle (Koto *et al*., 1972). When the fruit shows severe symptoms, all parts of the fruit will have the spots and the necrotic cells will form a cavity (Kawamata, 1982). Cavities are usually dry and surrounded by apparently healthy tissue (Crisosto, 2002). They can vary in shape and size in the flesh of the fruit, principally at the stem end (Lallu, 1989). This disorder may be restricted to a few cells, in which case a brown spot in the flesh is observed and cavities are not apparent (Lallu, 1989). The spot is not visible on the surface of the fruit because it occurs only in the flesh (Kawamata, 1982; Raese, 1989; Crisosto, 2002). Therefore, there is no external indication of FSD, making it impossible to predict this disorder without cutting the fruit open (Crisosto, 2002).

Furthermore, fruit with this disorder have a bitter taste and are not suitable for consumption (Kawamata, 1982).

It has been suggested that in individual cultivars FSD is more prevalent in large size fruit (more than 250 g) (Koto *et al*., 1972; Lallu, 1989; Crisosto, 2002) and increases during storage with increasing maturity. Thus, this disorder is related to cultivar, size and maturity (Marsh, 1989; Lallu, 1989).

Incidence of FSD varies markedly between seasons (Lallu, 1989; Marsh, 1989). However, Ferguson *et al.,* (1989, as cited in Joublan *et al.,* 1998) alleged that the initial symptoms of this disorder could be attributed more to management practices during fruit growth rather than postharvest treatments. Climatic conditions such as either a hot or cool summer (Crisosto, 2002) or high rainfall (Marsh, 1989) before harvest may increase the incidence of this physiological disorder. In addition, other conditions such as later picking (advanced maturity), low crop load (large fruit), extreme temperature changes during the maturation season, erratic irrigation or precipitation (timing, frequency and amount), sunburn, harvesting fruit under warm temperatures and quick cooling of the fruit might induce FSD (Crisosto, 2002).

Differences in susceptibility to FSD have been observed among cultivars. Joublan *et al.* (1998) carried out research using the varieties 'Shinseiki', 'Nijiseiki', 'Hosui' and 'Shinko' (Asian pears). In the 'Nijiseiki' and 'Shinseiki' varieties, FSD appeared as small brown spots in the flesh between the equatorial and the calyx cavity of the fruit, but cavities were not observed in the fruit. On the other hand, no symptoms of FSD were observed in the varieties 'Hosui' and 'Shinko' (Joublan *et al.,* 1998). In addition, Lallu (1989) found similar results where varieties 'Shinseiki' and 'Nijisseiki' showed the highest incidence of FSD whereas 'Kosui' and 'Hosui' showed less susceptibility to FSD.

Burge *et al.* (1991) also found a low incidence of FSD in the variety 'Hosui' and those fruit affected most showed only 1 to 3 spots. Thus, the variety 'Hosui' has been reported to be less susceptible to FSD (Ferguson *et al.,* 1989; White *et al.,* 1990, both as cited in Joublan *et al.,* 1998; Marsh, 1989). In addition, Lallu (1989) found that the variety 'Shinsui' barely develops the disorder.

It has been reported that FSD developed in the first 2 to 6 weeks of storage at 0^{o}C in the varieties 'Shinseiki' and 'Nijiseiki' (White *et al.,* 1990, as cited in Joublan *et al.,* 1998). An increase in the incidence of FSD was observed in these varieties during the storage period

at 0^{o}C, achieving 100% of incidence after 60 days (Joublan *et al.,* 1998). The same author stated that the best cold storage treatment to decrease FSD was to store the fruit at 0-3^{o}C. Furthermore, Szczerbanik (2007) found that this disorder decreased in fruit stored at low levels of ethylene (0.1 µL/L).

Although, FSD is an important disorder affecting fruit quality and consequently profitability, the direct cause of this disorder remains unknown. Therefore, there is no efficient way to control FSD (White *et al.,* 1990, as cited in Joublan *et al.,* 1998; Crisosto, 2002).

3. Materials and Methods

3.1 Pear Population

Two adjacent populations, one planted in 2007 and the other in 2008, consisting of 20 families, were used for this research. These families were derived from interspecific hybrids between European, Japanese and Chinese pear (Table 1and 2). Almost all the families assessed were related and some families had low levels of inbreeding (Table 3).

Table 1. Founding ancestors from the pear breeding population.

Type of Pear	Cultivar	Species
European	'Bartlett'	*P. communis*
	'Beurre Hardy'	*P. communis*
	'Comice'	*P. communis*
	'Duchesse d'Angouleme'	*P. communis*
	'Leon Leclerc de Laval'	*P. communis*
	'Seckel'	*P. communis*
	'Winter Nellis'	*P. communis*
Asian	'Huobali'	Chinese (*P*. x *bretschneideri*)
	'Kikusui'	Japanese (*P*. *pyrifolia*)
	'Nijisseiki'	Japanese (*P*. *pyrifolia*)
	'Shiyuehuali'	Chinese (*P*. x *bretschneideri*)
	'Wasekouzou'	Japanese (*P*. *pyrifolia)*

Table 2. Parentage percentage of the families evaluated in the pear population, and the numbers of plots, trees and fruit assessed for each family.

	Parentage (%)			Number of		
Family	**European**	**Japanese**	**Chinese**	**Plots**	**Trees**	**Fruit**
C1	63	25	12	1	5	99
C2	63	25	12	2	10	98
C3	63	25	12	4	15	150
C4	63	25	12	6	15	144
C5	75	0	25	2	7	140
C6	50	25	25	3	11	110
C7	50	25	25	2	8	150
C8	31	38	31	1	5	93
C9	31	38	31	5	15	150
C10	38	50	12	4	15	148
C11	69	13	18	3	10	94
C12	44	13	43	3	16	154
C13	44	13	43	2	14	137
C14	31	38	31	2	8	157
C15	56	25	19	2	8	154
C16	38	25	37	5	15	150
C17	31	38	31	3	15	149
C18	38	25	37	3	6	106
C19	31	25	44	2	6	120
C20	50	38	12	2	7	137
Mean	***48***	***26***	***26***			
Total				***57***	***211***	***2640***

Table 3. Degree of relationship between the parents is in bold (lower triangle). Families originated from the crosses of the parents (upper triangle). Diagonal (in grey) shows the inbreeding coefficient. All values have been multiplied by 1000.

Parents	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	Conference	P12	P13	P14	P15	P16	P17
P1	0				C15													
P2	0	0			C11													
P3	0	0	63						C19									
P4	0	0	281	0								C5				C16		C18
P5	**0**	**0**	281	281	63					C14				C12/C13	C8	C9		C17
P6	63	0	105	78	105	31		C20										
P7	63	0	105	78	105	266	31									C10		
P8	31	0	193	289	193	**172**	297	78										
P9	0	0	**141**	125	141	133	133	129	0									
P10	0	0	125	63	**125**	133	133	98	156	0								
P11	0	0	0	0	0	0	0	0	0	0	0						C6	
Conference	0	0	0	**0**	0	0	0	0	0	0	250	0					C7	C1
P12	0	63	47	63	47	39	39	51	31	31	125	250	0			C4		C2/C3
P13	0	0	16	0	**16**	0	0	0	31	31	0	0	0	0				
P14	0	0	156	63	**156**	133	133	98	156	188	0	0	31	31	0			
P15	0	0	156	**63**	**156**	133	**133**	98	156	188	0	0	**31**	31	250	0		
P16	0	0	125	0	125	0	0	0	63	125	**0**	**0**	0	63	250	250	0	
P17	0	0	281	**63**	**281**	133	133	98	156	188	0	**0**	**31**	31	250	250	250	0

3.1.1 Experimental Design

The trees were planted at the Motueka Plant and Food Research Station (41°6'S 172°58'E) in full-sib plots of ten trees using an unbalanced randomized block design. Trees from both populations (2007 and 2008) were located close together in the same area of the plantation. Four rows (275, 277, 285 and 286), two for each population, were selected from which to choose the trees. All except for two trees were in the rows selected. For this study, the aim was to evaluate ten fruit from each of 10 to 15 trees per family, with the trees chosen from as many different plots as possible. However, fruit of closely planted seedlings is inconsistent and thus this was not always possible, although all families had at least 90 fruit evaluated (Table 2).

3.2 Management of the Experiment

3.2.1 Thinning of Trees

For each selected tree, the trunk circumference was measured and the total number of fruit counted to calculate the number of fruit per trunk cross-sectional area (TCA). The target crop load was set at 2.5 fruit per TCA and excess fruit were removed in mid-December. Thus, the range for fruit per TCA for the selected trees varied from 0.3 to 2.5.

3.2.2 Harvest

Harvest was carried out weekly from the end of January to March. When fruit was deemed mature according to a combination of indicators (skin colour, skin finish, seed colours and fruit firmness), 10 to 20 fruit from each tree were harvested into labelled paper bags (one bag per tree).

3.2.3 Handling of Fruit

Phenotypic variables (weight, maximum diameter and shape) were measured on each fruit immediately after fruit harvest. The paper bags containing fruit were then placed in a cool store for 30 days at 0°C ± 0.5°C. After this time, they were cut open to assess for FSD (see Section 3.3) and to sample for later chemical analyses.

3.2.4 Preparation of Samples for Chemical Analyses

Each fruit was cut in half horizontally along the equatorial plane (region of maximum diameter) and slices with a thickness of 1 cm were cut from the upper half. Using a stainless steel corer (1.1 cm of diameter), two pieces of flesh (without skin) were taken from the first slice cut. One subsample was taken from one of the sides and another subsample from the opposite side. In fruit affected by a physiological disorder, the first subsample was taken from the affected part and the second one from the opposite side.

The subsamples from all the fruit from a tree were immediately placed in small plastic containers (50 ml) that were filled with liquid nitrogen in order to freeze the subsamples. The frozen sample was ground using a batch mill (A11 basic analytical mill). Four 2 seconds pulsations (28000 rotations per minute) were made per sample and the final result was a fine powder. For the B analysis, approximately 4 grams of powder was returned to the original container and stored at -80^{o}C. For the sugar analysis, approximately 0.2 grams of powder was placed in tubes containing 5 ml of ethanol (80%) and stored at -20 ^{o}C.

3.3 Evaluation of the Disorder Symptoms

Fruit was cut open and sliced as detailed in Section 3.4.5. The slice most affected by disorder symptoms (browning or/and cavities) was selected to be photographed. Fruit pictures were categorised according to the description in Section 3.3.1. Using the software WinDIAS, the area (number of pixels) unaffected by symptoms and the area occupied by browning or/and cavities was calculated in each slice and transformed to a percentage.

3.3.1 Categorisation of the Symptoms

Different symptoms such as cavities, brown core, brown lines, small brown spots, big and small brown patches were observed when the fruit were cut open. However, the majority of the evaluated fruit (82%), and 53% of the seedlings, showed no disorder. Most of the symptoms observed corresponded to those described by Franck *et al.* (2006) and Han *et al.* (2006).

3.3.1.1 Cavities

Cavities were observed in both white (clean) tissue and brown tissue (Figure 1).

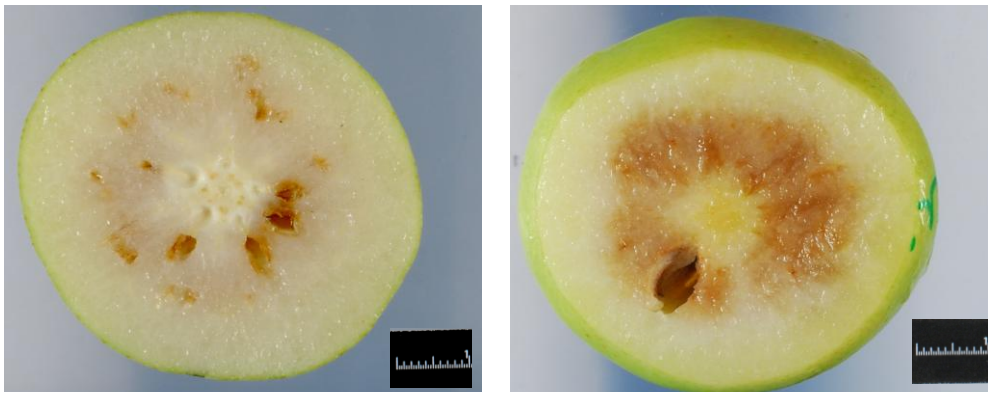

Figure 1. Fruit showing cavities in white tissue (left) and in brown tissue (right). Bar 1 cm.

3.3.1.2 Browning Symptoms

In terms of browning, different forms of distribution (whole patches and scattered spots) were observed. For example, fruit showed browning as whole patches, including small (Figure 2A) or large patches (Figure 2B and 2C). The latter included radial patches (Figure 2B) and asymmetrical patches (Figure 2C).

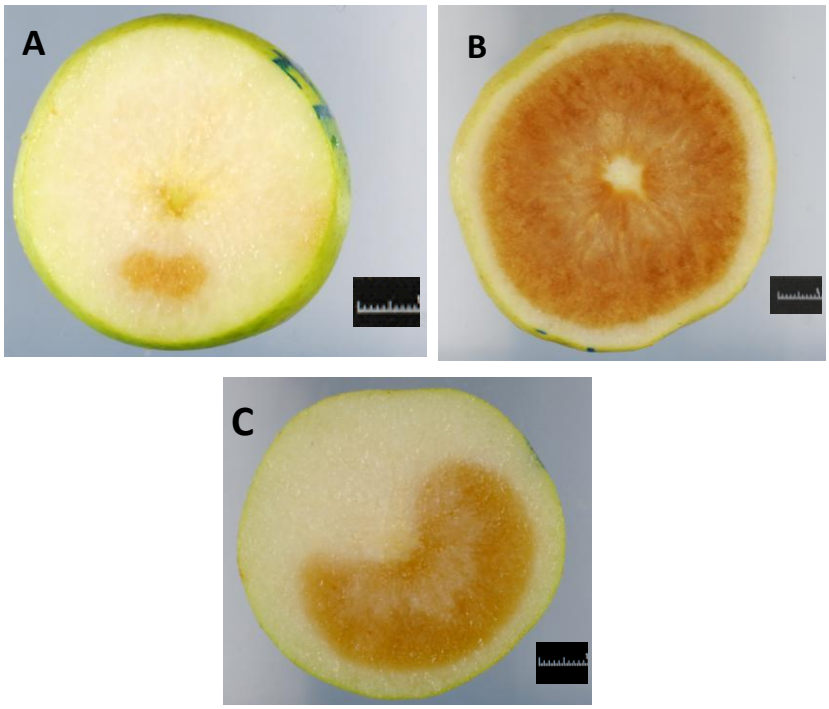

Figure 2. Fruit showing small patches (A), radial browning (B) and asymmetrical browning (C). Bar 1 cm.

On the other hand, some fruit showed brown spots scattered throughout the flesh (Figure 3). These spots may have coalesced to form single patches.

Figure 3. Fruit showing scattered spots in the flesh. Bar 1 cm.

A few fruit showed small dark brown spots and lines in the tissue affected by browning (Figure 3 and 4), and some fruit showed brown core (Figure 5).

Figure 4. Fruit showing dark brown small spots and lines in brown tissue. Bar 1 cm.

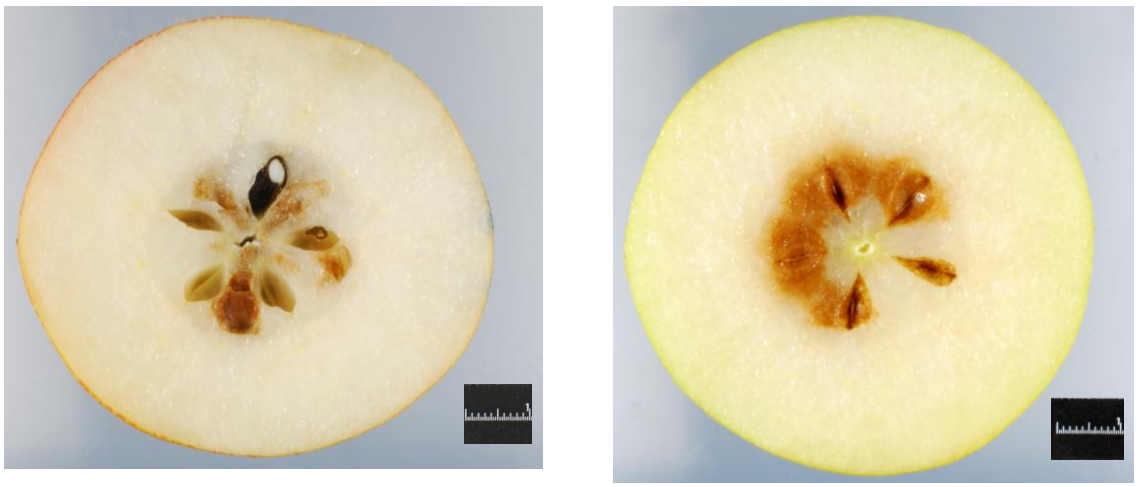

Figure 5. Fruit showing brown core. Bar 1 cm.

Finally, a few fruit affected by browning also showed flesh breakdown (soft tissue) (Figure 6) which can extend to the rest of the fruit. However, more frequently a small zone below the skin sometimes remained unaffected. These symptoms have been related to senescence (Larrigaudiere *et al*., 2004; Giraud *et al*., 2001). Thus, it was assumed that fruit showing these symptoms were overripe.

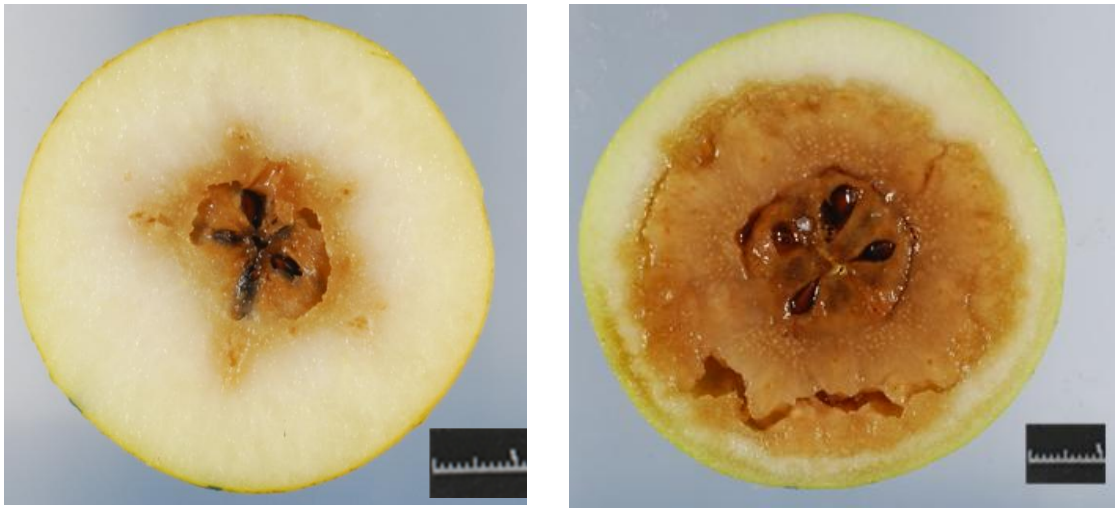

Figure 6. Fruit showing browning and flesh breakdown. The fruit on the left shows an initial stage while the fruit on the right shows an advanced stage of this symptom. Bar 1 cm.

3.4 Chemical Analyses

Mineral and sugar analyses were carried out at Lincoln University laboratories. An inductively coupled plasma optical emission spectroscopy method (ICP-OES) (Cartwright *et al.,* 1983; Harris, 2003) was used to carry out B analysis while a high performance liquid chromatography method (HPLC) (Wasik *et al*., 2007) was used for sugar analysis.

3.4.1 Boron Analysis

Approximately one gram of well mixed thawed powder sample was dispensed into tared microwave vessels (Xpress vessels, 55 ml PFA venting) using a plastic dropper. The weight was recorded to the nearest 0.001g. To each sample, 5 ml of nitric acid (HNO_3) (Aristar quality, trace element grade) and 1 ml of hydrogen peroxide (H_2O_2) was added and allowed to predigest in a fume cupboard overnight. This allowed the gases generated to

dissipate before digestion. The vessels were then capped and placed in the forty-place rotor inside a microwave digester (CEM MARS Xpress, England) for digestion. The microwave digester has an operator selectable output of 0 – 1600 watts ± 15% by ion-exchange chromatography (IEC) method. Microwave energy was used to heat the samples in a closed vessel microwave system. Samples were placed inside a Teflon PFA® and Kevlar shielded vessel. Once in the MARS Xpress, the samples were subjected to rapid heating and elevated pressures, causing the sample to digest or dissolve in a short time.

The microwave heating program was 10 minutes ramp to 150^{o}C and held at 150^{o}C for 10 minutes. A temperature of 150^{o}C was sufficient to completely mineralize the sample. After that, the vessels were cooled to room temperature (21^{o}C) and they were uncapped slowly to prevent excess venting. Then 14 ml of MilliQ (Barnsted EASYpure RF) water was added to make a final volume of 20 ml. Finally, the samples were stored in 30ml polypropylene vials. These vials were placed in the Varian 720 ICP-OES (with full PC control of all instrument settings, Australia Ply Ltd) to measure the B content in each sample. The ICP-OES features a custom-designed and patented charge-coupled device (CCD) detector with unique image mapping (I-MAP™) technology. Single and multi element standards (Merck) were used to obtain the measurements. In the spectrophotometer, the concentration of a specific element in a sample is related to the intensity of lines in its optical spectrum. The sample, in an aerosol form, was introduced into high energy plasma that dissociated the sample into atoms and ions which emitted electromagnetic radiation. The emitted light was spectrally resolved by diffractive optics, and the intensity of light was measured with a detector (Nolte, 2003). There was complete wavelength coverage from 167 to 785 nm with a resolution of 7 nm and all wavelengths were captured in one simultaneous reading. The final result was expressed in µg/g of fruit (fresh weight basis).

3.4.2 Sugar Analysis

The stored samples were filtered through a 0.45 µm nylon membrane to obtain a filtrate. After filtration, 1200 µl of Adonitol (Sigma-Aldrich, USA, ≥ 99.0%) at a concentration of 400 ppm was added to 400 µl of each sample to make a final volume of 1600 µl in a vial.

Finally, samples were placed in a high performance liquid chromatograph to obtain the sugar contents.

