# CONSUMER PREFERENCE (OF RED-FLESHED APPLES) AND QUANTIFICATION OF QUALITY RELATED TRAITS, PARTICULARLY SKIN AND FLESH COLOUR, IN APPLE BREEDING FAMILIES.

by

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# Declaration

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# Abstract

In order to develop a novel pink- or red-fleshed apple for the fresh consumer market, the red-fleshed genotype, 'KAZ 91' (*Malus niedzwetzkyana* Dieck.), was crossed with 'Meran' (*M. domestica* Borkh) at the Agricultural Research Council (ARC) apple breeding programme in South Africa. The objective of this study was to evaluate and quantify colour variability in the flesh and peel of this progeny (Family 1), to assess quality traits (i.e., acidity and total soluble solids) and phenolic levels in fruit peel and flesh, and to investigate consumer preference for the taste and appearance of red-fleshed apples. All data, except for consumer preference, were also collected on two white-fleshed *M. domestica* Borkh families, i.e., 'Reinette Burchardt' x 'Treco Red Gala' (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3).

Fruit of Family 1 seedlings were on average darker red with greater blush coverage and higher anthocyanin and phenolic levels compared to fruits of Family 2 and 3. The proportion of bearing trees with red-fleshed fruits in Family 1 increased from 25% in 2007 to 35% in 2008. The intensity and distribution of red pigmentation in the flesh varied considerably between seedlings and even between individual apples from the same tree. A high intraclass correlation coefficient was found for red-flesh coverage within Family 1, indicating a high level of genetic determination that can be used in breeding. When only red-fleshed seedlings were considered, an intermediate repeatability coefficient (0.54) for red-flesh coverage indicates that the extent of red flesh coverage varies to some extent between seasons. The effect of environmental factors on red flesh colour needs to be assessed and breeders need to take care to select for genotypes with stable flesh colour intensity and coverage. Small intraclass correlations were found between families for other traits. No correlation was found for anthocyanin and total phenolics in both peel and flesh or between anthocyanin levels in the peel and flesh of Family 1 fruit. This suggests that red-fleshed fruit will not necessarily be high in antioxidants – since phenolics is by far the greatest contributor to fruit antioxidant capacity. The lack of a correlation between peel and flesh anthocyanin levels also suggest that fruit with dark red flesh will not necessarily have a dark red skin colour. Family 1 fruit were more prone to flesh browning and were more acidic compared to fruit of Families 2 and 3.

With regard to consumer preference for flesh colour, 74% of South African consumers preferred white flesh while 64% preferred an attractive “floral” pattern created by the combination of a red cortex and white core. Consumers indicated a much lower liking for other distribution patterns and lower intensities of red flesh colour. Consumers preferred the taste of apples that were crisp, crunchy and high in apple flavour irrespective of flesh colour. Red-fleshed fruit were generally acidic or had poor texture, and some were also astringent. However, despite a general dislike in acidic fruit, consumers showed a preference for acidic fruit if that fruit also had high red-flesh coverage.

# Opsomming

Appeltelers van die Suid-Afrikaanse Landbounavorsingsraad (LNR) Infruitec-Nietvoorbij poog om unieke appelkultivars met ‘n pienk of rooi vleiskleur te ontwikkel. Vir hierdie doel het hulle die rooivleis genotipe, ‘KAZ 91’ (*Malus niedzwetzkyana* Dieck), gekruis met ‘Meran’ (*M. domestica* Borkh). Hierdie studie is uitgevoer ten einde kleurvariasie, interne kwaliteitseienskappe (i.e., suurheid en totale oplosbare vastestowwe) en vlakke van fenole in die vleis en skil van bogenoemde kruisingkombinasie se nageslag (Familie 1) te evalueer en te kwantifiseer asook om verbruikersvoorkeure vir die smaak en voorkoms van rooivleis appels te bestudeer. Buiten vir verbruikersvoorkeure, is alle data ook ingesamel vir twee witvleis *M. domestica* families, naamlik ‘Reinette Burchardt’ x ‘Treco Red Gala’ (Familie 2) en ‘Meran’ x ‘Treco Red Gala’ (Familie 3).

Familie 1 saailinge se vrugte was gemiddeld donkerder rooi met ‘n groter rooi blos en hoër antosianien- en fenoolvlakke in vergeleke met vrugte van Families 2 en 3. Van die Familie 1 saailinge wat wel vrugte gedra het, het 25% en 35% in onderskeidelik 2007 en 2008 vrugte met rooi vleis gehad. Die intensiteit en verspreiding van rooi pigmentasie in die vleis het aansienlik varieer tussen saailinge en selfs tussen individuele appels van dieselfde boom. ‘n Hoë intraklas korrelasie koëffisiënt is gevind vir die proporsie van die vleis met rooi pigmentasie in Familie 1 nageslag. Dit dui op ‘n hoë vlak van genetiese determinasie en vinnige vordering met teling vir hierdie eienskap. Indien net rooi-vleis saailinge egter oorweeg word, word ‘n intermediêre herhaalbaarheid koëffisiënt (0.54) vir die proporsie van die vleis wat rooi is verkry, wat dui op aansienlike variasie tussen seisoene in die omvang van rooi pigmentasie. Die effek van omgewingsfaktore op rooi vleiskleur behoort dus bestudeer te word en telers moet let daarop om te selekteer vir genotipes met stabiele vleiskleur intensiteit en bedekking. Die intraklas korrelasies tussen families vir ander vrugeienskappe was klein. Antosianienvlakke en totale fenole in die skil en vleis van Familie 1 vrugte het nie gekorreleer nie. Dit dui daarop dat rooivleis appels nie noodwendig ‘n hoër antioksidantkapasiteit het nie – fenole maak by verre die grootste bydrae tot die antioksidantkapisiteit van vrugte. Antosianienvlakke in die skil en vleis van Familie 1 vrugte het ook nie gekorreleer nie wat daarop dui dat vrugte met ‘n donker rooi vleis nie noodwendig ook ‘n donker skilkleur sal hê nie. Familie 1 vrugte