The HPLC analysis system consisted of a LC -20 AD Prominence Liquid Chromatograph (SHIMADZU CORP, Kyoto, Japan), a SiL-10 AF Auto Sampler (SHIMADZU CORP, Kyoto, Japan), a CTO - 10 Asvp Column Oven (SHIMADZU CORP, Kyoto, Japan) and an Alltech 3300 ELSD Detector (Grace Division Discovery Sciences, Australia). A Prevail Carbohydrate ES 5µ analysis column (250 mm x 4.6 mm) (Grace Division Discovery Sciences, Australia) and a Prevail Carbohydrate ES 5µ guard column (Grace Division Discovery Sciences, Australia) were used.

A multi-point calibration approach was applied to obtain standard calibration curves for the sugars. The range for the calibration was from 10 to 1000 ppm. The standards used for the sugars analysis were: D-Glucose (Merck, USA, ≥ 99.5%), Sucrose (FISONS, England, ≥ 99.0%), D- Fructose (Univar, New Zealand) and D-Sorbitol (Sigma-Aldrich, USA, ≥ 99.5%). Finally, chromatograms were registered per each sugar, and the final result was expressed in $mg.g^{-1}$ of fruit (fresh weight basis).

3.5 Variables

3.5.1 Variables Measured on Individual Fruit

Variables below were measured on individual fruit; however, the average per seedling was used for the statistical analysis.

1) Fruit weight (±0.1 g).
2) Fruit diameter (±0.01 mm): diameter at the widest point measured using callipers.
3) Fruit height (±0.01 mm): height of the fruit from the base of the stem to the calyx end, measured using callipers.
4) Fruit shape: categorised using the Plant and Food fruit shape chart (Appendix 4) based on the fruit height:diameter (H:D) ratio.

3.5.2 Variables Measured on Individual Seedlings

1) TCA (cm^{-2}).

2) Estimated yield (g): an estimate of the yield, was obtained multiplying the number of fruit per tree (after thinning) by the average weight of the fruit.

3) B content (µg/g^{-1}fruit, fresh weight basis): As detailed in Section 3.3.1.

4) Sugar content (mg/g^{-1}fruit, fresh weight basis): Glucose, sucrose, fructose and sorbitol contents were analysed as detailed in Section 3.3.2.

5) Percentage of the area affected by symptoms of FSD: the variables analysed were browning (proportion of the flesh affected by any category of browning, averaged over all the fruit), cavities (proportion of the flesh affected by any category of cavities, averaged over all the fruit) and unaffected by any disorder (proportion of the flesh unaffected by any disorder, averaged over all the fruit).

Different transformations (square root, log_{10} and trigonometric[1]) were applied to some variables to improve the distribution of the data before being analysed (Table 4).

Table 4. Transformations applied to the variables.

Traits	Units	Transformation
Production variables:		
Fruit weight	g	square root
Fruit diameter	mm	none
Fruit height	mm	log_{10}
Fruit shape	H:D ratio	none
TCA	cm^2	square root
Yield	g	Log_{10}
Chemical variables:		
B	$\mu g.g^{-1}$	Square root
Glucose	$mg.g^{-1}$	square root
Fructose	$mg.g^{-1}$	none
Sucrose	$mg.g^{-1}$	none
Sorbitol	$mg.g^{-1}$	square root
Total Sugars	$mg.g^{-1}$	none
Disorder variables:		
Unaffected area	%	trigonometric
Browning	%	trigonometric
Cavities	%	trigonometric

[1] If P is a percentage then the trigonometric transformation is arcsine(square-root(P/100)

3.6 Statistical Analysis

The linear mixed model approach was used to fit the plant model (Lynch and Walsh, 1998). The restricted maximum likelihood (REML) method was used to fit the linear mixed models, with the general mean as the only fixed effect. Two random effects models were tested: both had genotypes (seedlings) with pedigree information but one incorporates family to check for specific combining ability effects whereas the other did not. The likelihood ratio test (LRT) and graphical comparison (e.g., scatter plots of progeny means and mid-parental values) were carried out to compare the two models. Two types of analyses were carried out: univariate and bivariate (Lynch and Walsh, 1998). In matrix form, the model for the univariate analysis was $\mathbf{y}=\mathbf{X}\beta+\mathbf{Z}u+\mathbf{e}$ where y is a vector containing the phenotypic values for a variable measured in *n* individuals, β is a *p* x 1 vector of fixed effects, u is a *q* x 1 vector of random effects, **X** and **Z** are respectively *n* x *p* and *n* x *q* incidence matrices, and **e** is the *n* x 1 column vector of residual deviations assumed to be distributed independently of the random genetic effects. REML estimates of the variance components (Gilmour, Thompson & Cullis, 1995) for the random effects and empirical breeding values (eBV) were estimated directly from the model (asreml fits). Best linear unbiased predictors (BLUP) (Herderson, 1973) was used to estimate the eBV which were used as prediction of the relative genetic value in parents and progeny. A bivariate analysis was carried out to analyse the relationship between two variables simultaneously and obtaining a better prediction of the estimated genetic parameters. All pair-wise bivariate analysis was carried out: $\mathbf{y}_j=\mathbf{X}_j\beta_j+\mathbf{Z}_j\mathbf{u}_j+\mathbf{e}_j$ where there are q_j fixed effects associated with character *j* so that X_j and β_j have, respectively, dimensionality *n* x q_j and q_j x 1 for each trait, **u** is a q_j x 1 vector of random effects, **Z** is an incidence matrix, and **e** is the residual deviation. The variances from the univariate analysis were used to state initial values and an estimate of the residual variance covariance matrix was obtained by subtracting the genetic variance-covariance matrix from the phenotypic one.

Variance components obtained from univariate analysis were used to estimate narrow heritabilities: $h^2 = V_A/V_P = V_A/(V_A+V_R)$ where h^2 is the narrow heritability, V_A is the additive genetic variance and V_R is the residual variance (e.g., that attributable to non-additive genetic effects, environmental effects and procedural effects). Phenotypic correlations were computed as the Pearson's correlation coefficients between the observed data

(transformed where appropriate) in the univariate analysis; while they were estimated from the variance components in the bivariate analysis. Genetic correlations were approximated as the correlation between the eBV in the univariate analysis; whereas they were estimated from the additive genetic variance-covariance matrix obtained in the bivariate analyses: $r_G = Cov_{X,Y}/sqrt(V_X \cdot V_Y)$ where r_G is the genetic correlation, $Cov_{X,Y}$ is the covariance between both traits, Vx is the genetic variance of the first trait and V_Y is the genetic variance of the second trait. Standard errors for the estimates of heritabilities were obtained using the jackknife method (Buzas, 1997) which involved successively dropping each family from the calculations. This method was used because of the distribution of the data and its reliability.

The relative efficiency of indirect selection, where a breeder is interested in improving one trait Y but bases the selection decision on a different correlated trait X, is given by $|r_G| \cdot h_X/h_Y$ where r_G is the genetic correlation, h_X is the square root of the heritability of the trait to select for, and h_Y is the square root of the heritability of the other trait (Falconer, 1996). According to Piepho and Mohring (2007), the later way to estimate the selection response is not the best for an unbalanced design; however, Falconer's formula gives a good approximation. Since genetic correlation must be less than one, indirect selection is only more efficient than direct selection if $h_X > h_Y$ although there may be other reasons for choosing indirect selection (e.g., trait X may be cheaper to measure than trait Y or it may be possible to measure X considerably earlier in the process than Y).

All statistical analyses and graphs were carried out using R 2.13.0 (R Development Core Team, 2011), and the mixed models were fitted using the asreml-r package (Butler, 2009).

4. Results

Simple family means of the untransformed variables showed a high level of variability between the families for all variables (Tables 5a-5d). For the production variables the range in family means was between 32% (fruit diameter) and 165% (yield) of the mean of all families, for chemical variables this range was between 26% (total sugars) and 196% (sucrose) and for the disorders it was between 10% (unaffected area) and almost 500% (cavities).

Table 5a. Mean values per family for fruit weight, diameter and height. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom. Shape column corresponds to that most frequently observed. For description of the shapes see Appendix 4.

Family	Fruit Weight (g)	Fruit Weight (g)*	Fruit Diameter (mm)	Fruit Height (mm)	Fruit Height (mm)*	H:D Ratio	Shape
C1	132.3	11.48	63.10	61.37	1.79	0.98	convex
C2	170.2	12.80	67.15	69.93	1.84	1.04	convex
C3	139.5	11.70	63.76	67.90	1.83	1.06	straight
C4	148.9	12.06	65.07	69.23	1.83	1.06	straight
C5	203.2	14.04	70.03	**82.72**	**1.91**	1.18	straight
C6	125.1	11.13	60.83	65.42	1.81	1.08	convex
C7	106.8	10.27	56.94	65.80	1.81	1.15	straight
C8	134.9	11.47	62.41	60.82	1.78	0.96	convex
C9	146.8	12.04	65.28	63.73	1.80	0.98	straight
C10	147.8	12.12	63.93	71.69	1.85	1.12	straight
C11	143.6	11.73	63.56	66.30	1.81	1.03	straight
C12	119.6	10.79	57.54	73.36	1.86	1.28	straight
C13	**105.6**	**10.12**	**55.11**	70.94	1.85	1.30	straight
C14	142.5	11.82	62.20	70.77	1.85	1.13	straight
C15	133.1	11.38	59.46	77.67	1.89	**1.32**	straight
C16	**237.8**	**15.20**	**75.63**	74.90	1.87	0.99	convex
C17	121.5	10.89	61.09	**56.45**	**1.75**	0.92	convex
C18	196.6	13.95	72.30	66.34	1.82	0.92	convex
C19	167.7	12.90	67.32	67.59	1.83	1.01	convex
C20	178.2	12.88	68.20	61.23	1.78	**0.90**	convex
Mean	***150.1***	***12.04***	***64.05***	***68.21***	***1.83***	***1.07***	

* Transformed variables (see Table 4)

Table 5b. Mean values per family for TCA and yield. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	TCA (cm^2)	TCA (cm^2)*	Yield (g)	Yield (g)*
C1	30.14	5.46	5066	3.66
C2	20.54	4.40	3498	3.48
C3	30.77	5.45	5092	3.64
C4	28.51	5.27	4159	3.56
C5	**34.54**	**5.86**	7637	3.84
C6	31.65	5.53	4309	3.58
C7	28.31	5.07	4301	3.52
C8	**12.52**	**3.53**	**2814**	**3.43**
C9	22.90	4.73	6125	3.75
C10	22.49	4.66	5485	3.67
C11	13.20	3.57	3260	3.44
C12	21.28	4.56	3526	3.49
C13	17.79	4.19	3110	3.46
C14	30.18	5.46	7612	3.86
C15	19.60	4.35	4538	3.60
C16	30.31	5.36	**11124**	**3.95**
C17	22.61	4.68	5100	3.65
C18	19.76	4.43	5115	3.65
C19	12.60	3.54	4544	3.64
C20	19.19	4.27	4034	3.54
Mean	***23.44***	***4.72***	***5022***	***3.62***

* Transformed variables (see Table 4)

Table 5c. Mean values per family for chemical variables. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	B ($\mu g.g^{-1}$)	B ($\mu g.g^{-1}$)*	Glucose ($mg.g^{-1}$)	Glucose ($mg.g^{-1}$)*	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)*	Total Sugars ($mg.g^{-1}$)
C1	2.55	1.58	10.56	3.22	64.89	11.49	16.19	4.01	103.12
C2	2.78	1.65	12.26	3.48	64.28	9.65	25.73	4.99	111.92
C3	2.50	1.57	11.69	3.38	66.89	16.56	17.95	4.11	113.10
C4	2.88	1.68	**10.17**	**3.17**	75.12	12.51	19.53	4.29	**117.33**
C5	2.38	1.54	14.29	3.77	65.50	2.84	26.00	5.05	108.63
C6	3.58	1.88	16.02	3.95	**75.35**	5.86	18.46	4.20	115.68
C7	3.19	1.77	12.60	3.52	71.99	4.75	**15.84**	**3.91**	105.18
C8	3.05	1.74	13.82	3.67	56.44	**2.30**	18.13	4.22	**90.69**
C9	3.58	1.87	12.62	3.53	53.16	9.08	18.88	4.32	93.75
C10	3.06	1.72	12.81	3.54	63.96	7.55	23.57	4.79	107.88
C11	**4.67**	**2.11**	13.66	3.66	54.96	11.19	18.50	4.22	98.32
C12	4.54	2.12	13.16	3.60	55.03	12.56	**27.92**	**5.23**	108.68
C13	3.98	1.98	12.66	3.52	**50.08**	**17.91**	23.79	4.81	104.43
C14	4.22	2.02	12.59	3.53	50.63	6.22	25.90	5.07	95.34
C15	**2.33**	**1.52**	10.64	3.23	60.57	7.93	22.48	4.68	101.62
C16	3.36	1.81	17.99	4.22	67.15	4.54	24.94	4.93	114.62
C17	3.47	1.85	18.07	4.23	63.72	2.88	23.62	4.77	108.30
C18	4.04	2.00	18.04	4.17	55.16	3.02	16.37	4.00	92.59
C19	3.08	1.74	**18.89**	**4.33**	56.38	6.86	23.64	4.81	105.77
C20	3.83	1.95	15.71	3.92	56.00	3.41	17.34	4.10	92.46
Mean	***3.35***	***1.80***	***13.91***	***3.68***	***61.36***	***7.96***	***21.24***	***4.53***	***104.47***

* Transformed variables (see Table 4)

Table 5d. Severity and incidence mean values per family for disorder variables. Percentage of incidence was calculated according to the presence or absence of symptoms in each seedling per family, values are independent to each other. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

	Percentage of the fruit affected by symptoms (Severity)						Incidence (%)		
Family	Unaffected Area	Unaffected Area *	Browning	Browning *	Cavities	Cavities *	Unaffected Area	Browning	Cavities
C1	97.67	1.42	2.27	0.1512	0.06	0.0245	20	**80**	40
C2	96.82	1.39	3.11	0.1773	0.02	0.0141	70	30	20
C3	**89.95**	**1.25**	**9.63**	**0.3155**	0.18	0.0424	47	47	27
C4	90.84	1.26	9.09	0.3063	0.02	0.0141	47	47	33
C5	95.09	1.35	4.72	0.2190	**0.003**	**0.0055**	57	29	14
C6	97.31	1.41	2.27	0.1512	0.02	0.0141	55	18	46
C7	99.75	1.52	0.24	0.0490	0.01	0.0100	50	50	**13**
C8	99.51	1.50	0.36	0.0600	0.13	0.0361	60	40	40
C9	99.32	1.49	0.60	0.0775	0.08	0.0283	47	20	53
C10	99.73	1.52	0.22	0.0469	0.05	0.0224	67	13	33
C11	92.87	1.30	6.98	0.2674	0.14	0.0374	50	30	50
C12	98.96	1.47	0.65	0.0807	0.39	0.0625	44	31	56
C13	95.12	1.35	4.38	0.2108	**0.50**	**0.0708**	43	29	50
C14	99.91	1.54	**0.00**	**0.0000**	0.09	0.0300	**88**	**0**	**13**
C15	99.81	1.53	0.11	0.0332	0.08	0.0283	63	25	38
C16	97.33	1.41	2.65	0.1635	0.01	0.0100	67	13	27
C17	**99.94**	**1.55**	0.03	0.0173	0.03	0.0173	67	7	27
C18	99.58	1.51	0.36	0.0600	0.06	0.0245	**17**	50	67
C19	95.71	1.36	4.19	0.2062	0.10	0.0316	**17**	50	**83**
C20	95.78	1.36	4.16	0.2054	0.06	0.0245	43	29	57
Mean	***97.05***	***1.42***	***2.80***	***0.1399***	***0.10***	***0.0274***	***51***	***32***	***39***

* Transformed variables (see Table 4)

In addition, the percentage of incidence of cavities, browning and unaffected area was calculated according to the presence or absence of symptoms in each seedling per family (Table 5d), taking into consideration that fruit from different seedlings showed both browning and cavities together or independently, and some fruit showed none of them. Therefore, percentages do not add up 100% because all are independent of each other.

Rank correlations between severity and incidence were moderately positive (0.41, 0.57 and 0.59 for browning, cavities and non-affected area respectively), indicating variability depending on each family. However, a few families showed low percentages of severity and incidence in both browning and cavities (Figure 7a and 7b). Families C10, C14 and C17 were among the best families for both incidence and severity of browning, and C2, C5, C7 and C16 were among the best for both incidence and severity of cavities.

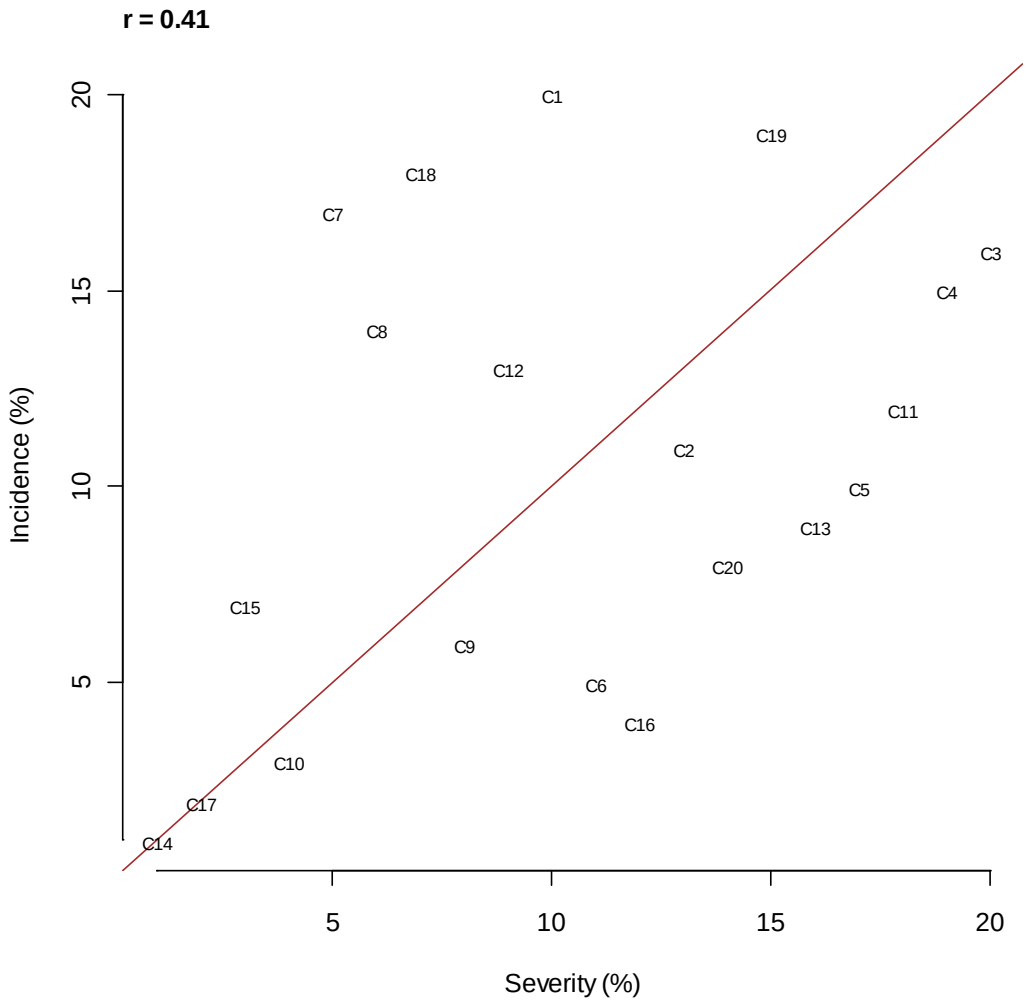

Figure 7a. Scatter plots showing the ranks for each family with regard to incidence of the disorder on the ranks for severity for browning. The rank correlation is given at the top left of each plot, and the points are represented by the family identification.

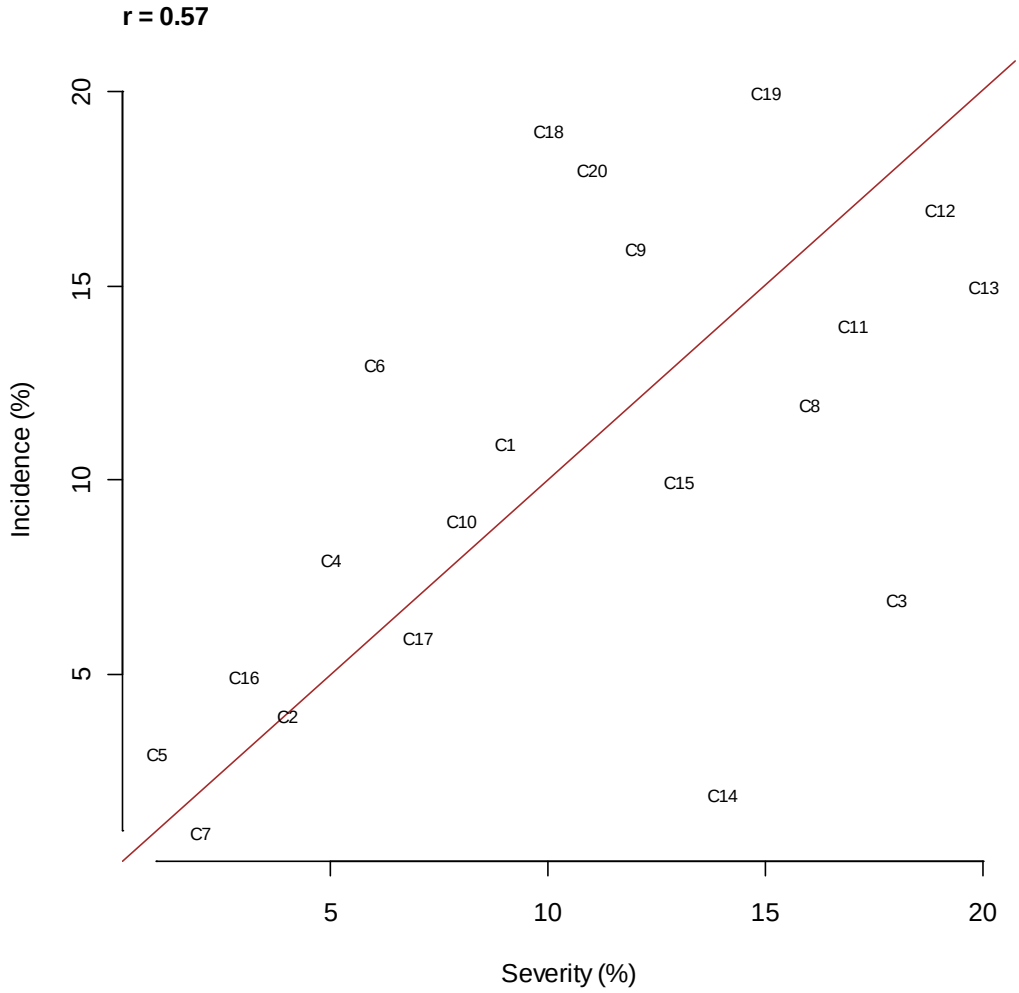

Figure 7b. Scatter plots showing the ranks for each family with regard to incidence of the disorder on the ranks for severity for cavities. The rank correlation is given at the top left of each plot, and the points are represented by the family identification.

4.1 Univariate Analyses

4.1.1 Random Effects

For most variables, the likelihood ratio test indicated that the simpler model (e.g., that without family in the random effects) was adequate (P>0.05). However, for fructose, sucrose, sorbitol and total sugars, the LRT indicated that including family resulted in a statistically significant improvement (P = 0.005, 0.008, 0.023 and 0.020 respectively). However, even for these four variables, there was a strong correlation between mid-parent eBV and the progeny mean eBV when the model excluding family from the

random effects was used (Figure 8). Therefore, the simple model (without family) was deemed adequate for the analysis of all variables.

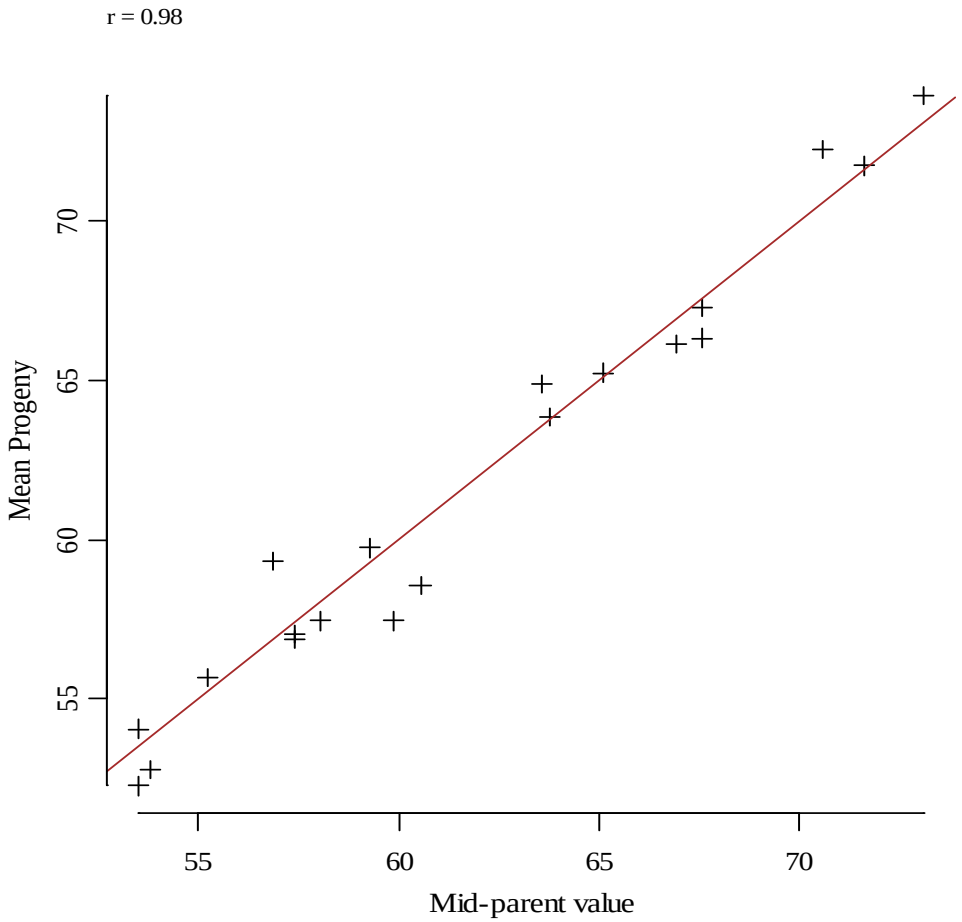

Figure 8. Scatter plot showing the relationship between the fructose eBV for the progeny means versus the mid-parent eBV for each family using the model without family in the random effects. The brown line is that of equality, and the correlation coefficient is shown at the top left.

4.1.2 Empirical Breeding Values

Empirical breeding values (Figures 9a-9e) were estimated for the parents of the families. For the production variables the range (from untransformed data) was between 41% (fruit height) and 128% (yield) of the eBV mean of all parents; for chemical variables this range was between 21% (total sugars) and 281% (sucrose); and for the disorders it was between 9% (unaffected area) and almost 500% (cavities).

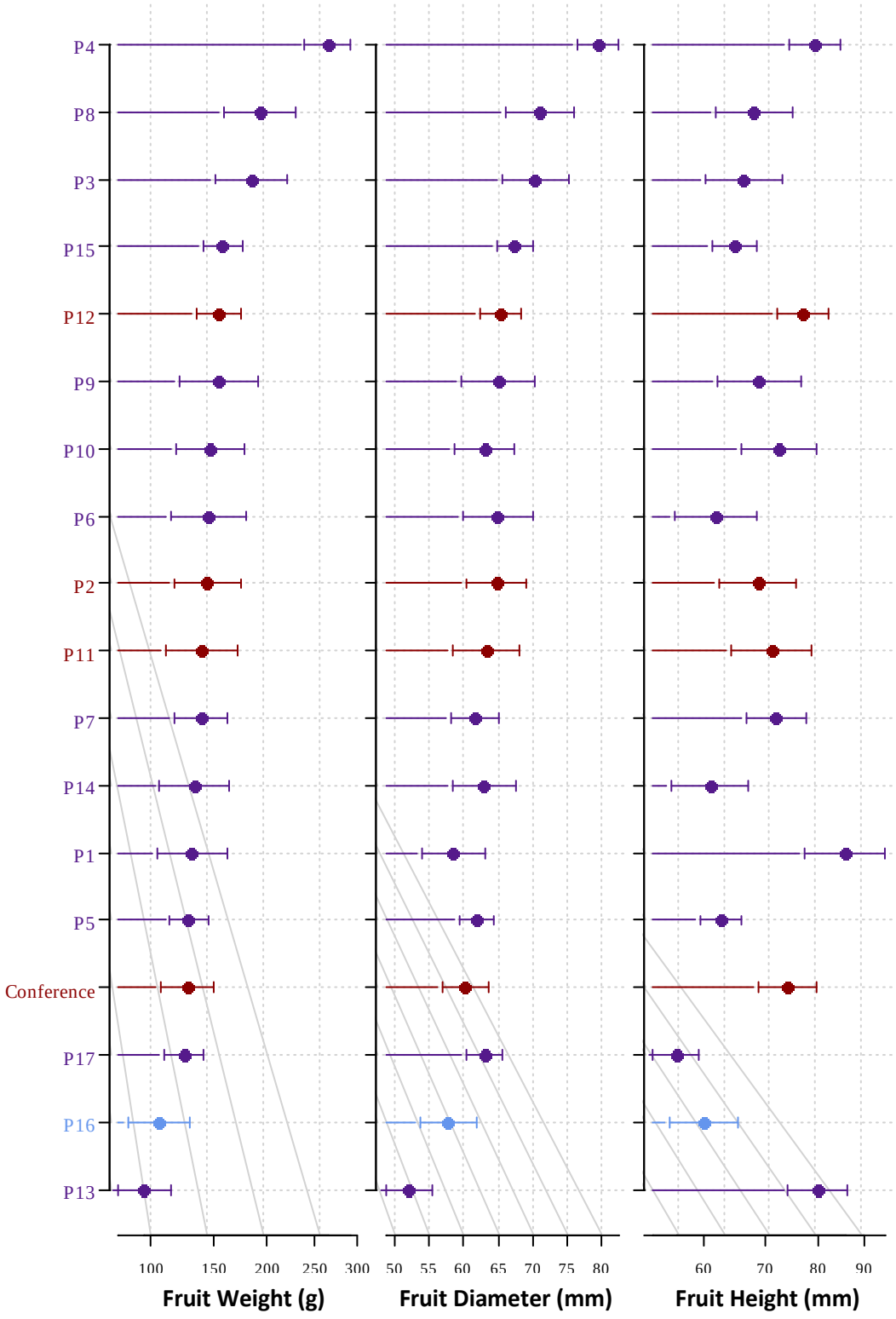

Figure 9a. Parent eBV for fruit weight, diameter and height. Bars show the standard error. Production variables are sorted by fruit weight. Scale is based on back transformed data for weight and height. Colours represents the parentage: 100% Asian (blue), 100% European (red) and different percentages of Asian and European (purple).

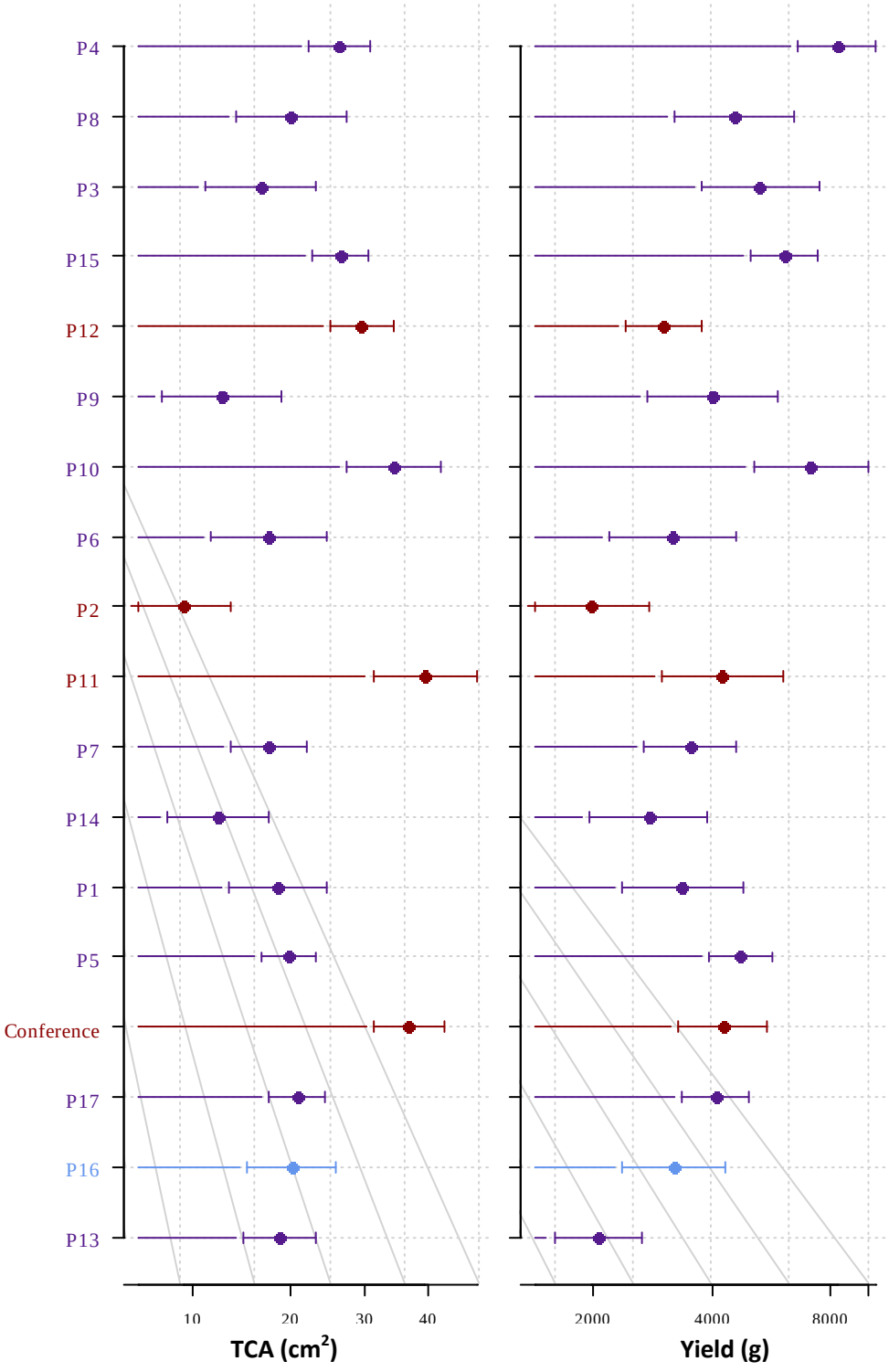

Figure 9b. Parent eBV for TCA and yield. Bars show the standard error. Production variables are sorted by fruit weight. Scale is based on back transformed data for both variables. Colours represents the parentage: 100% Asian (blue), 100% European (red) and different percentages of Asian and European (purple).

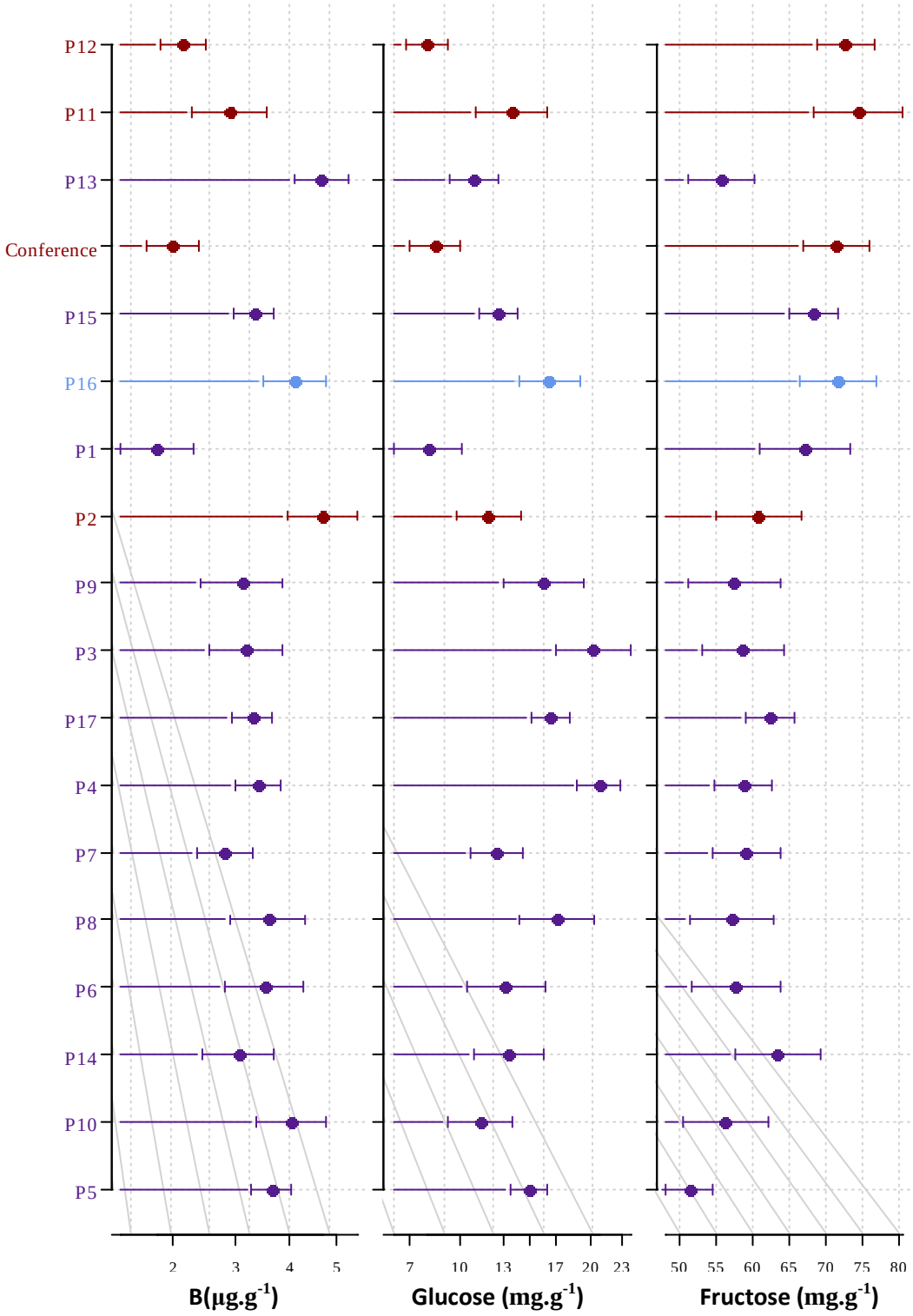

Figure 9c. Parent eBV for B, glucose and fructose. Bars show the standard error. Chemical variables are sorted by total sugars. Scale is based on back transformed data for B and glucose. Colours represents the parentage: 100% Asian (blue), 100% European (red) and different percentages of Asian and European (purple).

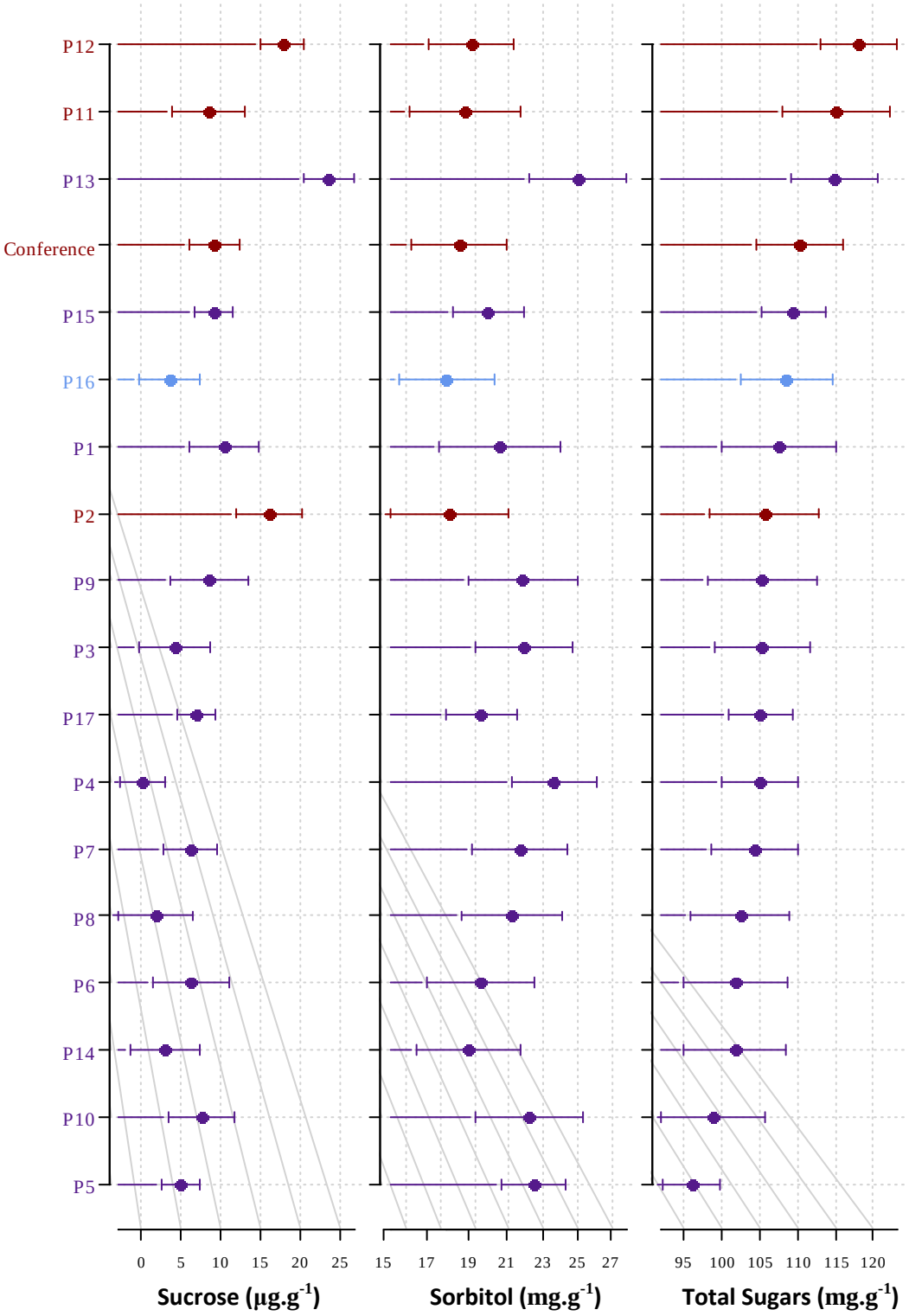

Figure 9d. Parent eBV for sucrose, sorbitol and total sugars. Bars show the standard error. Chemical variables are sorted by total sugars. Scale is based on back transformed data for sorbitol. Colours represents the parentage: 100% Asian (blue), 100% European (red) and different percentages of Asian and European (purple).

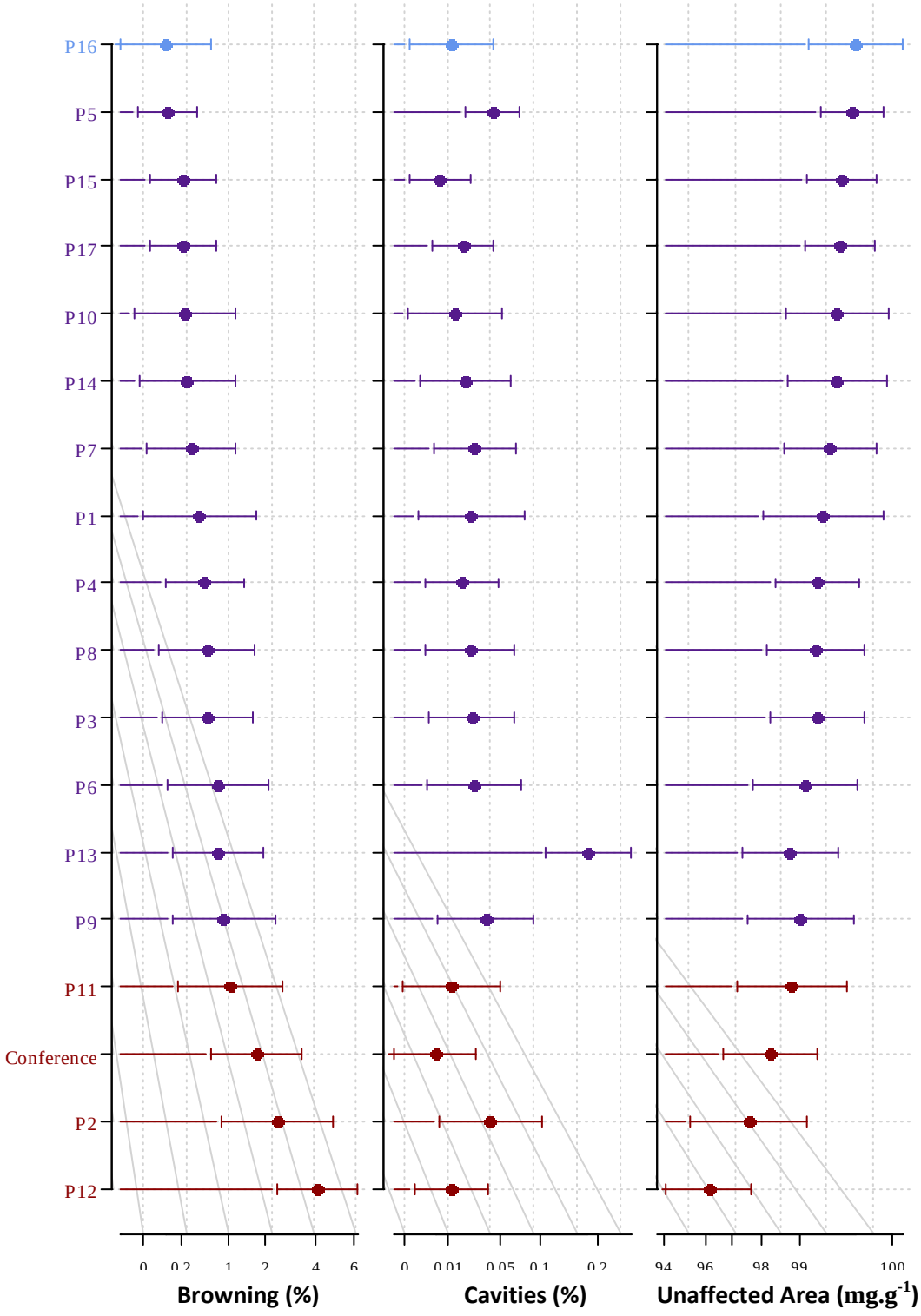

Figure 9e. Parent eBV for disorder variables. Bars show the standard error. These variables are sorted by browning. Scale is based on back transformed data for all the variables. Colours represents the parentage: 100% Asian (blue), 100% European (red) and different percentages of Asian and European (purple).