was gemiddeld suurder as vrugte van Families 2 en 3 en Familie 1 vrugte se vleis het gemiddeld ook meer verbruining ondergaan.

Ten opsigte van verbruikersvoorkeur vir vleiskleur is gevind dat 74% van verbruikers ‘n wit vleiskleur verkies het terwyl 64% gehou het van die aantreklike “blom” patroon gevorm deur ‘n rooi korteks en wit kern. Verbruikers het aansienlik minder gehou van enige ander verspreiding van rooi pigment in die vleis of van ‘n laer intensiteit rooi kleur. In terme van smaak is geurige appels met ‘n bros tekstuur verkies ongeag hul vleiskleur. Rooivleisappels was oor die algemeen suur met ‘n swak tekstuur. Sommige rooivleisappels was ook frank. Tog, ten spyte van ‘n algemene afkeur in suur appels, het verbruikers ‘n voorkeur getoon vir vrugte met ‘n kombinasie van hoë suur en ‘n hoë proporsie vleis met rooi pigmentasie.

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# GENERAL INTRODUCTION

Breeding for red flesh colour in apples has become a major objective of various government funded and private breeding programs, e.g. Plant & Food Research in New Zealand (Volz *et al.,* 2006) and Next Fruit Generation in Switzerland (Freshplaza, 2008). Breeders at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij in South Africa aim to develop a novel cultivar with pink or red flesh colour making use of 'KAZ91' as a breeding parent. 'KAZ91' is a selection of *Malus niedzwetzkyana* Dieck., a red-fleshed wild apple species from the mountain forests of Kazakhstan (Dzhangaliev *et al.,* 2003).

The first objective of this study was to study colour variability in the flesh and peel of a 'KAZ 91' x 'Meran' progeny. Peel colour variability was also studied in 'Reinette Burchard' x 'Treco Red Gala' and 'Meran' x 'Treco Red Gala'. Quality-related traits, i.e., total soluble solids (TSS), titratable acidity and firmness were assessed within and between seedlings of these families. Variance in the distribution of red pigmentation within the flesh of red-fleshed progenies was also determined. Phenolic concentrations in the peel and flesh were assessed in order to correlate flesh colour with phenolic level (antioxidant potential) and peel colour. Since the flesh of *M. niedzwetzkyana* Dieck. fruits browns easily (Mulabagal *et al.,* 2007), we also assessed the browning potential of 'KAZ91' progeny. Heritability estimates and intraclass correlation coefficients for the various traits were calculated. Broad sense heritability indicates the extent to which an individual's phenotype is determined by its genotype (Falconer, 1989) and, therefore, the rapidity at which a breeder can select for the trait.

Despite the availability of red-fleshed cultivars originating from mutations of *M. domestica* Borkh (USDA, ARS, National Genetic Resources Program, 2009) or through crossbreeding with *M. niedzwetzkyana* Dieck. (Dzhangaliev *et al.,* 2003), none of these cultivars are among the major cultivars produced around the world (O'Rourke, 2008). In the case of cultivars derived from *M. niedzwetzkyana* Dieck., this may be due to the co-inheritance of undesirable traits, e.g. high acidity and poor texture, present in wild apples (Dzhangaliev, 2003). It may also be that due to the

predominance of white-fleshed apples, consumers are not inclined to prefer red-fleshed apples. Harker *et al.* (2003) found that consumers generally prefer apples that they are familiar with. Hence, the second objective of this study was to assess consumer preference for the pattern and intensity of red pigmentation in apple flesh as well as for the taste of red- and white-fleshed 'KAZ91' progeny.

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# LITERATURE REVIEW: ORIGIN AND CHARACHTERISTICS OF WILD *MALUS* SPECIES WITH EMPHASIS ON BREEDING FOR RED-FLESHED APPLES.