4.1.3 Variance Components and Heritabilities

Values for genetic variances were superior to the environmental ones for most of the variables except for sorbitol, total sugars and the disorders variables (Table 6). Heritability values (Table 6) varied widely from 0.15 (unaffected area) to 0.91 (TCA). When seedling eBV and seedling means (raw data) were plotted, most of the variables showed low shrinkage (e.g., Figure 10a) which was reflected in their high or relatively high heritability and also indicates that the data showed a normal distribution; whereas the degree of shrinkage was higher for the variables with low heritability, indicating that the data did not showed a normal distribution (e.g., Figure 10b where data showed skewed distribution). Furthermore, a linear aggregation of the families was observed in the scatter plots.

Table 6. Variance components and heritabilities for the variables evaluated in the pear population. Heritability minimum and maximum values for each trait in the whole population (20 families) are shown.

	Variances			**Heritability (h^2)**			
Variables	**Phenotypic**	**Genetic**	**Environmental**	**h^2**	**s.e.**	**Min**	**Max**
Fruit Weight*	5.896	3.987	1.909	0.68	0.27	0.44	0.78
Fruit Diameter	79.02	62.67	16.35	0.79	0.23	0.61	0.89
Fruit Height*	0.00736	0.00433	0.00303	0.59	0.36	0.27	0.69
TCA*	1.659	1.511	0.148	0.91	0.33	0.75	0.98
Yield*	0.0876	0.0588	0.0288	0.67	0.27	0.49	0.77
B*	0.1244	0.0900	0.0344	0.72	0.24	0.52	0.78
Glucose*	0.4429	0.3725	0.0704	0.84	0.2	0.74	0.94
Fructose	145.6	78.63	66.96	0.54	0.24	0.49	0.74
Sucrose	70.57	53.67	16.91	0.76	0.27	0.66	0.96
Sorbitol*	0.8260	0.1471	0.6788	0.18	0.17	0.07	0.26
Total Sugars	274.4	83.86	190.6	0.31	0.31	0.23	0.57
Unaffected Area*	0.03046	0.00464	0.02582	0.15	0.12	0.09	0.22
Browning*	0.02996	0.00527	0.02469	0.18	0.12	0.11	0.24
Cavities*	0.000935	0.000182	0.00753	0.19	0.09	0.14	0.22

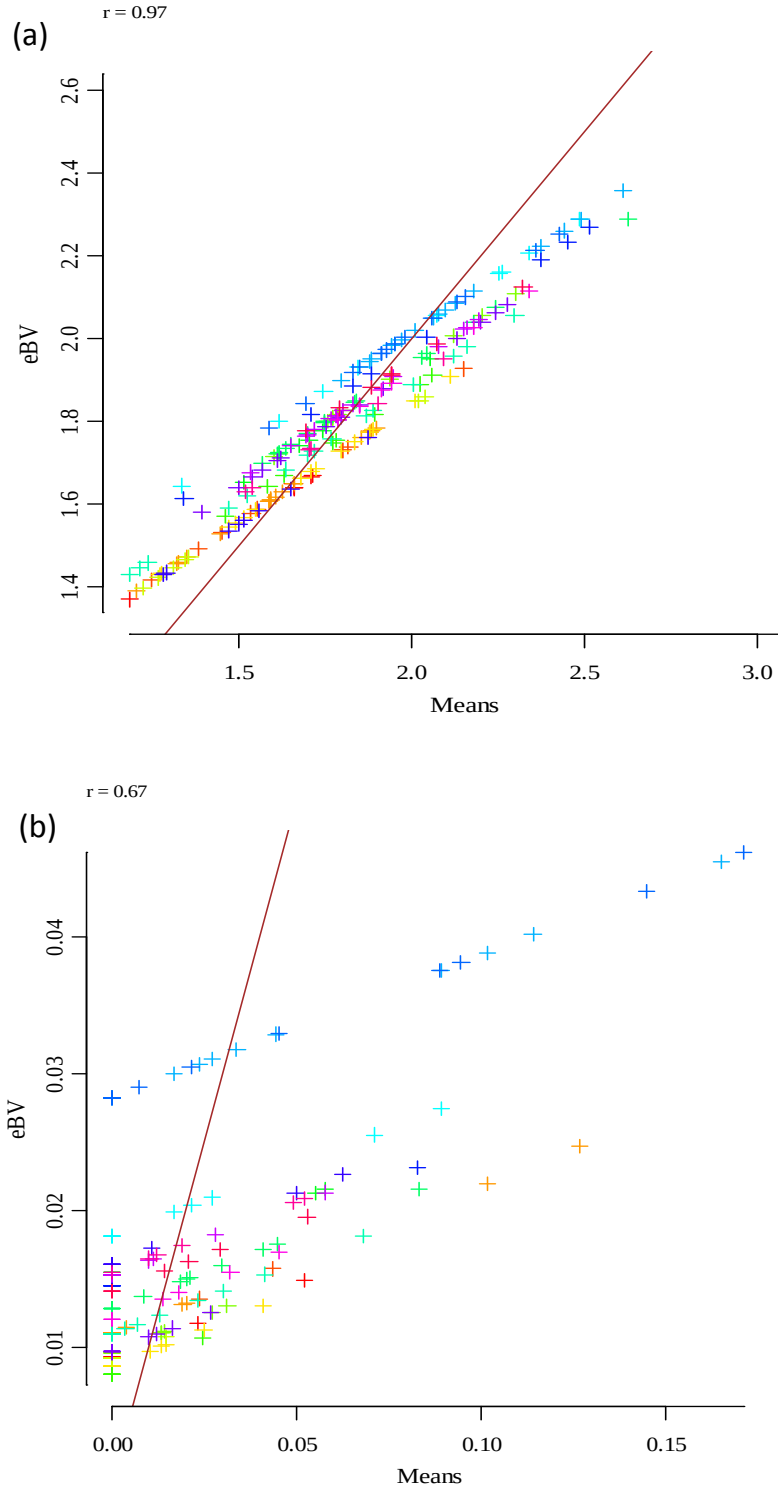

Figure 10. Scatter plots showing the seedling eBV with regard to seedling means for (a) B and (b) cavities. The rank correlation is given at the top left of each plot. The red line shows the shrinkage degree and the different colours represent the 20 families evaluated.

4.1.4 Genetic and Phenotypic Correlations

In most of the cases (74%), genetic correlations (Table 7) were larger than the phenotypic ones. The two sets of correlations showed a relatively high correlation (0.72) (Figure 11). The high positive genetic correlations (0.97) was obtained between fruit weight and fruit diameter whereas the high negative genetic correlation (-0.99) were obtained between browning and the unaffected area.

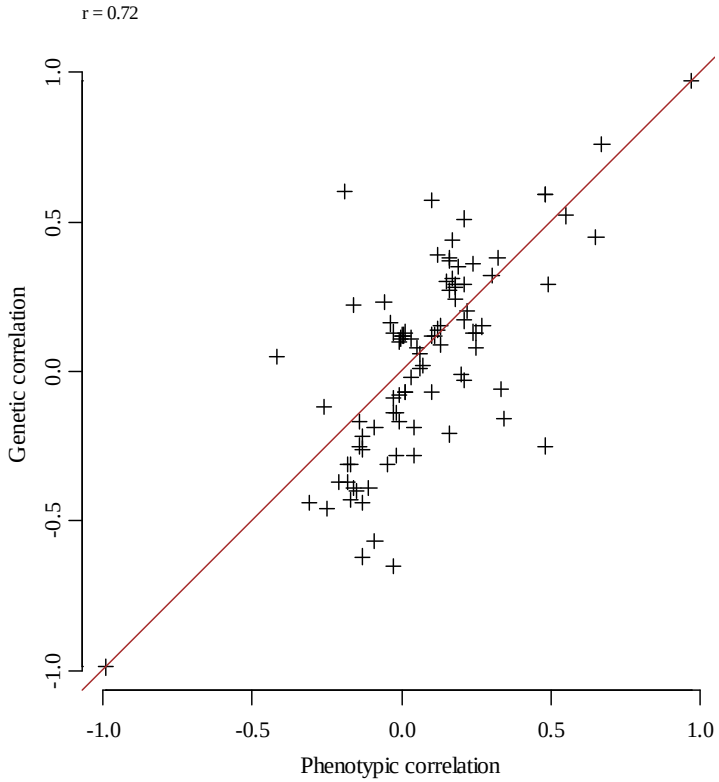

Figure 11. Scatter plot showing the relationship between genetic versus phenotypic correlations. The brown line is that of equality, and the correlation coefficient is shown at the top left.

4.1.5 Efficiency of the Indirect Selection

The ratio for the correlated response between fructose and the occurrence of cavities was 1.04. Thus, this ratio (greater than 1.0) confirmed the efficiency of the indirect selection by fructose.

Table 7. Genetic correlations (upper triangle) and phenotypic correlations (lower triangle) between all the pair traits estimated from the univariate analysis.

	Fruit Weight*	Fruit Diameter	Fruit Height*	TCA*	Yield*	B*	Glucose*	Fructose	Sucrose	Sorbitol*	Total Sugars	Unaffected Area*	Browning*	Cavities*
Fruit Weight*	**1.00**	0.97	0.45	0.14	0.59	-0.14	0.27	0.17	-0.31	0.08	0.13	-0.07	0.11	-0.39
Fruit Diameter	0.97	**1.00**	0.29	0.15	0.59	-0.14	0.29	0.20	-0.31	-0.01	0.13	-0.07	0.12	-0.44
Fruit Height *	0.65	0.49	**1.00**	0.12	0.15	-0.09	-0.19	0.09	0.13	0.32	0.29	-0.19	0.16	0.12
TCA*	0.12	0.13	0.10	**1.00**	0.52	-0.22	0.02	0.35	0.01	-0.17	0.31	-0.08	0.11	-0.37
Yield*	0.48	0.48	0.27	0.55	**1.00**	-0.17	0.28	0.06	-0.31	0.12	-0.07	0.30	-0.25	-0.40
B *	-0.02	-0.03	-0.03	-0.13	-0.14	**1.00**	0.24	-0.46	0.08	0.38	-0.28	0.23	-0.28	0.51
Glucose *	0.16	0.18	-0.09	0.07	0.18	0.18	**1.00**	-0.03	-0.37	0.13	-0.06	0.22	-0.21	-0.02
Fructose	0.21	0.22	0.13	0.19	0.06	-0.25	0.21	**1.00**	-0.12	-0.65	0.76	-0.39	0.44	-0.62
Sucrose	-0.17	-0.18	-0.03	0.06	-0.05	0.05	-0.21	-0.26	**1.00**	0.10	0.36	-0.43	0.37	0.39
Sorbitol *	0.25	0.20	0.30	-0.01	0.11	0.16	0.01	-0.03	-0.01	**1.00**	-0.25	0.38	-0.44	0.60
Total Sugars	0.25	0.24	0.21	0.17	0.10	-0.02	0.33	0.67	0.24	0.48	**1.00**	-0.57	0.57	-0.26
Unaffected Area*	0.01	0.01	0.04	-0.01	0.15	-0.06	-0.16	-0.16	-0.17	0.32	-0.09	**1.00**	-0.99	0.05
Browning*	-0.004	0.003	-0.04	0.03	-0.14	0.04	0.16	0.17	0.16	-0.31	0.10	-0.99	**1.00**	-0.16
Cavities*	-0.11	-0.13	0.004	-0.18	-0.15	0.21	0.03	-0.13	0.12	-0.19	-0.13	-0.42	0.34	**1.00**

* Transformed variables (see Table 4)

4.2 Bivariate Analyses

4.2.1 Genetic and Phenotypic Correlations

Atypical results were obtained for genetic and phenotypic correlations in the bivariate analysis (Table 8). For instance, values close to 1.0 were obtained in a few variables such as those between fruit weight and fruit diameter, and between fructose and cavities. For 17 pairs of variables, the bivariate analysis failed to converge, even after setting sensible initial estimates. Furthermore, when genetic and phenotypic correlation coefficients obtained from the variance components were plotted, almost no correlation (0.02) and an atypical dispersion was observed (Figure 12), confirming the abnormality of these results.

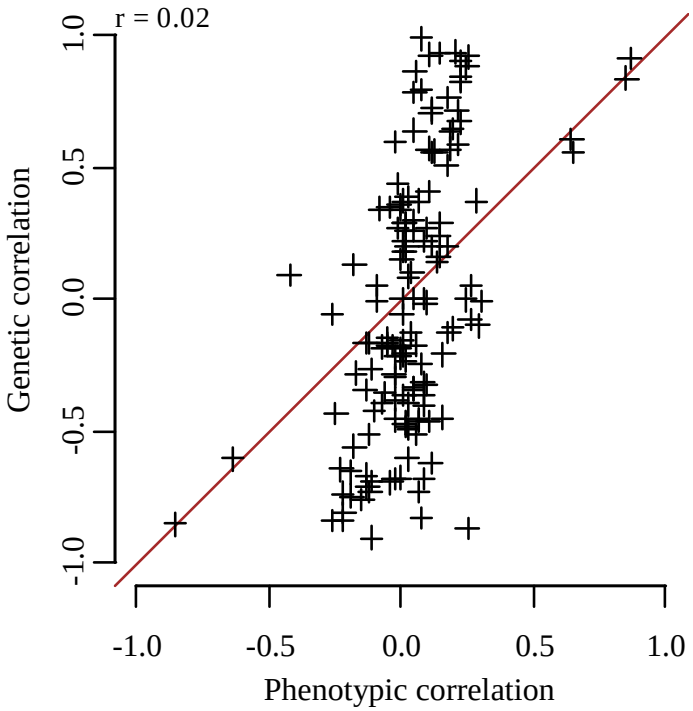

Figure 12. Scatter plot showing the relationship between genetic versus phenotypic correlations. The brown line is that of equality, and the correlation coefficient is shown at the top left.

Table 8. Genetic correlations (upper triangle) and phenotypic correlations (lower triangle) between all the pair variables estimated from the bivariate analysis. The values that did not converge are shown in grey.

	Fruit Weight*	Fruit Diameter	Fruit Height*	TCA*	Yield*	B*	Glucose*	Fructose	Sucrose	Sorbitol*	Total Sugars	Unaffected Area*	Browning*	Cavities*
Fruit Weight*	**1.00**	0.98	0.76	0.34	0.76	-0.20	0.53	0.18	-0.44	0.61	0.15	-0.16	0.22	-0.66
Fruit Diameter	0.97	**1.00**	0.64	0.28	0.67	-0.12	0.57	0.22	-0.46	-0.17	0.13	-0.19	0.26	-0.76
Fruit Height *	0.76	0.64	**1.00**	0.40	0.28	-0.48	-0.45	-0.001	0.41	0.81	0.44	-0.36	0.27	0.18
TCA*	0.17	0.15	0.19	**1.00**	0.77	-0.23	-0.03	0.64	-0.17	0.37	0.49	0.38	-0.35	-0.84
Yield*	0.51	0.48	0.28	0.64	**1.00**	-0.21	0.32	0.13	-0.75	0.48	-0.18	0.65	-0.56	-0.81
B *	-0.01	0.15	-0.15	-0.10	-0.15	**1.00**	0.43	-0.61	0.10	0.08	-0.47	0.35	-0.48	0.88
Glucose *	0.23	0.26	-0.18	0.08	0.18	0.24	**1.00**	-0.02	-0.68	-0.03	0.51	-0.46	-0.68	-0.22
Fructose	0.20	0.20	0.12	0.20	0.06	-0.24	0.25	**1.00**	-0.11	-0.67	0.89	-0.17	0.25	-0.90
Sucrose	-0.18	-0.19	0.51	0.01	-0.13	0.05	-0.24	-0.25	**1.00**	0.46	0.55	-0.83	0.72	0.77
Sorbitol *	0.36	0.19	0.37	0.01	0.17	0.11	-0.01	-0.09	0.03	**1.00**	0.83	0.88	-0.19	0.41
Total Sugars	0.25	0.23	0.23	0.18	0.07	-0.04	0.42	0.75	0.27	0.66	**1.00**	-0.88	0.92	-0.05
Unaffected Area*	0.003	-0.01	0.01	0.07	0.19	-0.05	-0.28	-0.14	-0.22	0.41	-0.11	**1.00**	-0.99	0.09
Browning*	0.01	0.16	-0.02	-0.06	-0.18	0.02	0.09	0.14	0.21	-0.30	0.11	-0.99	**1.00**	0.82
Cavities*	-0.13	-0.15	0.16	-0.26	-0.22	0.25	-0.01	-0.11	0.18	0.004	-0.09	-0.42	0.49	**1.00**

* Transformed variables (see Table 4)

5. Discussion

This chapter will mainly discuss the results generated from the univariate analysis for the different variables: production, chemical and disorders. In terms of the chemical variables, individual results for B and sugars are discussed. In relation to the disorder symptoms, the influence of B, sorbitol and fructose is a particular focus. Finally, the utility of eBV for further selection, and suggestions for initial selection methods depending on the disorder (browning and cavities) are covered.

5.1 Statistical Approach

5.1.1 Family

The pear seedlings evaluated in this study constituted a breeding population. All the parents were related to at least one of the others and 58% of possible pairings showed some degree of relationship. Thus, almost all the families assessed had some degree of relationship between them, except family C20 with families C6 and C7. Although only six families were not inbred, the level of inbreeding of the others was generally low (median inbreeding coefficient = 0.10).

The non-significance obtained by incorporating family as a random effect indicates that specific combining effects were unimportant for breeding which may suggest that most of the genetic variance is additive. This validates the use of eBV in assessing parents and progeny.

For all variables examined, at least one of the top ranked parents was a parent of the top ranked families and similarly for the bottom ranked parents and families.

5.1.2 Univariate versus Bivariate Analyses

It is generally considered better to estimate genetic correlations from bivariate rather than univariate analyses (Lu *et al*., 2001). However, with the models fitted to this pear data the genetic correlations from the bivariate analyses seemed improbable. For example, there were many instances where the magnitude of the genetic correlations was close to unity even though the phenotypic correlations were negligible. In some

cases the apparent unreliability of the bivariate analyses could be explained by convergence issues or poor residual plots. However, in other instances, this was not so and the reason for the suspect values from these bivariate analyses requires further study.

When the univariate analyses were used to estimate genetic correlations, the relationship between the genetic and phenotypic correlations was as might be expected with 74% of the genetic correlations being of greater magnitude than the corresponding phenotypic ones (Waitt & Levin, 1998). Therefore, the genetic parameters (variance, heritability, genetic and phenotypic correlation and eBV) discussed in this chapter will be those estimated from the univariate analysis.

5.2 Production Variables

Genetic variability was observed in all the production variables, which is useful for breeding. Environmental variances were low for these variables; thus, there was not a substantial influence of things like harvest time, soil variation, neighbour-tree effects (e.g. competition).

There is considerable variability for fruit size in *Pyrus* (Bell *et al*., 1996). Among the most important production variables, parents P4 and P8 showed eBV over 200 g for fruit weight and over 70 mm for diameter. Parents showed a diameter range from 52 to 79 mm; however, many genotypes of *P. communis* and *P. pyrifolia* can exceed 120 mm (Bell *et al*., 1996). For yield, parents P4 and P10 showed eBV over 8000 g.

The most common shape of Asian pears is round and most European pears are pyriform (Bell *et al*., 1996). However, their segregating populations usually show round and oblate shapes, suggesting that this shape is dominant over pyriform and turbinate shapes (Zielinski *et al.*, 1965; Wang & Wei, 1987, both as cited in Hancock & Lobos, 2008). In this study, convex (round) and straight (pyriform) shapes were observed in similar frequencies in families which had greater than 50% Asian parentage while the straight shape was more common for families which had mostly European parentage. Overall, 45% of the families evaluated predominantly showed convex shape whereas the remaining 55% showed straight shape.

In this study, all the production variables had relatively high heritabilities (more than 0.5). These results confirm those obtained by other researchers. For instance, high heritabilities have been reported for fruit weight in pear (Abe *et al.*, 1993; Abe *et al.*, 1995; Shin, *et al*., 2008) and other fruit crops (Durel *et al*., 1998; Brettell, *et al*, 2004) and also for fruit shape (White *et al*., 2000a). Inheritance of pear size and fruit shape has been reported to be under polygenic control (Crane & Lewis 1949; Zielinski *et al.*, 1965, Shen *et al*., 1979; Wang & Wei, 1987; White and Alspach, 1996, all as cited in Hancock & Lobos, 2008). Moreover, fruit set, yield and environmental factors such as water availability can influence pear size (Hancock & Lobos, 2008).

One of the characteristic features of quantitative variables is the commonly experienced association of phenotypic values for different characters (Bos & Caligari, 2008). Significant phenotypic and genetic correlation among fruit weight and other quantitative and qualitative variables have been reported in pear and other fruit crops (Abe *et al.*, 1993; Abe *et al.*, 1995; Lavi *et al.,* 1998). In this study, both phenotypic and genetic correlations were relatively high (more than 0.5) and positive between fruit weight, fruit diameter and yield.