## 1. Introduction

O'Rourke, (2008) grouped apple cultivars into three groups; traditional majors which include Red Delicious, Golden Delicious and Granny Smith; new majors which include Gala/Royal Gala, Fuji, Braeburn and Jonagold/Jonagored; and all other cultivars. Traditional majors are produced in large volumes world-wide and are fully red, green or yellow in colour when ripe. The new majors are produced in increasing volumes and are predominantly full red or red blushed. Most of the new majors and new releases differ from the traditional majors in some or other characteristic, such as an attractive and striking appearance or an improved taste that should increase their attractiveness to consumers. Consumers are willing to pay more for novel cultivars with attractive fruit (O'Rourke, 2008). The pink blush cultivar, Cripp's Pink, is a point in case. Although only released to the market during the early 1970's, Cripp's Pink has become the 24$^{th}$ most produced cultivar in 2000 and is predicted to reach the 8$^{th}$ position in 2010 making it a new major cultivar (O'Rourke, 2005; O'Rourke, 2008). New improved selections of 'Cripp's Pink' that produce apples with better pink blush colour, i.e., 'Rosy Glow', 'Ruby Pink' and 'Lady in Red', have already been developed (Freshplaza, 2008).

Granny Smith, Golden Delicious and red cultivars such as Red Delicious have long dominated the South African apple industry. In recent years, the contribution of these cultivars to total production has decreased steadily in favour of more lucrative blush cultivars such as Fuji and Cripp's Pink (DFPT, 2008). The share of 'Cripp's Pink' to total plantings reached 7% in 2008 in South Africa (DFPT, 2008)

Novel cultivars must have unique quality characteristics such as a distinct and preferred colour, flavour, texture, etc. and should also make an impression on the

market and consumers (Reid and Buisson, 2001). Gamble *et al.* (2006) showed that consumers were willing to buy a novel red pear cultivar even though it had less than optimum flavour. The authors suggested that a bright red or spotted red pear will stand a good chance to be preferred by consumers due to its novelty and ease of recognition among the traditional green, yellow and brown pears (Gamble *et al.,* 2006; Jaeger *et al.,* 2003). Following the same line of thinking, many apple breeding programmes aim to breed a cultivar that differs markedly in appearance from existing full red, blushed, green and yellow cultivars. An avenue of breeding for novelty that is pursued by the apple breeding program of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij of South Africa is the development of cultivars with a red or pink flesh colour. The breeders hope to achieve this by using a selection of the red-fleshed wild apple species, *Malus niedzwetkyana* Dieck., collected in 1996 from the wild apple forests of Kazakstan, as a breeding parent.

In this review, the origin and variation of *Malus* species in Kazakhstan, the positive and negative characteristics of these species, red pigmentation in apples and factors that influence anthocyanin biosynthesis will be discussed. The history of breeding for increased red pigmentation in flesh of apples and other fruit crops, health benefits of anthocyanins, consumer preference for apples and factors that influence consumer preference will also be discussed.

## 2. Wild *Malus* species

### 2.1. Origin and collection of *Malus* species.

Many fruits, most notably apples, pears and apricots are thought to have their origin in central Asia, China, Caucasia and Asia Minor (Forsline *et al.,* 2003; Sanada and Sato, 2003). The genus *Malus* contains 27 to 30 species (Janick *et al.,* 1996; Luby, 2003). In addition, inter-crossing and self incompatibility in some species resulted in interspecific hybrids (Forsline *et al.,* 2003) while some species are classified as cultivated species because there is no information indicating their origin as wild species (Forsline *et al.,* 2003). *M. domestica* Borkh, the domesticated apple as we know it today, originated through interspecific hybridization of various *Malus* species (Forsline *et al.,* 2003; Janick *et al.,* 1996; Luby, 2003). The centre of origin of *M.*

*domestica* is a controversial issue among taxonomists. The Caucasus mountain range of South-West Asia, the slopes of the Tien Shan mountain range between China, Kazakhstan and Kyrgyzstan in South-central Asia and western Asia are mentioned as possible centres of origin (Tromp *et al.,* 2005). However, *M. sieversii* Lebed M. Roem., the wild apple of Tien Shan, is regarded by most as the major progenitor of the domestic apple (Luby, 2003; Janick *et al.,* 1996). Wild apple seeds were transferred from the Tien Shan range along the "silk road" to all the areas mentioned above (Forsline *et al.* 2003). Three of the most prominent wild apple species with potential for inclusion in breeding programmes are discussed below.


**Fig.1** A map of central Asia indicating geographic sites where *M. sieversii* Lebed. M.Roem. is found along the Tien Shan mountain range. Adapted from (Karychev *et al.,* 2005; Forsline *et al.,* 2003).

## 2.2. Sieversii apples (*M. sieversii* (Lebed). M.Roem).

*M. sieverisii* grows 5-12 m tall in moist soils between 800-1500 m above sea level along the Tien Shan mountain range on the border between Kazakhstan and China. It occupies most of the fruit forests in Tarbagatai, Dzhungarskei, Zaliliyskei, Kungei,

Ters-kei, Kirghiz, Tallaskei Alatau, Ketmentau and Karatau (Fig. 1; Dzhangaliev *et al.,* 2003). *M. sieversii* fruit are rounded or oblate, rounded-cylindrical, cylindrical or round conical in shape while fruit skin and flesh colour varies from yellow to red and white to yellow, respectively. Mean fruit mass varies between 20-60 g with a maximum of 120 g. Fruit ripens from the end of July until early October (Northern Hemisphere) (Dzhangaliev *et al.,* 2003). Sieversii apples are juicy and tender, with a flavour that ranges from insipid and sweet, sour-sweet, sour to very sour with a bitter taste (Dzhangaliev *et al.,* 2003).

## 2.3. Kirghiz apple (*M. kirghisorum* Al. etAn.Theod.)

These apples are similar to Sieversii apples (Dzhangaliev, 2003). It grows well in deep, rich forest soils between 1200-1800 m on the northern slopes of Zailiyskei and Dzhungaskei Altau along the Tien Shan range (Fig. 1) and is resistant to frost. Fruits are globular or cylindrical in shape, narrow at the stalk and ripen from August until September. The fruit skin colour is green, yellow or reddish and the flesh colour is white with a flavour that is sour-sweet, acid to bitter and astringent. These apples also vary in size; they are about 3-8 cm long and 3-8 cm in diameter. These apples are used to make ciders, fruit paste, jam, stewed fruits, puree and juice (Dzhangaliev, 2003).

### 2. 4. *M. niedzwetzkyana* Dieck

Isolated trees of *M. niedzwetzkyana* Dieck. were found in the mountain forests of Karatau (Berkara Gorge), Western Tien–Shan (Mashat mountains), and Dzhungarskei (Pikhtovoe Gorge) during the 1996 collection of wild apples (Fig.1; Forsline *et al.,* 2003). These apples are winter hardy and drought resistant (Dzhangaliev *et al.,* 2003). The fruits of *M. niedzwetzkyana* Dieck. are violet-purple in colour with a bluish, waxy bloom (Fig. 2) while their flesh is pink to purple in colour (Dzhangaliev *et al.,* 2003). The fruit are normally large sized (>400 g) (Mulabagal *et al.,* (2007) and ripen from mid February in the Western Cape. These apples have different flavours ranging from sweet, sour, sweet-sour and are also astringent (personal communication I. Labuschagné). Mulabagal *et al.,* (2007) indicated that fruits of *M. niedzwetzkyana*

Dieck. are susceptible to browning and they are not juicy, however *M. niedzwetzkyana* Dieck. has been used as breeding parent in breeding programs i.e. some of its accession include cultivars such as Redfield and Redflesh.

**Fig. 2** Selection of *M. niedzwetzkyana* Dieck. (Picture courtesy of I. Labuschagné, ARC Infruitec – Nietvoorbij, 2008).

## 2.5. Positive and negative characteristics of wild *Malus* species.

Wild apples are used in breeding programs due to their resistance to a range of diseases such as apple scab, apple mildew, and crown and collar rot (Forsline *et al.,* 2003). They are also used to incorporate novel colour, aroma, flavour, size, and texture into breeding programs (Forsline *et al.,* 2003). In addition, wild apples may also be used to impart genes for reduced vigour and resistance against environmental stress such as drought and sun burn into breeding programmes (Forsline *et al.,* 2003).

Wild apples are often acidic and high in tannins which make them astringent (Dzhangaliev, 2003). Some of the wild apples have low sugar levels. Flavour ranges from sweet, sweetish-sour, sour, sweetish-bitter, bitter to sourish–bitter (Dzhangaliev, 2003). The difference in flavour is due to the differences in tannin levels and high

acidity. Wild apples also vary in fruit size ranging from small to large fruits (Dzhangaliev, 2003).

### 2.6. History of breeding for red pigmentation in apple flesh

Little research has been done on red-fleshed apples. In 1944, a new cultivar, Pink Pearl, was released for commercial cultivation by Albert Etter, an apple breeder from California, after crossing the pink/red-fleshed cultivar Surprise with a white-fleshed cultivar (Greenmantlenursery, 2009). Research on red-fleshed apples has been conducted by Plant & Food Research in New Zealand since 1998 (Volz *et al.,* 2009); red-fleshed apple varieties were introduced to Hort Research gene pool from wild apple forest of Kazakhstan and Krygyztan (The orchardist, 2006). Plant & Food Research reported that some of these apple cultivars were characterized by red-flesh and bitter taste (The orchardist, 2006). High quality white fleshed apples were crossed with accessions of red-fleshed cultivars to give rise to progenies with various colours white, pink to full purple (The orchardist, 2006, Volz *et al.,* 2006, Volz *et al.,* 2009). Plant & Food Research reported that there are some health benefits associated with the red-fleshed apple (The orchardist, 2006). No red-fleshed cultivar has yet been released from this breeding programme. In 2008, Next Fruit Generation (NFG) introduced a red-fleshed cultivar (Fig. 3) after 12 years of breeding by Swiss researchers starting off with a small, bitter red-fleshed apple from eastern Germany (Freshplaza, 2008). NFG indicated that they aim to commercialize the apple for different markets in partnership with interested parties.

## 3. Anthocyanins in red-fleshed apples.

Anthocyanins are vacuolar, water-soluble flavonoids produced via the shikimate pathway that are responsible for pink, red, purple and blue colours in fruits (Dong *et al.,* 1995; Gil *et al.,* 1997; Takos *et al.,* 2006). Anthocyanins are mostly found in epidermal tissue and sometimes in the sub-epidermal tissue of fruit. However, in red-fleshed apple (Fig. 3), the whole fruit may contain anthocyanins (Mazza and Velioglu, 1992).