TCA is used by researchers and growers to regulate and estimate crop load. High phenotypic and genetic correlations (around 0.7) were observed between TCA and yield. This result is related to the thinning carried out in the trees to standardise the crop load.

5.3 Chemical Variables

Limited information is available concerning the content of minerals and sugars in pears, especially in the fruit. Moreover, range values for adequate concentration of minerals in pear fruit are not available. Most of the chemical variables (except for sorbitol and total sugars) showed genetic variability and relatively high heritabilities (more than 0.5).

5.3.1 Boron

Among the minerals, B was the focus of this study. This mineral is required for cell wall synthesis, lignification and cell wall structure (Hansch & Mendel, 2009). Buwalda & Meekings (1990) found that B concentration in pear fruit decreased while B accumulation increased during the period of fruit development. Parents P2, P13, P16 and P10 showed

the highest eBV (around 4 $\mu g.g^{-1}$) while Conference and P1 showed the lowest B eBV (closer to 2 $\mu g.g^{-1}$). Yokomizo (1977) reported B content around 8 ppm in fruit flesh of two pear varieties ('Chojuro' and 'Kikusui') which were not supplied with B fertilisers. This is considerably more than the family means reported herein, although some individual seedlings had B contents around 9 ppm (e.g., comparable to Yokomizo, 1977). Although soil fertilization (25 kg.ha of B as Borate) was applied in the pear seedling, Motueka's soils are characterised by low natural B and a tendency to bind this mineral into forms which are not accessible for the plant (During, 1972).

The exact cellular allocation (cell walls or cytoplasm) of the B in the fruit was not determined. Consequently, B measurements corresponded to total B accumulated in the fruit.

B is unique amongst all essential minerals in that species vary considerably in their ability to retranslocate within the plant. It is supplied to fruit tissue primarily by the phloem and its movement in the phloem occurs irrespective of the source (soil, solution or foliar) (Brown & Shelp, 1997). There is evidence of a genetic basis for the ability of plants to translocate B during the reproductive growth phase (Brown & Shelp, 1997). Brown and Hu (1996) and Hu *et al*. (1997) suggested that sorbitol facilitates phloem B transport by the formation of B-sorbitol complexes in rosaceous trees. In this study, B and sorbitol showed a positive genetic correlation (0.38) which would be expected given that this alcohol-sugar is a carrier of B (Hu *et al*., 1997; Brown & Shelp, 1997). Brown and Shelp (1997) also suggested that the production and distribution of polyols (alcohol-sugars) and, consequently B-mobility, are influenced by environmental and phenological factors. In addition, it has been reported that tobacco plant genetically modified to produce sorbitol showed a significantly greater rate of B uptake, suggesting that the expression of sorbitol is enough to influence the metabolism of B (Brown & Shelp, 1997).

5.3.2 Sugars

Sugars and their signalling have regulatory functions related to growth, development and cellular activity in plants (Rolland *et al*., 2006; Yamaki 2010). Sorbitol occurs in leaves and is loaded into phloem and then translocated to the fruit (Hirai, 1979) where it is unloaded into the parenchyma tissue (Yamaki, 2010). Sorbitol is actively converted into other

sugars after its unloading in the fruit (Moriguchi *et al*., 1990; Yamaki & Moriguchi, 1989, both as cited in Moriguchi *et al*., 1992). Its conversion to fructose or glucose is facilitated by the action of the enzyme sorbitol dehydrogenase (Hirai, 1979; Kanayama, 2009). Sorbitol, sucrose, fructose and glucose are the major storage sugars in mature pear fruit (Kajiura *et al*., 1979; Yamiki & Moriguchi, 1989).

Sugar accumulation is important for sweetness and fruit quality (Yamaki, 2010). The genetic variability observed in this research for the different sugars will allow the selecting and recombining of these desirable variables which contribute to the tastiness of the fruit and are appropriate for diabetic nutrition, especially fructose and sorbitol (Hudina & Stampar, 2000b).

High sorbitol eBV were showed by parents P13 and P4 (around 24 $mg.g^{-1}$) while parents which showed the lowest eBV (close to 19 $mg.g^{-1}$) were P2 and P16.

Sorbitol showed a relatively high negative genetic correlation (-0.65) with fructose but little with glucose (0.13). The correlation between sorbitol and fructose could indicate that there was effective sorbitol dehydrogenase activity. Moreover, it could be suggested that the main product of sorbitol conversion was fructose, as has been reported in apple (Beruter, 2004) and other fruit crops (Bieleski & Redgwell, 1980, as cited in Hu *et al*., 1997). Yamaki and Moriguchi (1989) found that the conversion of translocated sorbitol to fructose in Japanese pear fruit showed a fluctuating pattern. It was high in young fruit, decreased with fruit enlargement and increased again with fruit maturation.

Another translocated sugar towards the fruit is sucrose. It is generally generated in leaves, translocated to fruit flesh through the phloem and unloaded into the parenchyma tissue (Yamaki 2010). However, its accumulation in fruit has different sucrose-metabolic pathways (Yamaki, 2010). The highest parental eBV for sucrose (over 16 $mg.g^{-1}$) were registered by P2, P12 and P13 while the lowest eBV (less than 3.5 $mg.g^{-1}$) were shown by P4, P14 and P8.

Kajiura *et al*. (1979) defined some varieties of Japanese pear as high-sucrose-accumulating types and some varieties of Chinese pear as low-sucrose-accumulating types. In this study, two of the parents (P2 and P12) which showed the highest eBV for sucrose had 100% European percentage but parent P13 had more Chinese than Japanese parentage. On the other hand, one of the parents which showed the lowest eBV for sucrose had more Chinese parentage (P4), another one had more Japanese parentage

(P14) and the third one (P8) had the same proportion of Japanese and Chinese parentage. Therefore, the tendency observed by Kajiura *et al*. (1979) cannot be applied to these results.

Sucrose is also converted into fructose and glucose by the action of invertase enzyme (Rolland *et al*., 2006; Yamaki, 2010). Sucrose showed a negative phenotypic and genetic correlation with glucose (-0.21 and -0.37 respectively) which would be expected given that when the amount of glucose increases in the fruit, sucrose decreases due to its conversion. Phenotypic and genetic correlations with fructose, whilst still negative (-0.26 and -0.12 respectively), were minor which is consistent with the highly negative correlation between sorbitol and fructose which indicates that much of the fructose originated from sorbitol.

Fructose influences the perception of sweetness in the fruit (Itai *et al*., 2010). Parents P16, P11, P12 and Conference obtained the highest eBV (over 75 $mg.g^{-1}$) for this sugar whereas P5 showed the lowest eBV (around 50 $mg.g^{-1}$).

In terms of glucose, the highest eBV (over 17 $mg.g^{-1}$) were registered by parents P4 and P3 while the lowest eBV (around 7 $mg.g^{-1}$) were showed by Conference, P1 and P12.

Moriguchi *et al*. (1992) and Hudina and Stampar (2000b) assessed different cultivars of European, Japanese and Chinese pears for sugar content. In comparison to the results obtained in this study, both authors found lower percentages of fructose (44% and 49% *cf* 59%), higher levels of sucrose (25% and 14% *cf* 8%) and similar levels of glucose (13% and 11% *cf* 13%). However, Moriguchi *et al*. (1992) reported lower sorbitol levels (18%) than either Hudina and Stampar (2000b) (23%) or this study (20%). Yamada *et al*. (2006) studied the cultivar 'La France' and reported similar levels to those found in this study.

Overall, fructose was the predominant sugar in the pear fruit as has also been reported in apple (Suni, 2000). Sorbitol has been reported as the predominant translocated sugar (65-70%) while sucrose is the second one (30-35%) in other fruit crops such as apple (Klages *et al*., 2001). These sorbitol:sucrose ratios are similar to those found in the fruit in this study; however, sugar composition in fruit pear should not be related to phloem exudates in apple. Thus, further research is needed to determine the sugar composition of phloem exudates in pears.

5.4 Evaluation of Flesh Spot Decay

High fruit quality is one of the main objectives in pear breeding programmes, and the appearance of physiological disorders such as FSD has a negative impact on quality. FSD could be considered as a poly-defined term because the appearance of single symptoms or a combination of symptoms (browning and cavities) comprises this disorder. It is usually assumed that flesh browning and the occurrence of cavities are one and the same disorder. In fact, both browning and cavities have usually been reported as part of FSD (Kawamata, 1982; Lallu, 1989; Crisosto, 2002). However, various authors prefer to make distinctions between them, and biochemical data have given evidence of a difference between the metabolic origins of flesh browning and cavities (Franck *et al*., 2007). According to the results obtained in this study, symptoms (browning and cavities) observed in the occurrence of FSD in the fruit should be treated independently because there was a low genetic correlation (-0.16) between browning and cavities. The phenotypic correlation was negligible (0.34) which indicate that environmental factors are involved in this result.

There were fruit which showed only browning or only cavities or both combined in the fruit flesh (Figures 1, 2 and 3). Similar symptoms were reported by Koto *et al*. (1972), who reported that the presence of the flesh decay (browning) in the pear variety 'Kikusui' (Japanese pear) was composed of two types of symptoms, with cavities and without cavities. According to observations during the slicing of the fruit, it could be suggested that small brown spots appear first in the flesh and then the cavity is formed in the same area. However, cavities were not always observed in fruit with brown spots and also the size of the spots varied. Moreover, it cannot be assumed that all the browning symptoms observed in the fruit belonged specifically to FSD itself because there is no a clear delineation in the literature allowing for this differentiation.

Parents showing the highest eBV (almost 99%) for the percentages of unaffected area in the fruit were P16 and P5. In terms of browning, parents P16 and P5 obtained the lowest eBV (less than 0.5%) whereas parents 'Conference', P2 and P12 showed the highest eBV (close and more than 2%). The parent which showed the lowest eBV for browning (P16) had 100% Asian parentage while parents which showed the highest eBV had 100% European parentage, suggesting that susceptibility for this disorder comes from the

European rather than the Asian parentage. Furthermore, these results confirm the susceptibly of the cultivar 'Conference' to browning disorders (Xuan *et al*., 2001; Wojcik & Wojcik, 2003; Franck *et al*., 2007).

For cavities, parents 'Conference' and P15 showed the lowest eBV (less than 0.01%) whereas parents P13 and P5 showed the highest eBV (around 0.05 and 0.2% respectively). No family was totally free from cavities; all of them showed at least a minimum percentage of the occurrence of this symptom. Moreover, there was no clear to tendency for susceptibility derived from the parentage. Three of the parents ('Conference', P15 and P12) from the top five parents showing the lowest eBV for cavities are 100% European. Moreover, parents P15 and P12 have the cultivar 'Conference' involved in the crosses that generate them.

Parent P16 (100% Asian) which showed the lowest eBV for browning was among the top four parents showing the lowest eBV for cavities; whereas 'Conference' (100% European) which showed the lowest eBV for cavities was among the top three parents showing the highest susceptibility to browning. Thus, parents showing high susceptibility to browning and less susceptibility to cavities, or vice versa and parents showing less susceptibility to both symptoms were found in this study.

The high environment variances indicate that non-genetic conditions influenced the occurrence of the disorder variables and this is supported by the theory that browning disorders and FSD are affected by the environment (Crisosto, 2002; Franck *et al.,* 2007). Moreover, the occurrence of pear disorders has been reported to be very variable from year to year (Franck *et al*., 2007; Morgan *et al*., 2010).

For both browning and cavities, heritabilities were relatively low with 0.18 and 0.19 respectively. Although these estimates are tentative because of the poor residual distributions (e.g., about half of the seedlings showed no disorder), they are similar to that obtained by Morgan, *et al.* (2010) (0.18).

It has been reported that, within any particular cultivar, the incidence of FSD is highest in large fruit (Koto *et al*., 1972; Lallu, 1989; Marsh, 1989; Crisosto, 2002). These results were mostly achieved in research carried out on specific susceptible cultivars. For instance, Koto *et al*. (1972) reported that in the variety 'Kikusui' (Japanese pear), fruit weight and the occurrence of FSD was closely related with an appearance ratio of 100% in fruit above 350 g and 50% in fruit less than 200g. The same tendency was reported by Lallu (1989) in

the cultivars ‘Nijisseiki’, ‘Shinseiki’, ‘Kosui’ and ‘Hosui’ (the two latter being less susceptible to FSD). This present study demonstrates that this correlation between fruit size and disorder within genotypes does not apply across genotypes. The correlation coefficients obtained between fruit weight and the presence of browning and cavities in this experiment (Table 8) were mostly negligible. Furthermore, symptoms were observed in small as well as larger fruit (range from 54 to 441 g).

5.4.1 Influence of Boron

The initial hypothesis was that high B concentration in the fruit could prevent or reduce FSD symptoms in the fruit. However, this tendency was observed only in relation to browning, but not for the occurrence of cavities: a negative genetic correlation (-0.28) was observed between B and browning, but a positive one (0.51) was recorded between B and cavities.

Parents that showed the lowest eBV for browning were among the top five parents for B eBV. This apparent effect of B in the decrease of browning could be attributed to the fact that B plays an essential role in the structural integrity of cell wall and biomembranes (Hu & Brown, 1994; Xuan *et al*., 2001; Hansch & Mendel, 2009). In addition, internal browning has been correlated to the membrane permeability of fruit cortex cells (Wojcik and Wojcik 2003). When this element is not present in the required amounts in the fruit, changes in cell wall and membrane structure are produced and thus browning symptoms may occur. Indeed, Xuan *et al*. (2001) suggested that B improves the ability of fruit tissue to avoid typical browning disorders. Moreover, Xuan *et al*. (2001), and Wojcik and Wojcik (2003) found that foliar application of this mineral decreased the occurrence of internal browning in the cultivar ‘Conference’. Similar results were found by Khoshghalb *et al.* (2008), who stated that B applications significantly reduced browning in the core and flesh of Asian pears. The latter results are mostly related to the expression of phenotypic symptoms. The phenotypic correlation between B and browning was negligible (0.04). For this reason more research is needed to confirm the link found in this study between this mineral and browning. It is worth reiterating that in this research only the overall B content of fruit was measured, not its presence in particular cellular structures or

solutions. Thus, a high B content results from B in the sorbitol-B complex rather than B in cell walls and biomembranes.

It must be pointed out that the occurrence of browning is also related to other chemical compounds such as ascorbic acid, phenolic concentrations and peroxidases (Amoit *et al.*, 1992; Nicolas *et al.*, 1994, both as cited in Franck *et al.,* 2007). Furthermore, the oxidative process which produces cell collapse can be involved in the appearance of this disorder because the normal cellular compartmentalisation is lost and substrates may be oxidised when membrane damage occurs (Franck *et al*., 2007), producing browning symptoms. B increases the concentration of antioxidant compounds such as ascorbic concentrations (Xuan *et al.* 2001; Keles *et al*., 2004) and increases the activity of antioxidant enzymes (Aftab, 2010), thus, B could help to prevent the oxidative processes which cause browning symptoms. Nevertheless, an excess of this element can produce a decrease in other antioxidant compounds such as α-tocoperol (Keles *et al*., 2004) and it has also been reported that B applications reduced phenolic compounds and their antioxidant activity in blueberry fruit (Eichholz, 2011) thereby potentially enhancing browning symptoms.

Within the family C13, the two seedlings with the highest eBV for B also showed the highest eBV for browning. This is counter to the tendency for high B to be correlated with low browning and suggests that this mineral may have limited use in predicting the susceptibility of individual seedlings to browning disorders.

5.4.2 Influence of Sugars

It has been reported that sugar signalling enhances cell expansion and is also related to the repression of senescence-associated genes (Rolland, 2006). However, there is no evidence in the literature that sugars are involved in the prevention of physiological disorders.

A negative genetic correlation (-0.44) was observed between browning and sorbitol while a positive correlation (0.60) was observed between cavities and sorbitol. These results indicated an opposite effect of sorbitol in the occurrence of both symptoms. On the other hand, a positive correlation (0.44) was observed between the percentage of browning and fructose; however, the opposite occurred between the percentage of cavities and this sugar where a negative correlation (-0.62) was estimated, suggesting that this sugar

might have a link with the appearance of the latter disorder. Furthermore, parents that showed the lowest eBV for cavities were among the top five parents for fructose eBV.

There is evidence of an apoplasmic phloem unloading pathway for sugars in the fruit (Zhang, *et al*., 2004) and that the movement of sugars into the cell, which involves membrane transport, generates osmotic pressure (Gao *et al*., 2005). Therefore, it may be hypothesized that fructose is predominantly inside the cell and does not affect the apoplastic osmotic potential, consequently it is not producing osmotic pressure that may cause cells to separate, forming cavities (Johnston, 2011). Furthermore, it may also be speculated that sorbitol could be a potential factor for osmotic pressure in the apoplast forcing the cells to separate due to the high mean sorbitol (in comparison to apple) as well as the positive correlation observed between this sugar and the occurrence of cavities, (Johnston, 2011). More research is needed to find out the relationship between these two sugars, the occurrence of cavities and the specific location of B in the fruit.

5.5 Breeding Values and Initial Selection

Pear breeders try to combine complementary parental variables to obtain good tasting and texture fruit without physiological disorders in the progeny. The choice of parents for the production of the seedlings is thus an important decision for the breeding process. According to the results of this study, it would be desirable to combine parents with high B and fructose to reduce the occurrence of browning and cavities in the fruit. The basis of this approach is the assumption that much of the genetic variance is additive (Bos & Calagari, 2008); therefore, it is feasible to obtain progeny showing the desired traits from both parents.

Breeding values can be considered a tool to compare the progeny with their parents in order to determine if genetic gain has been achieved in a particular variable. Because most of the genetic variance was additive in this population, individual seedlings showing highest eBV for B, sorbitol and fructose should be superior parents with respect to these traits.

5.5.1 Selection of the Best Individuals

5.5.1.1 Browning

Although B had a negative genetic correlation (-0.28) with browning, it had a positive one (0.51) with cavities. Thus, more research into B is needed before a selection strategy based on this mineral can be devised to reduce browning. However, browning can be reduced by simply direct selection, taking individuals from the best families which have little or no browning symptoms. Therefore, the best seedlings from families C8, C14 and C17, which showed the lowest eBV for browning (Appendix 3e), can be selected as further parents.

5.5.1.2 Cavities

Selecting by fructose would enhance fruit sweetness and potentially would reduce the formation of cavities. This would also have the added benefit of fructose being the sugar which is more suitable for diabetics (Hudina & Stampar, 2000b), therefore beneficial for human health.

Whereas fructose might be desirable due to its negative genetic correlation with cavities (-0.62), it is less desirable due to its positive genetic correlation with browning (0.44). However, three different methods of selecting by fructose to reduce cavities can be suggested. Firstly, indirect selection would be the best option from the genetic point of view and based on the efficiency of this method shown in the results chapter (Section 4.1.5). Thus, the best seedlings from families that showed the highest eBV (C4, C6 and C7, Appendix 3c) can be selected for further crosses. A second method would be threshold selection, considering families which showed few or no cavities as the threshold, and then selecting the individuals which showed high fructose within these families. Therefore, the best seedlings showing the highest eBV for fructose must be selected from families C4, C5, C6, C7, C10, and C16 which showed the lowest eBV for cavities. Finally, an index selection method (Falconer, 1986) could be carried out. This index will indicate the best individuals from all the families in terms of high eBV for fructose and low eBV for cavities, and consequently, those belonging to the top group will be selected as further parents.

6. Conclusions

1. Internal browning and cavities should not be considered as one and the same disorder (FSD).
2. B and sorbitol contents showed a positive genetic correlation (0.38) which would indicate that they are correlated, supporting the argument that the translocation of B is facilitated by the formation of complexes with sorbitol.
3. High heritability was estimated for B (0.72). However, low heritability was observed for sorbitol (0.18) which may reflect that in the fruit it is broken down into fructose and glucose, releasing B. Furthermore, a moderate heritability was estimated for fructose (0.54).
4. A negative genetic correlation was estimated between both B and sorbitol and the area of the fruit affected by browning (-0.28 and -0.44). However, the opposite effect was observed in relation to the occurrence of cavities.
5. In terms of cavities, a negative genetic correlation (-0.62) was estimated between fructose and the occurrence of cavities, suggesting that there might be a link between high levels of this sugar and lower percentages of cavity formation in the fruit.
6. Overall, the heritability observed for the occurrence of browning and cavities was low, indicating that the additive genetic variance is low. Nevertheless, it must be considered that other chemical compounds and environmental factors are involved in the occurrence of these physiological disorders.
7. Parent P16 (100% Asian) was the individual showing most potential for consideration for further crosses due to its low susceptibility to both browning and cavities.
8. Selection by fructose to reduce the occurrence of cavities is a good option based on the high genetic correlation (-0.62) between them.
9. The best option for reducing browning in further populations is by selecting individuals (future parents) from families that showed little or no browning.

7. Recommendations

In examining the occurrence of browning and cavities in pear genotypes, this study relied on only a single year's data on a restricted number of families from two commercial pear breeding populations. Furthermore, the residual plots from the disorders indicated severe deviations from the Normality assumption. Storage time could be regarded as a limiting factor, and thus longer periods should be considered in order to enhance the development of the disorder symptoms. Although further work is desirable, some tentative recommendations can be made:

A. So-called FSD is not a single disorder but should be separated into browning and cavities.
B. B may be linked to one or other of these disorders but to properly investigate this one would need to measure where in the fruit the B is located.
C. Fructose seems to be correlated with cavities and can thus be used to choose amongst genotypes without symptoms of this disorder.

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Finally, I want to dedicate this work to my family, friends and myself.