Different patterns of flesh pigmentation occur depending on whether the cortex or core or both these tissues contain anthocyanins (Fig 4A & B); Chagne *et al.,* 2007).

**Fig. 3** Picture of red-fleshed apple bred by Next Fruit Generation (Freshplaza, 2008)

**A** **B**

**Fig. 4** (A) A fully red apple (cortex and core) and (B) red apple with (red cortex and white core) derived from a cross between 'KAZ 91' (*M. niedzwetzkyana* Dieck.) and 'Meran' (*M. domestica* Borkh.) (Picture by F. Thovhogi, ARC Infruitec – Nietvoorbij, 2008).

The major anthocyanin found in apple skin is cyanidin 3-galactoside (Lancaster *et al.,* 1994; Ubi *et al.,* 2006). Other anthocyanins that occur in small quantity include cyanidin 3-arabinoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, and cyanidin 3-glucoside (Ubi *et al.,* 2006). Mazza and Velioglu (1992) reported that the red-fleshed cultivar Scugog contained these same anthocyanins in its flesh. However, the anthocyanin profile in the flesh of 'Scugog' differs from that of apple skin in that the proportion of cyanidin 3-galactose was low while levels of cyanidin 3-glucoside, 3-arabinoside and 3-xyloside were high (Mazza and Velioglu, 1992). Mulabagal *et al.* (2007) found high levels of cyaniding-3-*O* –glucosyl rutinoside in three red-fleshed cultivars, *Malus* cv. Cranberry, Kerr and *M. niedzwetzkyana* Dieck.. The highest anthocyanin concentration was found in 'Cranberry (1.12 mg $g^{-1}$ FW) followed by 'Kerr' (0.55 mg $g^{-1}$ FW) and *M. niedzwetzkyana* Dieck. (0.36 mg $g^{-1}$ FW)

## 4. Regulation of anthocyanin synthesis.

According to Mazza and Miniati (1993), the distribution and heritability of anthocyanin pigmentation in apples are influenced by a number of genes. Honda *et al.* (2002) and Kim *et al.* (2003) found that red-skinned cultivars had high anthocyanin biosynthetic gene activity compared with non-red cultivars. High anthocyanin levels in the peel correlate with high expression levels of these genes. According to Allan *et al.* (2008), different MYB transcription factors control colour development in fruits. Three regulatory genes involved in red colour development in apple, namely MdMYB10, MdMYB1 and MdMYBA, were identified in different studies. Higher transcript levels of MdMYB1 were detected in red-skinned 'Cripp's Pink' compared to non-red 'Golden Delicious' (Takos *et al.,* 2006). In addition, re-exposure of fruit to sunlight after bagging resulted in an increase in the level MdMYB1 transcript after a lag period of one day and this resulted in an increase in anthocyanin accumulation in the apple skin (Takos *et al.,* 2006). Low temperature and UV-B irradiation stimulated the production of MdMYBA in the skin of apple and the dark red cultivar Jonathan had high level of MdMYBA expression compared to pale red Tsangaru (Ban *et al.,* 2007). Espley *et al.* (2007) found that anthocyanin synthesis in apple is enhanced by the over-expression of MdMYB10 resulting in plants with high levels of red

pigmentation in all their tissues including flesh. Ban *et al.* (2007) found that the cortex and skin of the red-fleshed cultivar Red Field also had high expression levels of MdMYB10. Espley *et al.* (2007) ascribed the difference in flesh pigmentation between red-fleshed and white-fleshed apple to differences in the activity of MdMYB10. Chagne *et al.* (2007) found that the fruits harvested from trees with red leaves were also red-fleshed indicating that a single gene controls the two traits and the *Rni* allele responsible for the red flesh and leaves was identified.

## 5. Environmental factors that influence anthocyanin production

No information is available on how climate and site may affect anthocyanin synthesis in the flesh of red or pink-fleshed apples. The influence of environmental factors on red colour development in apple skin has however been studied.

### 5.1. Light

The most effective wavelength in the induction of anthocyanin in apple skin is UV-B light (Lancaster, 1992; Ubi *et al.,* 2006). Blue and red light are less effective in stimulating anthocyanin synthesis (Arakawa *et al.,* 1985). Apple fruits that are not exposed to light are poorly coloured compared to fruits that receive sufficient sunlight (Sielgelman and Hendricks, 1958; Lancaster, 1992). The minimum sunlight required to form red colour is about 50%, while maximum red colour development is achieved at about 70% sunlight (Gurnsey and Lawes, 1999). Apple fruit skin from the shaded side of the tree accumulated high level of anthocyanin when irradiated at 20 °C (Lancaster *et al.,* 2000; Reay and Lancaster, 2001).

### 5.2. Temperature

Anthocyanin degradation in ‘Cripp’s Pink’ skin was stimulated by high temperature ≥30 ºC (Marias *et al.,* 2001; Steyn *et al.,* 2004) whereas anthocyanin synthesis is enhanced by low temperature (Curry, 1997; Lancaster *et al.,* 2000). High anthocyanin synthesis was found in ‘Red Chief ‘Delicious’ after pre-cooling for 48 h at 15 ºC (Curry, 1997). No anthocyanin synthesis occurred below 15 ºC and above 35 ºC

(Curry, 1997). Cold temperature (18 ºC) exposure during the night and moderate temperature during the day (20-25ºC) stimulates red colour formation, but high day temperatures can negate the effect of cold night temperature (Iglesias *et al.,* 2002). In this study, it was found that anthocyanin accumulation in 'Cripp's Pink' was enhanced by alternating temperature of 6/20ºC. Curry (1997) indicated that temperature affects pre-climacteric and post-climacteric fruits differently in red colour development. The optimum temperature for pre- and post-climacteric fruit was 25 ºC and 27 ºC, respectively.

## 6. Health benefits of anthocyanins

### 6.1. In humans

Anthocyanin extracts or pure anthocyanin have a wide range of positive effects on human health such as improving eye sight and brain cognitive function, and enhancing weight loss, decreasing ulceration and cardiovascular risk (Boyer and Liu, 2004; Espin *et al.,* 2007; Kong *et al.,* 2003). Pawlowicz *et al.* (2000) found that oxidative stress that causes complications during pregnancy can be controlled by natural anthocyanins. It was also shown that bilberries (*Vaccinium myrtillus)* containing high levels of anthocyanins can be used to control circulatory disorders and improve visual acuity (Pawlowicz *et al.,* 2000). In a study conducted *in vitro* with multiple cell types, anti-inflammatory effects were displayed through the ability of anthocyanin to inhibit mRNA and various interleukins (Wang and Stoner, 2008). Beattie *et al.* (2005) found that delphinidin and malvidin glycosides extracted from bilberry inhibit the growth of colon cancer cells. Extracts of pure anthocyanin and anthocyanin-rich fruits and vegetables used *in vitro* showed anti-proliferative activity towards multiple cancer cell types by blocking different stages in the cell cycle by exerting an effect through the cell cycle regulator protein (Wang and Stoner, 2008). Dried berries extract inhibited tumor initiation and progression. The initiation stage is inhibited through the reduction of carcinogen-induced DNA damage through the influence that is exerted on the carcinogen metabolism and the regression stage through the reduction of the pre-malignant cells growth (Stoner *et al.,* 2007).

### 6.2. In animals

Anthocyanin extracts from *Vaccinium* berries were found to enhance the memory of mice (Barros *et al.,* 2006, Shin *et al.,* 2006). The risk of diabetes and obesity may be reduced in animals that are given anthocyanins in their diet (Tsuda *et al.,* 2003). Kong *et al.* (2003) found that tart cherry inhibits tumor growth in mice. It has been indicated in laboratory test that apples have strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation and lower cholesterol (Boyer and Liu, 2004). Apples consumed with peel where able to reduce cancer cell proliferation better than apples consumed without peels (Boyer and Liu, 2004).

## 7. Sensory evaluation of apple and other fruits

### 7.1 The use of sensory analysis in fruit breeding

Sensory analysis is used to determine the degree of liking, preference and acceptability of different products by consumers. It is a scientific tool used to investigate; measure and explain the difference in the properties of food as these are recognized through sight, hearing, taste, smell and touch (Lawless and Heymann, 1998). Two types of sensory analysis may be used: 1) Analytical sensory evaluation is used to study the degree of variation in products or food through a trained panel, and 2) consumer sensory analysis is used to determine the degree of liking, preference and the preference and willingness to purchase a product by using the target consumers (Lawless and Heymann, 1998).

Sensory attributes mostly evaluated for apple include texture traits (crispness, hardness, firmness, crunchiness, juiciness, mealiness, skin toughness) and flavour traits (sweetness, sourness, astringency, aroma, odour) (Table 1; Kuhn and Thybo, 2001; Mehinagic *et al.,* 2004). Texture is based on changes in crispness, hardiness, crunchiness and juiciness during fruit maturation (Watkins, 2003). The re-arrangement of the primary wall and middle lamella in apple cells is the primary cause of loss in texture. A decrease in starch content and turgor may also result in

texture loss (Watkins, 2003). Flavour is a combination of taste and odor. Taste is characterized by the ability of the taste buds to sense sweetness, sourness, bitterness, and saltiness in the mouth. Consumer preference for taste comprises of a mixture of complex characteristics including concentrations of sugar, phenolics and organic acids (Watkins, 2003).

Descriptive sensory analysis enables breeders to select superior selections using limited resources and can also provide a good prediction of the consumer's response to the selections (Hampson *et al.,* 2000a; Kellerhals *et al.,* 1999). Hampson *et al.* (2000b) found that sensory results obtained from trained panels are more reliable when compared to evaluations by one or two individuals. When a sensory panel was used to predict liking of flavour for sweetness and sourness of apples, it was more useful when compared to the use of the instrumental measurements of total soluble solids (TSS) and titratable acidity (TA) (Harker *et al.,* 2002). In studies conducted to evaluate whether instrumental measurement and sensory analysis by trained or consumer panel are comparable, Harker *et al.* (2002) and Mehinanagic *et al.* (2006) found that although it is sometimes difficult to identify a difference in texture using an instrumental test, trained panelist were able to perceive the difference in texture.