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Appendices

Appendix 1a. Data per seedlings for production traits.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
1	P310	110.63	57.88	67.50	38.52	8521.94
2	P310	122.40	60.21	59.79	28.43	2203.11
3	P310	137.81	64.54	60.61	33.44	5512.40
4	P310	139.64	65.29	58.10	19.12	5166.50
5	P310	150.91	67.58	60.83	31.20	3923.67
6	P333	110.48	59.12	58.41	40.29	4640.16
7	P333	90.74	55.01	55.58	20.37	3697.08
8	P333	129.23	64.32	57.85	22.19	2455.37
9	P333	124.44	64.56	61.83	19.62	1368.84
10	P333	170.98	68.27	65.42	19.00	2051.76
11	P333	122.00	60.15	62.16	19.62	2196.00
12	P333	273.17	78.11	95.90	35.09	8468.27
13	P333	246.91	77.35	83.24	11.27	5185.11
14	P333	148.77	61.82	73.53	7.80	1785.24
15	P333	285.14	82.79	85.36	10.16	3136.51
16	P336	87.72	56.83	56.56	18.63	3268.15
17	P336	117.88	61.80	57.71	25.78	6078.62
18	P336	136.42	64.33	60.97	50.13	6138.90
19	P336	80.29	52.89	64.36	25.50	1605.80
20	P336	145.36	65.61	63.74	39.93	7704.08
21	P336	109.29	58.77	61.80	33.61	3715.86
22	P336	126.25	63.98	59.93	47.96	12625.00
23	P336	126.83	62.90	62.87	22.73	2917.09
24	P336	155.86	65.23	78.98	26.94	2182.04
25	P336	99.66	57.71	53.72	54.21	2790.48
26	P336	146.14	65.39	69.92	28.58	8183.84
27	P336	195.38	71.48	78.65	32.63	8987.48
28	P336	193.10	67.23	108.70	15.82	4055.10
29	P336	207.77	72.49	75.20	16.16	2493.24
30	P336	165.06	69.72	65.42	23.00	3631.32
31	P337	130.64	63.64	56.81	39.22	7315.84
32	P337	88.11	55.83	53.77	18.51	2114.70
33	P337	138.84	65.05	64.48	45.46	3471.00
34	P337	102.18	58.32	68.06	26.94	2963.22
35	P337	139.43	65.31	65.84	28.73	4601.19
36	P337	114.70	59.41	64.55	16.50	1720.50
37	P337	137.86	62.52	64.36	37.30	8133.57
38	P337	115.74	61.19	55.15	25.78	1273.14

Continuation Appendix 1a.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
39	P337	190.09	71.12	93.62	32.31	2090.98
40	P337	131.87	66.51	57.31	13.14	4879.19
41	P337	110.23	59.73	63.49	19.37	4269.42
42	P337	157.72	67.54	65.13	38.87	5677.92
43	P337	263.05	79.10	95.10	23.27	5524.05
44	P337	160.96	66.03	76.64	27.53	3058.24
45	P337	251.91	74.82	94.22	34.76	5290.11
46	P339	92.00	54.24	67.09	38.69	4784.00
47	P339	138.85	62.10	64.04	26.07	7239.48
48	P339	240.71	75.92	87.43	40.11	5536.22
49	P339	290.72	77.53	111.15	39.93	5232.87
50	P339	177.60	66.44	85.66	26.07	11366.08
51	P339	216.68	74.29	75.19	34.10	14776.43
52	P339	266.17	79.70	88.50	36.78	4524.89
53	P342	88.14	53.68	58.54	24.09	2908.62
54	P342	139.36	65.17	60.81	36.10	4320.16
55	P342	93.44	55.74	54.24	40.11	2803.20
56	P342	107.47	57.65	64.92	9.80	2107.43
57	P342	140.25	64.59	76.32	53.18	7854.00
58	P342	116.01	60.33	58.64	23.13	5367.40
59	P342	106.46	58.11	59.61	33.44	1809.82
60	P342	149.30	64.97	67.31	26.07	7784.61
61	P342	131.09	62.00	64.49	30.26	1966.35
62	P342	167.96	63.76	89.61	43.95	4199.00
63	P342	136.51	63.08	65.11	27.98	6279.46
64	P346	60.95	49.02	51.03	7.80	950.67
65	P346	112.68	58.95	69.26	47.96	3380.40
66	P346	132.73	61.60	72.71	11.65	3092.86
67	P346	97.66	55.13	60.70	19.00	1464.90
68	P346	138.69	61.31	71.35	17.20	3189.87
69	P346	97.95	56.06	66.52	48.94	9588.00
70	P346	95.97	55.68	57.01	21.80	3646.86
71	P346	118.09	57.77	77.82	52.15	9092.93
72	P355	125.09	61.44	56.22	11.65	2914.72
73	P355	93.74	57.17	52.83	13.45	2521.34
74	P355	86.79	54.49	51.09	9.63	1909.27
75	P355	174.41	69.38	62.67	12.04	4199.42
76	P355	194.39	69.55	81.31	15.82	2527.10
77	P356	146.96	64.64	61.97	34.76	11903.76
78	P356	114.14	60.92	58.23	17.31	3952.23
79	P356	140.86	65.14	60.92	26.07	7344.55

Continuation Appendix 1a.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
80	P356	85.95	55.60	52.64	24.79	4261.43
81	P356	144.76	66.28	63.47	24.37	7527.52
82	P356	148.34	66.28	61.69	13.76	4082.53
83	P356	99.79	56.62	63.78	14.18	4349.20
84	P356	124.28	62.13	58.02	14.29	3107.00
85	P356	151.81	65.48	69.69	25.78	7828.27
86	P356	144.07	66.28	59.08	21.93	6318.44
87	P356	224.15	74.53	78.91	37.30	2913.95
88	P356	141.81	63.26	70.73	23.00	7090.50
89	P356	176.75	69.14	67.99	23.00	8129.75
90	P356	163.05	69.34	59.58	26.80	8738.02
91	P356	195.16	73.59	69.30	16.16	4321.08
92	P357	183.85	69.72	79.85	30.26	8457.10
93	P357	138.28	60.99	71.59	25.21	7328.84
94	P357	122.32	58.58	69.03	16.85	4770.48
95	P357	154.94	64.15	78.81	21.66	6713.54
96	P357	133.85	59.96	79.17	19.12	3346.25
97	P357	153.27	64.20	73.93	21.27	4598.10
98	P357	140.21	64.11	59.67	10.52	3365.04
99	P357	143.04	63.24	62.12	40.83	11679.23
100	P357	122.13	61.36	62.78	16.62	1709.82
101	P357	180.08	69.43	82.82	21.66	7802.85
102	P357	162.21	63.96	83.79	27.38	1784.34
103	P357	201.78	67.88	103.69	21.66	8878.32
104	P357	148.99	68.20	60.36	38.69	6853.54
105	P357	120.35	62.04	54.35	9.98	2402.72
106	P357	112.19	61.13	53.41	15.60	2580.37
107	P358	79.60	54.22	47.37	8.36	1331.01
108	P358	100.26	59.58	58.83	14.18	2843.87
109	P358	65.93	53.26	42.11	8.94	1179.00
110	P358	106.46	54.93	78.75	11.84	1703.31
111	P358	137.96	63.62	65.58	22.73	7311.88
112	P358	189.02	73.17	64.23	12.24	4625.63
113	P358	154.01	63.62	77.72	24.09	4620.30
114	P358	230.38	74.08	83.67	12.43	2534.18
115	P358	123.54	62.62	58.03	8.52	1976.69
116	P358	248.51	76.50	86.72	8.69	4473.18
117	P361	125.42	56.69	91.63	27.24	2257.56
118	P361	59.18	45.75	61.69	29.49	3490.25
119	P361	122.90	61.34	70.25	7.96	1956.01
120	P361	142.04	60.43	79.27	18.87	5361.33

Continuation Appendix 1a.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
121	P361	157.14	62.57	94.14	24.93	3928.50
122	P361	98.85	52.92	74.55	23.41	3657.45
123	P361	63.47	47.49	61.78	18.87	2395.69
124	P361	184.52	70.11	76.76	22.19	3505.88
125	P361	114.18	57.70	73.32	17.31	1826.88
126	P361	77.63	50.52	67.45	25.93	4025.35
127	P361	72.65	50.84	48.53	11.17	1623.65
128	P361	113.22	56.15	66.86	21.01	4758.28
129	P361	156.31	62.84	77.02	26.80	1875.75
130	P361	135.91	62.48	75.11	25.36	8426.42
131	P361	182.87	66.93	81.89	25.50	6034.71
132	P361	107.64	55.97	73.50	14.50	1291.68
133	P362	54.44	45.53	54.84	18.75	2041.53
134	P362	139.39	62.40	77.45	19.12	1906.33
135	P362	83.02	49.18	84.66	22.33	3569.86
136	P362	109.24	56.39	83.90	24.37	5324.68
137	P362	84.43	52.38	67.46	20.37	2110.75
138	P362	97.74	55.19	63.59	21.53	4209.44
139	P362	95.62	51.27	73.61	19.12	3656.22
140	P362	135.51	61.43	72.46	16.27	4410.25
141	P362	128.84	61.76	64.71	12.73	3281.35
142	P362	75.51	49.75	71.30	13.24	2642.94
143	P362	59.20	46.11	54.58	10.89	1289.77
144	P362	105.26	54.36	78.12	19.12	3052.54
145	P362	92.22	52.63	65.55	19.74	3640.88
146	P362	218.22	73.14	80.88	11.46	2400.42
147	P365	163.20	65.93	79.21	31.20	10182.57
148	P365	98.55	57.02	57.36	24.23	4775.79
149	P365	107.17	56.93	59.11	30.26	6485.48
150	P365	108.14	58.04	69.26	34.43	7446.17
151	P365	200.63	68.96	83.16	21.66	8693.07
152	P365	114.44	57.71	61.87	44.70	10230.45
153	P365	143.54	62.77	74.21	28.73	9616.85
154	P365	204.09	70.26	81.97	26.21	3469.50
155	P366	84.89	51.65	62.64	22.46	3813.25
156	P366	133.33	60.08	92.25	25.78	6875.32
157	P366	117.37	55.58	80.64	22.33	5240.91
158	P366	100.23	53.50	72.82	17.90	3589.04
159	P366	106.37	54.66	76.23	6.88	1464.22
160	P366	128.85	60.13	78.36	16.16	2448.06
161	P366	140.74	60.31	76.73	29.03	4644.51

Continuation Appendix 1a.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
162	P366	252.86	79.80	81.72	16.27	8229.48
163	P369	271.73	81.68	69.53	35.77	16303.80
164	P369	186.82	71.91	62.37	52.15	19486.03
165	P369	121.29	63.19	52.34	68.78	9945.78
166	P369	203.06	77.00	59.78	27.83	11301.28
167	P369	185.34	68.79	78.03	14.50	6301.56
168	P369	249.03	80.05	71.72	19.87	2739.33
169	P369	106.66	59.79	51.60	17.90	3819.48
170	P369	252.87	77.55	72.21	44.70	29080.05
171	P369	240.75	75.97	79.67	31.36	16130.25
172	P369	236.22	76.96	71.41	28.43	7086.60
173	P369	232.13	76.99	77.74	33.12	5338.99
174	P369	188.53	69.41	78.21	19.74	4524.72
175	P369	307.58	79.08	106.52	18.26	8919.82
176	P369	440.96	94.01	85.52	29.64	20725.12
177	P369	344.03	82.10	106.89	12.53	5160.45
178	P386	83.50	53.79	55.97	24.09	4023.51
179	P386	112.27	59.37	59.41	19.99	4378.53
180	P386	101.72	58.65	50.97	8.61	2136.12
181	P386	139.08	64.52	58.58	20.76	5773.38
182	P386	159.91	65.05	67.11	17.90	5726.36
183	P386	132.01	62.28	57.23	16.50	4356.64
184	P386	102.89	58.92	50.59	28.43	5849.48
185	P386	64.99	50.20	45.11	11.27	1464.74
186	P386	115.20	59.78	65.82	20.63	1843.20
187	P386	64.82	52.20	41.53	23.82	3087.60
188	P386	110.43	62.25	50.99	30.26	6683.08
189	P386	118.79	61.40	51.89	38.52	7127.33
190	P386	203.95	71.53	77.36	18.87	7698.13
191	P386	179.22	70.59	60.69	29.18	10460.30
192	P386	133.81	65.84	53.42	30.26	5887.64
193	P433	226.61	77.90	67.32	19.12	4532.20
194	P433	154.27	67.67	58.75	17.08	5269.59
195	P433	238.65	76.44	67.50	21.80	3579.81
196	P433	199.48	74.77	66.92	20.37	4986.88
197	P433	135.98	63.16	63.34	15.82	1767.70
198	P433	224.57	73.86	74.23	24.37	10554.56
199	P437	164.89	64.78	72.81	15.82	5606.09
200	P437	164.66	68.15	68.06	13.66	4497.15
201	P437	173.17	68.28	71.53	15.05	5714.61
202	P437	153.33	66.22	64.37	6.30	2431.19

Continuation Appendix 1a.

Seedling Number	Family	F. Weight (g)	F. Diameter (mm)	F. Height (mm)	TCA (cm^2)	Yield (g)
203	P437	124.79	60.90	59.74	14.61	3646.37
204	P437	225.32	75.59	69.06	10.16	5369.14
205	P438	62.59	48.87	48.70	16.50	2065.62
206	P438	147.34	66.24	58.19	30.73	3683.50
207	P438	136.44	65.17	55.07	11.46	3126.97
208	P438	157.87	69.08	57.88	13.66	2683.80
209	P438	297.53	82.86	76.00	31.51	5950.50
210	P438	343.69	86.64	78.72	21.66	8935.81
211	P438	102.17	58.54	54.05	8.77	1792.76

Appendix 1b. Data per seedlings for chemical traits.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
1	P310	2.75	7.71	45.46	18.39	12.64	84.19
2	P310	2.94	7.45	51.09	15.53	17.61	91.69
3	P310	2.75	14.26	77.28	7.40	18.86	117.80
4	P310	2.91	13.08	75.77	14.16	16.61	119.62
5	P310	1.40	10.29	74.83	1.94	15.23	102.29
6	P333	1.55	10.87	66.00	2.84	23.79	103.50
7	P333	2.35	17.31	56.14	6.70	11.96	92.10
8	P333	1.91	11.46	64.10	10.82	18.78	105.16
9	P333	3.24	10.28	56.77	5.66	13.19	85.90
10	P333	2.10	16.37	81.42	11.96	24.76	134.52
11	P333	2.41	9.63	62.95	27.05	33.99	133.62
12	P333	2.74	12.74	50.50	16.19	25.68	105.11
13	P333	3.52	8.78	66.20	2.12	34.09	111.19
14	P333	3.30	11.76	79.33	10.18	35.28	136.55
15	P333	4.61	13.37	59.42	2.97	35.79	111.55
16	P336	2.53	9.08	43.78	30.61	5.76	89.22
17	P336	1.74	9.84	64.40	11.74	20.31	106.28
18	P336	3.59	9.58	52.85	25.49	13.88	101.80
19	P336	3.44	18.52	80.40	8.29	10.49	117.70
20	P336	1.44	9.37	58.70	24.64	19.92	112.63
21	P336	2.57	11.08	76.62	16.51	9.51	113.72
22	P336	1.82	7.81	48.12	29.49	9.94	95.36
23	P336	2.53	16.46	69.47	23.89	10.02	119.84
24	P336	2.09	8.72	53.74	10.86	25.82	99.13
25	P336	1.75	11.01	71.01	25.79	17.46	125.27
26	P336	2.63	17.34	78.68	19.59	16.22	131.83
27	P336	2.51	14.25	86.86	5.21	24.06	130.39
28	P336	2.51	15.02	71.35	5.39	33.65	125.40
29	P336	2.75	9.27	80.50	2.50	15.27	107.55
30	P336	3.54	8.03	66.87	8.42	36.99	120.31
31	P337	2.91	10.75	62.51	33.49	15.67	122.42
32	P337	2.16	9.32	88.51	5.48	9.58	112.90
33	P337	2.28	8.41	72.51	7.05	20.60	108.56
34	P337	3.21	12.60	83.08	5.27	9.54	110.49
35	P337	2.96	7.35	67.97	7.42	16.31	99.04
36	P337	2.82	8.77	83.74	3.03	10.51	106.06
37	P337	2.22	15.68	97.25	5.32	10.15	128.40
38	P337	3.52	12.54	68.91	21.45	13.17	116.06
39	P337	4.46	8.99	83.54	4.79	18.74	116.06
40	P337	3.56	8.82	69.97	16.85	31.70	127.34
41	P337	1.63	11.73	82.14	14.82	13.25	121.94

Continuation Appendix 1b.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
42	P337	1.61	10.30	80.93	26.73	17.81	135.77
43	P337	2.39	8.56	56.30	7.65	29.86	102.37
44	P337	4.03	9.52	60.79	12.37	28.57	111.26
45	P337	3.36	9.16	68.69	15.98	47.45	141.28
46	P339	4.14	11.83	60.45	1.76	17.09	91.13
47	P339	4.08	15.33	59.74	2.96	31.93	109.96
48	P339	1.80	12.86	72.99	1.90	17.98	105.73
49	P339	1.71	15.05	76.47	3.11	22.46	117.09
50	P339	1.49	13.03	65.10	1.25	26.64	106.02
51	P339	1.60	14.10	66.35	2.54	27.23	110.22
52	P339	1.83	17.84	57.41	6.36	38.64	120.25
53	P342	2.56	10.87	58.95	1.87	11.47	83.17
54	P342	4.50	18.10	74.48	2.69	18.01	113.28
55	P342	4.86	27.83	84.65	3.38	18.07	133.93
56	P342	3.24	19.14	78.89	3.89	5.92	107.84
57	P342	3.18	15.86	81.38	2.35	16.30	115.88
58	P342	3.23	11.51	78.51	4.39	16.01	110.42
59	P342	3.13	20.76	82.30	10.57	11.73	125.36
60	P342	2.61	16.33	84.30	5.26	17.46	123.35
61	P342	3.08	15.79	78.52	4.33	29.24	127.88
62	P342	5.29	8.94	69.43	4.87	24.82	108.06
63	P342	3.75	11.09	57.39	20.85	33.99	123.32
64	P346	2.66	8.87	72.78	2.70	9.06	93.40
65	P346	3.13	12.33	68.73	5.63	12.35	99.04
66	P346	3.58	9.79	69.91	11.97	23.12	114.78
67	P346	4.24	10.11	69.80	3.08	27.16	110.15
68	P346	4.09	19.75	74.25	5.81	11.78	111.60
69	P346	2.13	13.24	62.77	2.75	15.82	94.57
70	P346	3.17	14.28	71.81	2.72	10.87	99.68
71	P346	2.51	12.43	85.85	3.35	16.55	118.19
72	P355	4.21	11.95	49.96	1.81	16.06	79.79
73	P355	3.03	16.20	47.69	2.06	11.31	77.26
74	P355	2.80	12.90	60.92	1.54	20.12	95.48
75	P355	2.89	20.47	57.96	2.57	18.57	99.57
76	P355	2.29	7.59	65.66	3.50	24.59	101.34
77	P356	4.11	12.88	56.18	13.62	12.86	95.54
78	P356	3.06	16.04	60.50	4.62	13.87	95.03
79	P356	2.61	11.97	55.41	10.32	15.87	93.57
80	P356	2.45	19.02	57.47	14.65	21.40	112.55
81	P356	2.71	11.93	55.76	8.89	17.59	94.17
82	P356	3.04	9.14	52.32	13.07	20.98	95.51

Continuation Appendix 1b.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
83	P356	3.36	17.05	59.60	12.30	19.20	108.15
84	P356	5.03	12.97	53.12	5.50	25.04	96.62
85	P356	2.67	10.65	45.63	5.57	17.55	79.39
86	P356	2.88	8.70	51.37	2.80	22.01	84.89
87	P356	6.89	15.35	55.71	5.53	19.20	95.79
88	P356	2.59	7.39	38.32	4.59	16.16	66.46
89	P356	4.18	11.30	49.26	15.49	13.41	89.46
90	P356	4.74	11.81	47.10	11.93	23.26	94.10
91	P356	3.38	13.17	59.64	7.37	24.79	104.97
92	P357	2.67	10.33	58.52	4.40	12.93	86.19
93	P357	1.40	3.93	60.45	2.42	22.26	89.06
94	P357	1.46	11.02	56.03	3.78	10.77	81.60
95	P357	1.52	12.94	60.59	13.09	24.15	110.76
96	P357	4.01	13.54	63.40	13.13	21.22	111.29
97	P357	3.12	11.56	60.00	8.27	40.09	119.92
98	P357	3.56	15.22	69.18	6.13	20.71	111.23
99	P357	5.28	17.65	59.74	4.41	14.89	96.70
100	P357	3.48	13.58	57.00	9.70	26.70	106.98
101	P357	2.94	10.05	58.12	16.30	25.07	109.54
102	P357	2.15	13.82	80.15	9.28	22.26	125.52
103	P357	2.31	9.61	72.08	5.50	24.04	111.24
104	P357	2.89	17.51	69.22	2.34	41.32	130.39
105	P357	4.49	16.13	70.03	8.31	25.03	119.49
106	P357	4.67	15.19	64.84	6.17	22.06	108.25
107	P358	3.04	8.08	57.28	3.27	23.71	92.34
108	P358	2.61	19.74	47.81	7.58	8.50	83.63
109	P358	1.78	10.07	47.45	2.80	18.47	78.79
110	P358	3.41	13.61	53.60	3.05	27.76	98.03
111	P358	4.30	10.57	50.00	22.13	12.19	94.89
112	P358	5.07	13.38	56.65	10.74	19.59	100.37
113	P358	5.47	20.87	46.77	24.21	10.17	102.03
114	P358	5.10	11.34	66.29	8.91	14.08	100.62
115	P358	9.70	11.31	56.71	19.22	28.61	115.85
116	P358	6.18	17.65	67.06	10.01	21.97	116.69
117	P361	6.83	14.61	54.76	6.44	22.73	98.54
118	P361	3.52	20.22	49.58	21.96	13.13	104.89
119	P361	4.74	13.26	73.65	10.54	21.77	119.22
120	P361	4.32	7.65	61.13	4.19	28.67	101.65
121	P361	3.55	15.82	62.18	3.22	41.69	122.91
122	P361	6.19	12.56	68.86	3.06	22.72	107.20
123	P361	4.40	12.30	55.09	8.61	27.24	103.24

Continuation Appendix 1b.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
124	P361	3.77	18.16	56.81	6.99	30.87	112.84
125	P361	4.26	12.98	51.13	6.32	31.94	102.36
126	P361	5.95	9.57	45.10	18.56	30.27	103.50
127	P361	3.22	9.28	47.19	35.34	20.27	112.07
128	P361	5.62	15.65	42.26	30.14	24.37	112.41
129	P361	3.80	14.55	44.34	7.84	21.82	88.53
130	P361	3.88	11.85	63.97	11.42	30.66	117.90
131	P361	4.03	11.18	49.46	17.19	42.23	120.05
132	P361	4.52	10.98	54.97	9.22	36.41	111.58
133	P362	5.55	17.05	43.48	27.31	34.64	122.48
134	P362	2.51	13.49	54.02	20.60	33.22	121.33
135	P362	4.54	17.19	51.07	11.48	23.98	103.72
136	P362	2.86	9.42	54.94	17.87	31.92	114.15
137	P362	3.80	17.92	43.68	23.78	14.52	99.90
138	P362	3.35	9.78	37.03	30.94	18.71	96.46
139	P362	5.89	13.90	62.64	6.16	17.55	100.24
140	P362	3.70	12.50	41.01	27.88	22.64	104.03
141	P362	3.65	10.38	45.32	14.98	15.56	86.24
142	P362	3.65	14.13	43.25	7.89	27.13	92.38
143	P362	4.24	5.46	60.98	7.61	13.06	87.11
144	P362	3.92	10.46	56.10	17.70	21.87	106.11
145	P362	4.64	11.54	45.30	21.01	20.87	98.72
146	P362	3.42	13.98	62.24	15.54	37.46	129.21
147	P365	1.79	13.65	60.47	2.58	24.27	100.97
148	P365	3.34	10.89	13.46*	1.34	32.89	58.58
149	P365	2.91	16.06	49.94	2.14	24.37	92.51
150	P365	4.16	14.24	49.05	2.43	22.15	87.87
151	P365	3.54	9.00	54.83	2.18	23.21	89.22
152	P365	6.01	10.45	38.74	10.76	18.87	78.82
153	P365	6.33	10.90	46.64	9.62	29.70	96.86
154	P365	5.63	15.55	54.74	18.71	31.72	120.72
155	P366	1.64	10.10	59.24	6.48	29.95	105.77
156	P366	2.42	10.51	51.71	1.76	25.02	89.00
157	P366	2.29	12.95	67.81	10.29	29.05	120.10
158	P366	1.66	4.62	64.85	1.25	28.81	99.52
159	P366	2.24	10.21	61.27	4.57	13.13	89.19
160	P366	3.50	11.01	44.30	14.17	15.72	85.20
161	P366	2.72	10.60	48.70	9.25	12.38	80.93
162	P366	2.16	15.16	86.68	15.65	25.79	143.29
163	P369	3.22	16.02	58.22	2.04	12.49	88.78

*This value was regarded as outliner and considered as missing value for the analysis.