## 7.2 Consumer preference for apples

According to Harker *et al.* (2003), quality is the combination of all the attributes that makes the product satisfactory or acceptable to the consumer. Consumer preference for apples is based on appearance and taste (Peneau *et al.,* 2006). A consumer preference study using apple cultivars showed that consumers base their decision to purchase an apple on its colour and price (pre-purchase) (McCracken *et al.,* 1994). Flavour and texture were ranked as the most important attributes in determining the after purchase evaluation. Daillant- Spinnler *et al.* (1996), Harker *et al.* (2008) and Pre-Aymard *et al.* (2005) found that interaction between texture and taste of apples plays an important role in consumer preference. They found that with 'Gala', consumer acceptability was related to high firmness and also high sugar level whereas high sugar and acid increased consumer acceptability for 'Braeburn'. Apparently, a combination of traits influences consumer preference for apples, such as sweetness and hardness, acidity and juiciness with each of these combination defining specific

cultivars (Pre-Aymard *et al.,* 2005). Consumers prefer apples which were acidic up to a certain level (McCracken *et al.*, 1994). For example, consumers preferred 'Hawaii' and 'Red Delicious' which contained 0.15-0.18 g of malic acid per 100 g juice while apples that contained 3.12-3.16 g malic acid were less preferred (McCracken *et al.,* 1994).

Table.1. List of attributes important in the sensory evaluation of apple and their definitions adapted from (Amos, 2007; Kuhn and Thybo, 2001; Mehinagic *et al.,* 2004; Mehinagic *et al.,* 2006; Harker *et al.,* 2002).

| **Sensory attributes** | | **Definition** |
|---|---|---|
| Flavour | Sweet taste (TSS) | Intensity of sweet taste (sucrose) |
| | Sour taste (TA) | Intensity of sour taste (malic acid) |
| | Bitterness | Characteristics of caffeine |
| | Astringency | The intensity of the dry sensation in the mouth |
| | Odour | Intensity of perfumed or aromatic apple flavour |
| Texture | Skin toughness | Intensity of skin residual in the mouth before swallowing |
| | Crispness | Sound produced when biting with the front teeth and chewing once |
| | Crunchiness | The amount of noise produced when chewing with the back teeth |
| | Ease of break down | The amount of time and number of chewing movements that is needed to grind the sample before swallowing |
| | Juiciness | Amount of juice released when chewing for a long time |
| | Mealiness | Intensity of granular dry structure |

With regard to flesh colour, Janick *et al.* (1996) indicated that consumers prefer a clear yellow flesh colour in apples, but showed intolerance towards red flesh and slight bleeding in apple flesh. Familiarity also seems to play a role in preference for flesh colour since in regions were 'McIntosh' is regarded as the standard apple, consumers prefer a white compared to the more yellow flesh colour of some other cultivars.

Limited research has been conducted on the effect of skin toughness on consumer preference. Even though Amos (2007) found a significant change in the amount of force required to chew an apple with peel in comparison with the apple without peel, Daillant- Spinnler *et al.* (1996) found that apple fruit skin does not affect fruit sensory properties and also does not influence acceptability of different cultivars. Skin toughness is defined as the amount of peel that is left in the mouth before swallowing (Kuhn and Thybo, 2003).

## 8. Conclusion

Development of a novel apple cultivar that has unique attributes may improve the competitiveness of the apple industry in South Africa. The success of new cultivars depends to a large extent on consumer preference for their sensory and physical attributes. For example, 'Cripp's Pink', which is a pink apple, is faring very well in the market due to good eating quality and its unique appearance. There may be a place on the market for a novel apple that has a pink or red flesh colour, good eating quality and an attractive external appearance. Red-fleshed apples may also have health benefits due to their high levels of anthocyanins and other phenolics in their skin and flesh providing the developers of these fruit with an additional and important marketing opportunity.

More research is needed on consumer preference for pink/red-flesh apple. For example, consumer preference may differ for the different patterns and intensities of anthocyanin accumulation in the flesh. We do not know how flesh pigmentation correlates with other quality traits, e.g., skin colour, taste, texture etc. as well as how easy or difficult it may be to breed for the preferred colours and quality parameters using *M. niedzwetzkyana* Dieck. as parent. In addition, little is known about the factors that regulate flesh pigmentation and patterns of internal pigmentation. It is possible that light and maybe even temperature does not play a role. It is not known whether any variation in flesh colour exists between apples on the same tree and whether colour intensity may vary from year to year. Variation in flesh colour between apples on the same tree and between seasons will make it difficult to set

standards for production and marketing. It may also result in non-conformance with these standards and with consumer expectation.

No research has been done in South Africa regarding red-fleshed apples. Therefore there is lack of scientific knowledge regarding the intensity and coverage of red colour in the flesh of red-fleshed apples, as well as consumer preference for taste and internal appearance of these red-fleshed apples.