Continuation Appendix 1b.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
164	P369	3.18	25.27	69.57	3.13	19.44	117.41
165	P369	4.54	22.20	70.48	8.85	35.89	137.43
166	P369	3.41	17.39	69.53	2.23	29.85	119.00
167	P369	2.45	15.96	70.06	5.97	20.79	112.78
168	P369	4.84	19.30	83.54	1.92	21.80	126.56
169	P369	2.37	14.31	52.24	2.28	18.56	87.39
170	P369	2.59	26.92	71.06	3.09	22.62	123.69
171	P369	3.07	18.98	68.28	1.95	25.34	114.55
172	P369	3.67	13.95	59.22	10.51	22.37	106.06
173	P369	5.02	16.50	45.23	3.11	47.39	112.23
174	P369	2.24	14.82	82.02	4.67	20.24	121.74
175	P369	1.94	14.96	69.07	13.56	31.25	128.83
176	P369	5.18	18.30	58.37	2.29	26.96	105.92
177	P369	2.63	14.93	80.39	2.57	19.04	116.93
178	P386	2.86	15.32	56.92	4.50	34.36	111.10
179	P386	2.35	19.36	54.15	1.52	13.16	88.20
180	P386	2.95	18.24	51.27	2.63	9.60	81.74
181	P386	3.17	14.21	53.28	3.15	11.54	82.18
182	P386	2.72	14.21	63.95	1.79	18.58	98.54
183	P386	4.66	21.53	65.80	2.50	35.99	125.83
184	P386	4.31	20.85	61.61	2.09	21.46	106.00
185	P386	3.16	24.41	65.05	3.26	21.06	113.79
186	P386	3.79	14.01	66.58	2.31	14.06	96.95
187	P386	3.34	17.89	49.19	2.17	20.90	90.15
188	P386	2.88	13.12	61.96	2.78	24.78	102.64
189	P386	3.11	23.84	75.43	2.90	32.03	134.21
190	P386	3.25	14.04	71.96	4.49	31.07	121.56
191	P386	4.63	23.88	82.55	4.27	37.37	148.07
192	P386	4.82	16.15	76.17	2.88	28.34	123.54
193	P433	3.77	12.41	42.01	1.15	12.87	68.44
194	P433	3.19	17.77	59.29	3.88	12.34	93.28
195	P433	3.65	14.71	59.30	6.49	21.34	101.84
196	P433	5.47	15.65	52.34	1.92	16.52	86.42
197	P433	4.75	13.27	38.22	1.58	11.29	64.36
198	P433	3.41	34.45	79.81	3.09	23.85	141.20
199	P437	2.36	20.32	64.77	15.15	25.64	125.88
200	P437	2.31	14.19	51.17	1.27	11.19	77.81
201	P437	2.92	20.86	55.08	4.09	21.12	101.16
202	P437	2.90	17.07	52.73	2.88	30.63	103.30
203	P437	3.61	22.70	57.39	3.56	23.37	107.02

Continuation Appendix 1b.

Seedling	Family	B ($\mu g.g^{-1}$)	Glucose ($mg.g^{-1}$)	Fructose ($mg.g^{-1}$)	Sucrose ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)	T. Sugars ($mg.g^{-1}$)
204	P437	4.38	18.20	57.17	14.19	29.89	119.45
205	P438	4.30	11.47	41.82	2.59	9.16	65.04
206	P438	2.86	15.17	58.58	7.65	16.34	97.74
207	P438	3.76	18.36	57.94	3.10	17.63	97.02
208	P438	3.21	18.45	57.83	2.20	18.38	96.86
209	P438	3.76	20.52	75.49	2.45	14.80	113.26
210	P438	3.55	18.54	66.96	2.47	13.69	101.66
211	P438	5.39	7.44	33.35	45.59*	31.43	117.80

*This value was regarded as outliner and considered as missing value for the analysis.

Appendix 1c. Data per seedlings for disorder traits.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
1	P310	100.00	0.00	0.00
2	P310	99.57	0.43	0.00
3	P310	96.02	3.98	0.00
4	P310	97.70	2.24	0.05
5	P310	95.06	4.67	0.27
6	P333	100.00	0.00	0.00
7	P333	70.67	28.66	0.19
8	P333	100.00	0.00	0.00
9	P333	97.52	2.42	0.06
10	P333	100.00	0.00	0.00
11	P333	99.96	0.04	0.00
12	P333	100.00	0.00	0.00
13	P333	100.00	0.00	0.00
14	P333	100.00	0.00	0.00
15	P333	100.00	0.00	0.00
16	P336	73.22	25.18	1.61
17	P336	100.00	0.00	0.00
18	P336	100.00	0.00	0.00
19	P336	58.68	38.57	1.03
20	P336	100.00	0.00	0.00
21	P336	79.80	19.77	0.00
22	P336	99.96	0.00	0.04
23	P336	50.74	47.70	0.00
24	P336	100.00	0.00	0.00
25	P336	99.77	0.23	0.00
26	P336	98.13	1.83	0.04
27	P336	100.00	0.00	0.00
28	P336	100.00	0.00	0.00
29	P336	88.88	11.12	0.00
30	P336	100.00	0.00	0.00
31	P337	59.28	40.70	0.02
32	P337	100.00	0.00	0.00
33	P337	100.00	0.00	0.00
34	P337	98.16	1.68	0.17
35	P337	99.94	0.06	0.00
36	P337	89.92	9.32	0.00
37	P337	68.57	31.43	0.00
38	P337	47.47	52.47	0.06
39	P337	99.37	0.62	0.01
40	P337	100.00	0.00	0.00
41	P337	100.00	0.00	0.00

Continuation Appendix 1c.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
42	P337	99.95	0.00	0.02
43	P337	100.00	0.00	0.00
44	P337	100.00	0.00	0.00
45	P337	100.00	0.00	0.00
46	P339	99.98	0.00	0.00
47	P339	100.00	0.00	0.00
48	P339	70.05	28.70	0.00
49	P339	95.59	4.33	0.02
50	P339	100.00	0.00	0.00
51	P339	100.00	0.00	0.00
52	P339	100.00	0.00	0.00
53	P342	100.00	0.00	0.00
54	P342	100.00	0.00	0.00
55	P342	70.88	24.74	0.02
56	P342	99.67	0.24	0.10
57	P342	100.00	0.00	0.00
58	P342	99.98	0.00	0.02
59	P342	99.93	0.00	0.07
60	P342	100.00	0.00	0.00
61	P342	99.98	0.00	0.02
62	P342	100.00	0.00	0.00
63	P342	100.00	0.00	0.00
64	P346	99.79	0.15	0.06
65	P346	100.00	0.00	0.00
66	P346	100.00	0.00	0.00
67	P346	100.00	0.00	0.00
68	P346	98.72	1.28	0.00
69	P346	100.00	0.00	0.00
70	P346	99.55	0.45	0.00
71	P346	99.98	0.02	0.00
72	P355	100.00	0.00	0.00
73	P355	98.57	1.12	0.30
74	P355	100.00	0.00	0.00
75	P355	98.97	0.66	0.33
76	P355	100.00	0.00	0.00
77	P356	99.96	0.00	0.04
78	P356	99.96	0.00	0.04
79	P356	99.99	0.00	0.01
80	P356	100.00	0.00	0.00
81	P356	100.00	0.00	0.00
82	P356	100.00	0.00	0.00

Continuation Appendix 1c.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
83	P356	93.44	5.87	0.69
84	P356	99.97	0.00	0.03
85	P356	100.00	0.00	0.00
86	P356	100.00	0.00	0.00
87	P356	100.00	0.00	0.00
88	P356	96.76	3.04	0.20
89	P356	99.87	0.04	0.09
90	P356	100.00	0.00	0.00
91	P356	99.83	0.00	0.17
92	P357	99.83	0.00	0.17
93	P357	100.00	0.00	0.00
94	P357	100.00	0.00	0.00
95	P357	99.98	0.00	0.02
96	P357	100.00	0.00	0.00
97	P357	100.00	0.00	0.00
98	P357	99.95	0.00	0.05
99	P357	99.13	0.40	0.46
100	P357	100.00	0.00	0.00
101	P357	100.00	0.00	0.00
102	P357	97.00	2.91	0.09
103	P357	100.00	0.00	0.00
104	P357	100.00	0.00	0.00
105	P357	100.00	0.00	0.00
106	P357	100.00	0.00	0.00
107	P358	99.95	0.00	0.05
108	P358	100.00	0.00	0.00
109	P358	100.00	0.00	0.00
110	P358	100.00	0.00	0.00
111	P358	100.00	0.00	0.00
112	P358	80.19	19.01	0.79
113	P358	55.66	44.27	0.07
114	P358	100.00	0.00	0.00
115	P358	99.97	0.00	0.03
116	P358	92.97	6.53	0.51
117	P361	98.85	0.12	1.03
118	P361	100.00	0.00	0.00
119	P361	97.30	0.00	2.70
120	P361	100.00	0.00	0.00
121	P361	100.00	0.00	0.00
122	P361	99.94	0.00	0.06
123	P361	100.00	0.00	0.00

Continuation Appendix 1c.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
124	P361	98.70	0.00	1.30
125	P361	100.00	0.00	0.00
126	P361	98.48	0.72	0.80
127	P361	99.89	0.00	0.11
128	P361	100.00	0.00	0.00
129	P361	90.42	9.38	0.20
130	P361	99.82	0.11	0.07
131	P361	100.00	0.00	0.00
132	P361	99.93	0.04	0.03
133	P362	71.74	27.48	0.79
134	P362	100.00	0.00	0.00
135	P362	100.00	0.00	0.00
136	P362	100.00	0.00	0.00
137	P362	100.00	0.00	0.00
138	P362	99.99	0.00	0.01
139	P362	64.25	32.84	2.91
140	P362	99.95	0.00	0.05
141	P362	96.94	0.98	2.08
142	P362	100.00	0.00	0.00
143	P362	100.00	0.00	0.00
144	P362	99.11	0.00	0.89
145	P362	99.79	0.00	0.21
146	P362	99.91	0.09	0.00
147	P365	100.00	0.00	0.00
148	P365	100.00	0.00	0.00
149	P365	100.00	0.00	0.00
150	P365	100.00	0.00	0.00
151	P365	99.32	0.00	0.68
152	P365	100.00	0.00	0.00
153	P365	100.00	0.00	0.00
154	P365	100.00	0.00	0.00
155	P366	100.00	0.00	0.00
156	P366	100.00	0.00	0.00
157	P366	100.00	0.00	0.00
158	P366	100.00	0.00	0.00
159	P366	99.19	0.42	0.39
160	P366	99.28	0.47	0.25
161	P366	99.99	0.00	0.01
162	P366	100.00	0.00	0.00
163	P369	99.96	0.01	0.03
164	P369	100.00	0.00	0.00

Continuation Appendix 1c.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
165	P369	100.00	0.00	0.00
166	P369	100.00	0.00	0.00
167	P369	100.00	0.00	0.00
168	P369	60.07	39.69	0.00
169	P369	100.00	0.00	0.00
170	P369	99.99	0.00	0.01
171	P369	100.00	0.00	0.00
172	P369	100.00	0.00	0.00
173	P369	99.99	0.00	0.01
174	P369	99.93	0.00	0.07
175	P369	100.00	0.00	0.00
176	P369	100.00	0.00	0.00
177	P369	100.00	0.00	0.00
178	P386	100.00	0.00	0.00
179	P386	100.00	0.00	0.00
180	P386	99.99	0.00	0.01
181	P386	100.00	0.00	0.00
182	P386	100.00	0.00	0.00
183	P386	100.00	0.00	0.00
184	P386	100.00	0.00	0.00
185	P386	99.57	0.43	0.00
186	P386	99.92	0.00	0.08
187	P386	100.00	0.00	0.00
188	P386	99.67	0.00	0.33
189	P386	100.00	0.00	0.00
190	P386	100.00	0.00	0.00
191	P386	99.99	0.00	0.01
192	P386	100.00	0.00	0.00
193	P433	99.98	0.00	0.02
194	P433	99.97	0.00	0.03
195	P433	99.69	0.10	0.21
196	P433	100.00	0.00	0.00
197	P433	99.81	0.09	0.10
198	P433	98.05	1.95	0.00
199	P437	98.28	1.44	0.27
200	P437	90.58	9.17	0.24
201	P437	99.99	0.00	0.01
202	P437	85.45	14.52	0.04
203	P437	100.00	0.00	0.00
204	P437	99.99	0.00	0.01
205	P438	99.98	0.00	0.02

Continuation Appendix 1c.

Seedling	Family	Non-affected Area (%)	Browning (%)	Cavities (%)
206	P438	99.72	0.00	0.28
207	P438	100.00	0.00	0.00
208	P438	100.00	0.00	0.00
209	P438	86.83	13.10	0.09
210	P438	83.95	16.01	0.04
211	P438	100.00	0.00	0.00

Appendix 2a. Breeding values for parents for fruit weight, diameter and height. Maximum and minimum values are in bold, and the parent means are given at the bottom.

Seedling/ Cultivar	Fruit Weight (g)	Fruit Weight (g)*	s.e.	Fruit Diameter (mm)	s.e.	Fruit Height (mm)	Fruit Height (mm)*	s.e.
Conference	130.1	11.34	0.92	60.18	3.37	75.16	1.87	0.03
P1	136.1	11.50	1.26	58.45	4.63	**83.97**	**1.93**	0.04
P2	152.3	12.05	1.19	64.75	4.36	70.48	1.84	0.04
P3	189.5	13.59	1.25	70.42	4.86	67.21	1.82	0.04
P4	**268.9**	**16.30**	0.80	**79.46**	2.91	80.42	1.90	0.03
P5	133.6	11.39	0.67	61.78	2.50	64.10	1.80	0.02
P6	153.4	12.07	1.32	64.88	5.10	62.94	1.79	0.04
P7	140.2	11.82	0.96	61.61	3.54	73.18	1.86	0.03
P8	203.8	13.89	1.26	71.07	4.88	69.84	1.83	0.04
P9	155.4	12.44	1.38	65.05	5.31	69.30	1.84	0.05
P10	149.4	12.13	1.20	63.01	4.43	72.50	1.86	0.04
P11	142.4	11.85	1.28	63.26	4.82	72.26	1.85	0.04
P12	160.8	12.44	0.80	65.33	2.91	77.82	1.89	0.03
P13	**97.8**	**9.81**	0.91	**52.02**	3.35	79.13	1.90	0.03
P14	137.3	11.59	1.24	62.95	4.65	61.63	1.79	0.04
P15	161.9	12.59	0.69	67.45	2.56	65.56	1.81	0.02
P16	106.3	10.30	1.09	57.69	4.08	59.93	1.78	0.04
P17	126.5	11.23	0.70	63.06	2.58	**55.53**	**1.75**	0.02
Average	***152.5***	***12.13***	***1.05***	***64.02***	***3.94***	***70.05***	***1.84***	***0.04***

* Transformed variables (see Table 4)

Appendix 2b. Breeding values for parents for TCA and yield. Maximum and minimum values are in bold, and the parent means are given at the bottom.

Seedling/ Cultivar	TCA (cm^2)	TCA (cm^2)*	s.e.	Yield (g)	Yield (g)*	s.e.
Conference	36.32	6.07	0.48	4513	3.63	0.11
P1	19.96	4.31	0.66	3875	3.53	0.15
P2	**10.32**	**3.05**	0.62	1971	3.30	0.15
P3	18.13	4.08	0.74	6331	3.73	0.15
P4	27.60	5.13	0.41	**10400**	**3.92**	0.10
P5	20.12	4.46	0.37	5190	3.68	0.08
P6	18.44	4.18	0.77	3121	3.50	0.16
P7	18.03	4.19	0.51	3441	3.55	0.12
P8	21.27	4.48	0.74	5887	3.66	0.15
P9	13.69	3.55	0.80	4024	3.60	0.17
P10	33.56	5.87	0.64	8100	3.85	0.15
P11	**36.95**	**6.29**	0.70	4276	3.63	0.16
P12	30.65	5.44	0.42	3118	3.48	0.10
P13	19.56	4.33	0.48	**1752**	**3.31**	0.11
P14	14.41	3.51	0.68	2955	3.44	0.15
P15	27.45	5.15	0.37	7616	3.79	0.08
P16	23.61	4.49	0.60	4391	3.51	0.13
P17	21.67	4.56	0.37	4656	3.61	0.09
Average	***22.87***	***4.62***	***0.58***	***4757***	***3.60***	***0.13***

* Transformed variables (see Table 4)

Appendix 2c. Breeding values for parents for B, glucose and fructose. Maximum and minimum values are in bold, and the parent means are given at the bottom..

Seedling/ Cultivar	Boron ($\mu g.g^{-1}$)	Boron ($\mu g.g^{-1}$)*	s.e.	Glucose ($mg.g^{-1}$)	Glucose ($mg.g^{-1}$)*	s.e.	Fructose ($mg.g^{-1}$)	s.e.
Conference	2.06	1.41	0.13	7.69	2.91	0.25	71.49	4.54
P1	**1.74**	**1.34**	0.18	7.82	2.84	0.35	67.19	6.16
P2	**4.92**	**2.17**	0.17	12.21	3.45	0.33	60.83	5.85
P3	3.23	1.78	0.19	20.28	4.50	0.37	58.66	5.71
P4	3.55	1.84	0.12	**20.64**	**4.56**	0.22	58.72	3.94
P5	3.78	1.91	0.10	15.09	3.86	0.19	**51.34**	3.18
P6	3.55	1.87	0.20	13.50	3.63	0.39	57.69	6.10
P7	2.99	1.67	0.14	12.73	3.54	0.26	59.12	4.66
P8	3.67	1.89	0.19	17.40	4.14	0.37	57.10	5.79
P9	3.18	1.76	0.20	16.33	4.00	0.40	57.44	6.38
P10	4.14	2.01	0.17	11.33	3.36	0.33	56.25	5.90
P11	3.05	1.70	0.19	13.87	3.68	0.36	**74.50**	6.07
P12	2.22	1.46	0.12	**7.27**	**2.83**	0.22	72.75	3.90
P13	4.63	2.16	0.13	10.96	3.30	0.25	55.67	4.49
P14	3.06	1.74	0.18	13.82	3.66	0.35	63.43	5.84
P15	3.33	1.82	0.10	13.02	3.55	0.19	68.43	3.36
P16	3.97	2.03	0.16	17.01	4.06	0.31	71.72	5.16
P17	3.28	1.81	0.10	17.14	4.07	0.19	62.35	3.40
Average	***3.35***	***1.80***	***0.15***	***13.78***	***3.66***	***0.30***	***62.48***	***5.03***

* Transformed variables (see Table 4)

Appendix 2d. Breeding values for parents for sucrose, sorbitol and total sugars. Maximum and minimum values are in bold, and the parent means are given at the bottom.

Seedling/ Cultivar	Sucrose (mg/g)	s.e.	Sorbitol (mg/g)	Sorbitol (mg/g)*	s.e.	Total Sugars (mg/g)	s.e.
Conference	9.25	3.19	19.52	4.31	0.28	110.3	5.73
P1	10.53	4.38	21.44	4.54	0.36	107.6	7.58
P2	16.07	4.13	19.23	4.25	0.35	105.6	7.28
P3	4.33	4.52	22.56	4.68	0.28	105.3	6.33
P4	**0.28**	2.76	23.99	4.86	0.25	105.0	5.04
P5	5.00	2.35	23.02	4.74	0.19	**96.0**	3.85
P6	6.35	4.83	20.55	4.43	0.31	101.8	6.89
P7	6.27	3.35	22.34	4.66	0.28	104.3	5.78
P8	1.91	4.60	22.04	4.62	0.30	102.4	6.53
P9	8.64	4.96	22.50	4.68	0.32	105.3	7.18
P10	7.67	4.18	22.73	4.72	0.31	98.9	6.84
P11	8.54	4.53	19.83	4.35	0.32	115.1	7.08
P12	17.84	2.76	20.40	4.38	0.25	**118.1**	5.00
P13	**23.61**	3.17	**25.45**	**4.99**	0.28	114.9	5.74
P14	3.13	4.37	19.92	4.36	0.30	101.7	6.70
P15	9.16	2.42	20.83	4.48	0.21	109.4	4.23
P16	3.69	3.84	**18.95**	**4.23**	0.28	108.5	6.06
P17	6.99	2.44	20.58	4.43	0.21	105.1	4.26
Average	***8.29***	***3.71***	***21.44***	***4.54***	***0.28***	***106.4***	***6.01***

* Transformed variables (see Table 4)

Appendix 2e. Breeding values for parents for disorder traits. Maximum and minimum values are in bold, and the parent means are given at the bottom.

Seedling/ Cultivar	Un affected Area (%)	Un affected Area (%)*	s.e.	Browning (%)	Browning (%)*	s.e.	Cavities (%)	Cavities (%)*	s.e.
Conference	94.64	1.44	0.05	5.18	0.132	0.053	0.044	0.008	0.010
P1	97.30	1.50	0.06	2.51	0.066	0.067	0.089	0.015	0.012
P2	92.63	1.42	0.06	7.33	0.157	0.065	0.136	0.020	0.012
P3	97.28	1.49	0.05	2.60	0.075	0.053	0.076	0.016	0.010
P4	96.79	1.49	0.06	3.11	0.072	0.047	0.079	0.013	0.008
P5	**98.89**	**1.53**	0.03	0.93	0.028	0.035	0.159	0.020	0.006
P6	96.62	1.48	0.06	3.29	0.087	0.059	0.082	0.016	0.011
P7	97.95	1.50	0.05	1.90	0.056	0.053	0.091	0.016	0.010
P8	97.06	1.49	0.05	2.85	0.074	0.056	0.077	0.015	0.010
P9	96.55	1.47	0.06	3.34	0.094	0.061	0.106	0.019	0.011
P10	97.86	1.51	0.06	1.98	0.049	0.059	0.072	0.012	0.011
P11	95.42	1.46	0.06	4.28	0.102	0.062	0.055	0.011	0.011
P12	**90.05**	**1.37**	0.05	**9.89**	**0.205**	0.047	0.103	0.011	0.008
P13	95.84	1.46	0.05	3.69	0.087	0.054	**0.515**	**0.043**	0.010
P14	98.12	1.51	0.05	1.71	0.051	0.057	0.064	0.014	0.010
P15	97.78	1.52	0.04	2.15	0.047	0.039	**0.004**	**0.007**	0.007
P16	98.85	1.53	0.05	**0.88**	**0.026**	0.053	0.051	0.011	0.010
P17	98.38	1.51	0.04	1.43	0.047	0.039	0.070	0.014	0.007
Average	***96.56***	***1.48***	***0.05***	***3.28***	***0.081***	***0.053***	***0.104***	***0.016***	***0.010***

* Transformed variables (see Table 4)

Appendix 3a. Breeding values per family for fruit weight, fruit diameter and fruit height. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	F. Weight (g)	F. Weight (g)*	s.e.	F. Diameter (mm)	s.e.	F. Height (mm)	F. Height (mm)*	s.e.
C1	130.5	11.39	1.02	62.59	3.34	63.82	1.80	0.037
C2	158.4	12.33	1.00	66.14	3.30	67.92	1.83	0.036
C3	141.3	11.77	1.00	63.91	3.30	67.15	1.82	0.036
C4	154.4	12.28	1.00	65.53	3.31	70.75	1.84	0.036
C5	201.6	13.93	1.03	69.96	3.35	**79.68**	**1.90**	0.037
C6	124.8	11.11	1.02	60.70	3.34	65.84	1.81	0.037
C7	111.9	10.54	1.02	57.62	3.34	66.88	1.82	0.037
C8	135.1	11.48	1.02	62.39	3.35	62.10	1.79	0.037
C9	147.2	12.01	1.00	65.05	3.29	64.42	1.80	0.036
C10	149.3	12.16	1.01	64.14	3.31	70.25	1.84	0.037
C11	143.3	11.72	1.02	63.46	3.33	66.92	1.81	0.037
C12	117.9	10.69	0.99	57.32	3.29	72.27	1.85	0.036
C13	**110.2**	**10.36**	0.99	**55.73**	3.29	71.36	1.85	0.036
C14	142.1	11.79	1.02	62.27	3.33	69.23	1.84	0.037
C15	133.9	11.41	1.02	59.69	3.34	75.40	1.87	0.037
C16	**227.9**	**14.83**	1.01	**74.89**	3.31	73.72	1.86	0.036
C17	125.4	11.10	1.00	61.56	3.29	**58.55**	**1.76**	0.036
C18	197.1	13.86	1.01	71.94	3.32	67.35	1.82	0.037
C19	169.8	12.96	1.03	67.47	3.37	68.01	1.83	0.038
C20	178.4	12.93	1.02	68.12	3.34	64.48	1.80	0.037
Average	***150.0***	***12.03***	***1.01***	***64.02***	***3.32***	***68.31***	***1.83***	***0.037***

* Transformed variables (see Appendix 4)

Appendix 3b. Breeding values per family for TCA and yield. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	TCA (cm^2)	TCA (cm^2)*	s.e.	Yield (g)	Yield (g)*	s.e.
C1	29.73	5.43	0.353	4881	3.64	0.125
C2	22.56	4.50	0.351	3647	3.51	0.122
C3	29.12	5.38	0.351	4630	3.59	0.122
C4	28.70	5.27	0.351	4622	3.60	0.123
C5	**33.61**	**5.81**	0.353	7568	3.81	0.125
C6	31.16	5.50	0.352	4319	3.57	0.125
C7	28.90	5.10	0.353	4359	3.54	0.125
C8	14.25	3.60	0.353	3306	3.50	0.125
C9	23.22	4.75	0.350	6233	3.74	0.122
C10	22.58	4.66	0.351	5502	3.67	0.123
C11	13.94	3.60	0.352	3385	3.46	0.124
C12	20.75	4.53	0.350	3505	3.49	0.121
C13	18.54	4.22	0.350	**3251**	**3.48**	0.121
C14	28.96	5.41	0.352	7235	3.81	0.124
C15	19.76	4.36	0.353	4536	3.60	0.125
C16	29.31	5.32	0.351	**10314**	**3.90**	0.123
C17	21.98	4.65	0.350	5031	3.65	0.122
C18	21.51	4.50	0.352	6039	3.71	0.123
C19	**13.81**	**3.56**	0.354	4792	3.65	0.126
C20	19.44	4.28	0.352	4220	3.56	0.125
Average	***23.59***	***4.72***	***0.352***	***5069***	***3.62***	***0.124***

* Transformed variables (see Appendix 4)

Appendix 3c. Breeding values per family for B, glucose and fructose. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	B ($\mu g.g^{-1}$)	B ($\mu g.g^{-1}$)*	s.e.	Glucose ($mg.g^{-1}$)	Glucose ($mg.g^{-1}$)*	s.e.	Fructose ($mg.g^{-1}$)	s.e.
C1	2.61	1.60	0.143	10.99	3.30	0.229	66.17	5.22
C2	2.76	1.64	0.141	12.24	3.47	0.227	66.34	5.09
C3	2.62	1.60	0.141	11.81	3.40	0.227	67.31	5.09
C4	2.82	1.66	0.142	**10.16**	**3.18**	0.228	72.27	5.12
C5	2.59	1.56	0.144	14.26	3.76	0.230	65.25	5.29
C6	3.55	1.87	0.143	15.89	3.93	0.229	**73.94**	5.24
C7	3.10	1.75	0.143	12.54	3.51	0.229	71.75	5.23
C8	3.24	1.78	0.144	13.97	3.70	0.230	57.04	5.26
C9	3.57	1.87	0.140	12.97	3.58	0.227	57.45	5.05
C10	3.11	1.73	0.142	12.82	3.54	0.228	63.84	5.15
C11	**4.51**	**2.11**	0.143	13.66	3.66	0.229	55.68	5.22
C12	4.37	2.08	0.140	13.13	3.60	0.226	54.06	5.05
C13	4.09	2.01	0.140	12.74	3.54	0.226	**52.26**	5.05
C14	4.09	1.99	0.143	12.74	3.55	0.229	52.79	5.44
C15	**2.55**	**1.56**	0.144	10.84	3.26	0.229	59.74	5.27
C16	3.40	1.82	0.142	17.72	4.18	0.228	64.90	5.13
C17	3.50	1.86	0.141	17.61	4.15	0.227	59.34	5.07
C18	3.73	1.93	0.142	18.24	4.21	0.228	58.55	5.15
C19	3.14	1.76	0.145	**18.75**	**4.31**	0.230	57.45	5.35
C20	3.72	1.92	0.144	15.65	3.91	0.229	56.89	5.30
Average	***3.35***	***1.80***	***0.142***	***13.94***	***3.69***	***0.228***	***61.65***	***5.19***

* Transformed variables (see Appendix 4)

Appendix 3d. Breeding values per family for contents of sucrose, sorbitol and T. sugars. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	Sucrose ($mg.g^{-1}$)	s.e.	Sorbitol ($mg.g^{-1}$)	Sorbitol ($mg.g^{-1}$)*	s.e.	T. Sugars ($mg.g^{-1}$)	s.e.
C1	10.18	3.29	19.73	4.34	0.29	106.9	6.40
C2	10.72	3.25	20.92	4.47	0.28	111.7	6.17
C3	14.96	3.25	20.28	4.38	0.28	111.9	6.17
C4	12.90	3.26	20.53	4.42	0.29	**114.4**	6.24
C5	3.58	3.31	22.10	4.63	0.31	107.8	6.60
C6	5.96	3.29	19.31	4.28	0.30	112.5	6.53
C7	5.42	3.29	**18.96**	**4.24**	0.30	108.6	6.47
C8	**2.99**	3.31	21.20	4.52	0.29	97.4	6.46
C9	8.29	3.24	21.68	4.58	0.28	101.1	6.10
C10	7.61	3.27	21.75	4.59	0.29	107.1	6.34
C11	10.93	3.28	20.91	4.47	0.30	100.4	6.48
C12	13.25	3.23	**24.53**	**4.90**	0.28	106.0	6.13
C13	**16.49**	3.23	24.20	4.86	0.28	105.3	6.13
C14	6.26	3.29	23.12	4.76	0.29	**96.2**	6.43
C15	7.86	3.30	22.25	4.65	0.30	101.7	6.56
C16	4.61	3.26	22.62	4.69	0.28	108.5	6.25
C17	4.11	3.24	21.94	4.61	0.28	101.9	6.16
C18	3.26	3.27	21.80	4.58	0.29	102.8	6.28
C19	6.71	3.32	22.62	4.69	0.31	105.4	6.73
C20	3.76	3.63	20.99	4.48	0.31	101.5	6.69
Average	***7.99***	***3.29***	***21.57***	***4.56***	***0.29***	***105.5***	***6.37***

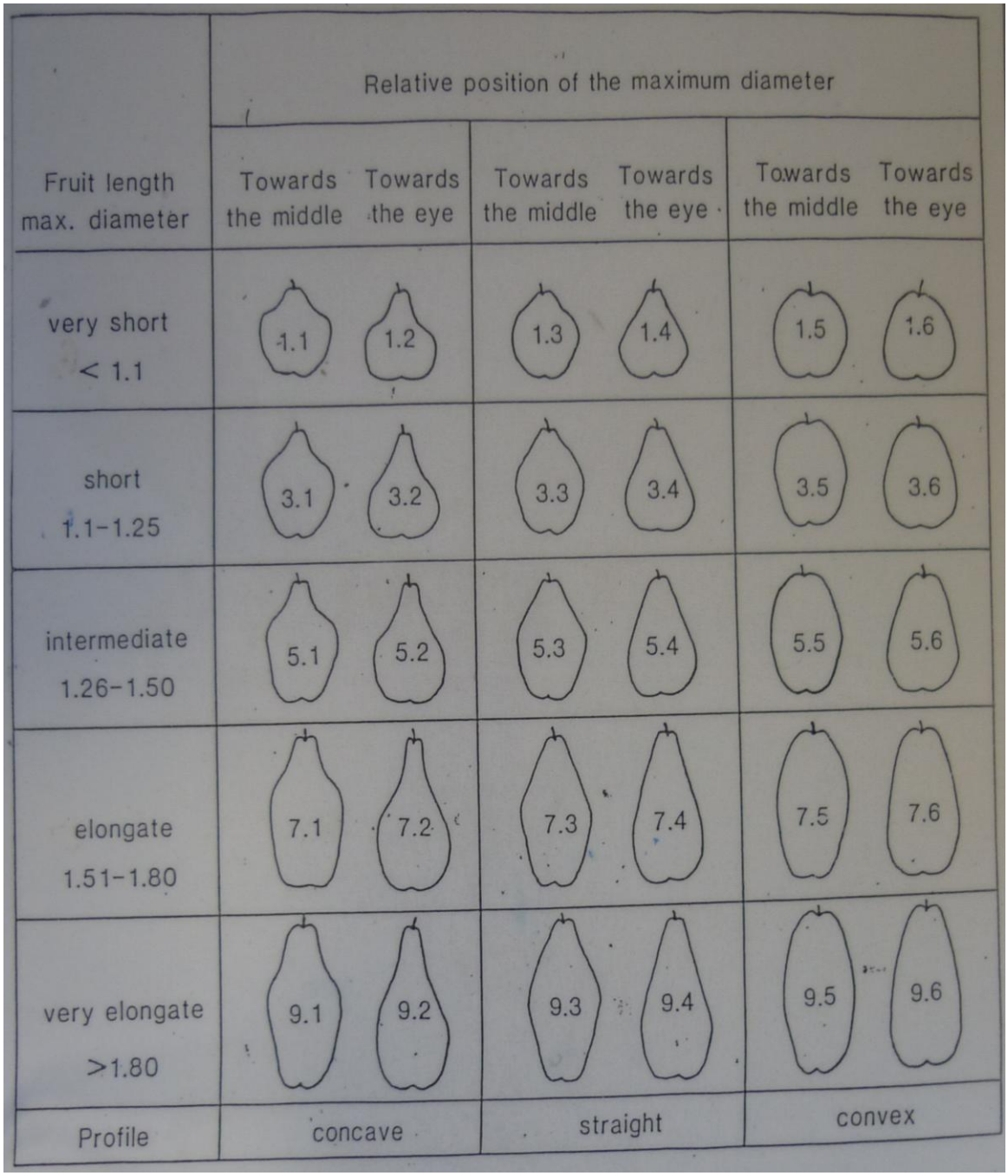
* Transformed variables (see Appendix 4)

Appendix 3e. Breeding values per family for disorder traits. Maximum and minimum values are in bold, and the mean of the family means is given at the bottom.

Family	Non-affected Area (%)	Non-affected Area (%)*	s.e.	Browning (%)	Browning (%)*	s.e.	Cavities (%)	Cavities (%)*	s.e.
C1	96.61	1.47	0.053	3.21	0.093	0.055	0.058	0.011	0.0102
C2	94.43	1.45	0.051	5.43	0.121	0.053	0.078	0.012	0.0098
C3	93.85	1.44	0.051	6.02	0.131	0.053	0.098	0.013	0.0098
C4	**93.66**	**1.43**	0.052	**6.30**	**0.133**	0.054	0.049	0.009	0.0099
C5	95.66	1.46	0.056	4.20	0.103	0.058	0.054	0.009	0.0106
C6	97.15	1.50	0.055	2.55	0.063	0.058	0.049	0.011	0.0105
C7	97.00	1.49	0.054	2.78	0.074	0.057	0.042	0.008	0.0104
C8	98.58	1.52	0.053	1.23	0.040	0.056	0.114	0.018	0.0102
C9	98.42	1.52	0.050	1.46	0.037	0.052	0.082	0.015	0.096
C10	98.02	1.51	0.053	1.86	0.048	0.055	0.048	0.012	0.0101
C11	95.52	1.47	0.054	4.38	0.097	0.057	0.147	0.020	0.0104
C12	97.49	1.50	0.051	2.16	0.055	0.053	0.344	0.032	0.0097
C13	97.18	1.49	0.051	2.49	0.061	0.053	**0.357**	**0.033**	0.097
C14	98.50	1.52	0.053	1.32	0.035	0.056	0.112	0.016	0.0102
C15	98.23	1.52	0.055	1.58	0.044	0.057	0.119	0.018	0.0105
C16	97.29	1.51	0.052	2.63	0.058	0.054	**0.037**	**0.010**	0.0099
C17	**98.74**	**1.53**	0.051	**1.08**	**0.034**	0.053	0.104	0.016	0.0098
C18	97.75	1.50	0.052	2.10	0.057	0.054	0.072	0.014	0.0099
C19	96.82	1.48	0.057	3.08	0.089	0.059	0.091	0.018	0.0108
C20	96.76	1.48	0.057	3.16	0.084	0.060	0.077	0.016	0.0109
Average	***96.88***	***1.49***	***0.053***	***2.95***	***0.073***	***0.055***	***0.107***	***0.016***	***0.0101***

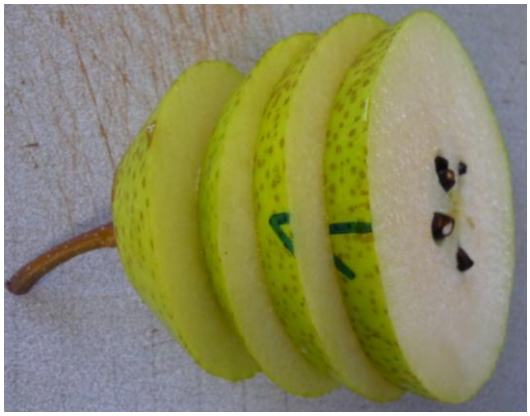
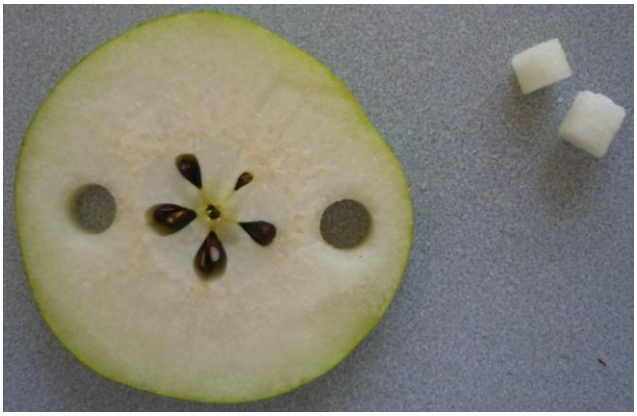
* Transformed variables (see Appendix 4)

Appendix 4. Plant and Food Shape Chart.



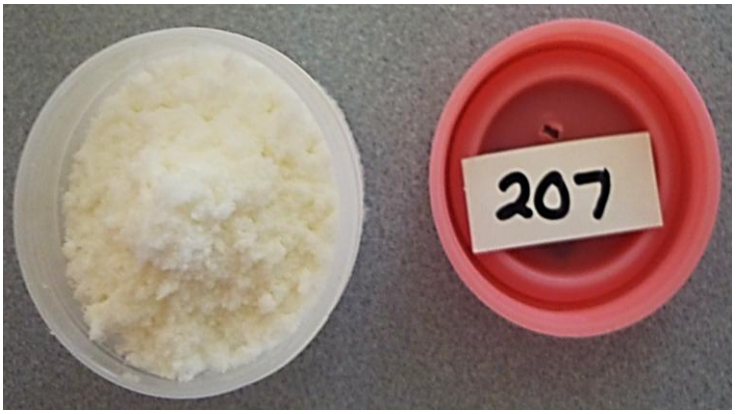
Appendix 5. Images of the phenotypic variability of the seedlings evaluated in the breeding population. Due to commercial sensitivity, the description of each pear genotypes below has been omitted.

Appendix 6a. Sampling of the pears during the evaluation of FSD. Slicing of the top stem from above the equator (left). Sampling of fruit flesh (right).

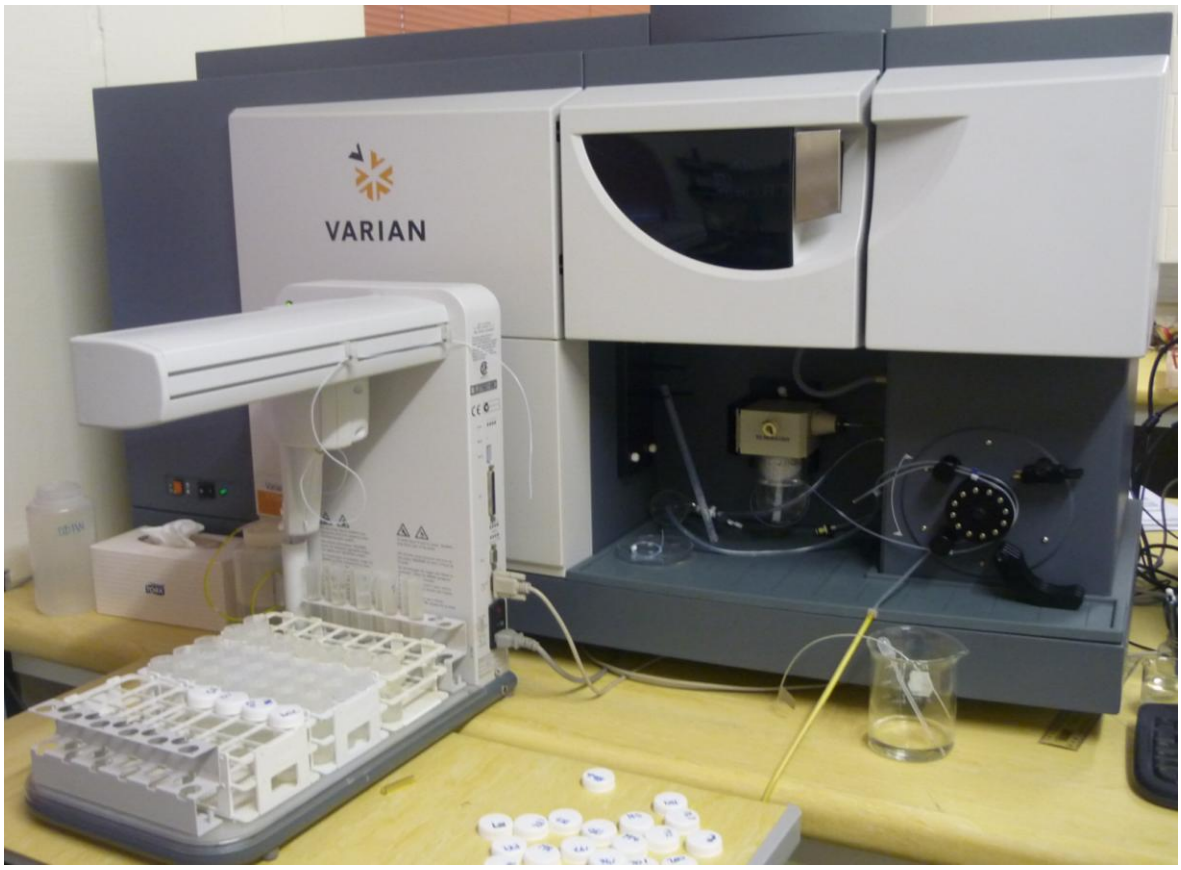



Appendix 6b. Grinding of samples to obtain fruit powder as final product.

Appendix 6c. Fruit flesh samples before and after the grinding process. Samples freeze by liquid nitrogen (left). Ground sample (right).

Appendix 7a. Spectroscopy equipment used for the mineral analysis.



Appendix 7b. HPLC equipment used for the sugar analysis.