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# PAPER 1: GENOTYPIC VARIATION OF COLOUR AND OTHER QUALITY RELATED TRAITS IN APPLE BREEDING FAMILIES

*Abstract:* **Internal flesh and peel colour variability of apple progenies derived from crosses between 'KAZ 91' and 'Meran' (Family 1), 'Reinette Burchard' and 'Treco Red Gala' (Family 2) and 'Meran' and 'Treco Red Gala (Family 3) were investigated in order to better understand the genotypic variability involved in the breeding process of novel pink / red-fleshed apple cultivars suitable for the fresh consumer market. 'KAZ 91' is a red-fleshed selection of *Malus niedzwetzkyana* Dieck. collected from Kazakstan in 1996 while Reinette Burchard, Meran and Treco Red Gala are white-fleshed cultivars of *Malus domestica* Borkh.. This study was conducted over a period of two years (2007 and 2008). External fruit colour, external and internal red colour coverage, flesh browning potential, total anthocyanins and total phenolics were measured. Fruit of Family 1 were generally darker red in peel colour with higher anthocyanin concentrations and greater blush coverage compared to fruit of families 2 and 3. As expected, only family 1 contained red-fleshed fruits (25% of bearing seedlings in 2007 were red fleshed and 35% in 2008). Varying intensities and patterns of red pigmentation, i.e., red cortex and core or white core and red cortex were observed. The intensity of red pigmentation in the flesh varied between seedlings as well as between individual apples of the same seedlings. About 29% of the red-fleshed progeny showed within-tree variance with regard to the intensity of red flesh colour and therefore it may prove difficult to set colour standards for red-fleshed cultivars. A higher total phenolic concentration was observed in the peel of Family 1 compared to Family 2 in 2007 and when compared to Family 2 and Family 3 in 2008. Flesh of apples from Family 1 seedlings also showed high total phenolics in both years. On average, red-fleshed Family 1 progeny had higher total phenolic levels compared to the white-fleshed progenies. However, no correlations were found for anthocyanin and total phenolics concentrations in the peel and flesh of Family 1 fruit, indicating that the antioxidant capacity of red-fleshed progeny does not relate to anthocyanin levels in their peel. Fruit of Family 1 seedlings showed a high degree of browning and were generally acidic**

**compared to Family 2 and Family 3. This may affect consumer preference for fruit selected from Family 1. High genetic determination for the presence of red flesh colour and high genetic variation in anthocyanin and total phenolics level among seedlings was found which indicates that this trait can be explored further for the purpose of breeding and selection. Within-tree variance for red flesh coverage and colour intensity can be ascribed to high levels of differentiation in pigment fixation and breeding and selection should be directed towards lower within tree variance.**

## INTRODUCTION

Fruit breeders are on the lookout for novel traits that can be used in the development of new high-value cultivars. Novelty in apple fruit is determined by the uniqueness of specific traits such as peel colour, taste, flavour, aroma and shape (Reid and Buisson, 2001). One of these novelty traits, namely red flesh colour, has recently become a major breeding objective of various apple breeding programmes, e.g. Plant & Food Research in New Zealand (Volz *et al.,* 2006; Volz *et al.,* 2009) and Next Fruit Generation in Switzerland (Freshplaza, 2008). While the flesh colour of the domestic apple, *Malus domestica* Borkh., typically ranges from white, cream to pale yellow to greenish-white (Janick *et al.,* 1996), the apple germplasm collection at Geneva contains 33 *M. domestica* accessions with pink or red flesh (USDA, ARS, National Genetic Resources Program, 2009). Apple breeders have focused on including the red flesh trait into their breeding programs through selections of *M. niedzwetzkyana* Dieck., a large-fruited wild apple species of the Tien Shan mountain range of Kazakhstan characterized by a pink to purple flesh colour (Dzhangaliev *et al.,* 2003).

Red-fleshed apples derive their colour from anthocyanins, water-soluble vacuolar pigments responsible for pink, red, purple and blue colours in fruit (Takos *et al.,* 2006). Very little is known about the segregation patterns of anthocyanin pigmentation in the flesh. Chagne *et al.* (2007) found that crossing 91.136 B6-77 (a red-fleshed selection of the red-fleshed cultivar Redfield originating from a cross between 'Wolfe River' and *M. niedzwetzkyana* Dieck.) with the white-fleshed *M. domestica* Borkh. cultivar, Sciros, gave rise to 19% seedlings bearing fruit with a red

core and red cortex, 23% seedlings with a white core and red cortex and 58% seedlings with white-fleshed fruits. Volz *et al.* (2006) also observed variation in red colour intensity and coverage of the cortex between red-fleshed seedlings. Colour intensity ranged from dark purple to pale pink while colour coverage ranged from only some vascular bundles displaying red colour to the entire cortex being fully red. In some fruit, red pigmentation was confined to layers of the cortex immediately below the skin. The proportion of red-fleshed seedlings in the family increased over time as these seedlings seem to take longer to become reproductive (Volz *et al.,* 2006). Hence, evaluation of red-flesh segregation ratios should be conducted over many years due to the low precocity of red-fleshed seedlings compared to white-fleshed seedlings (Volz *et al.,* 2006).

Apart from novelty, a further reason for the interest in red-fleshed apples is the potential health benefits associated with high anthocyanin levels in the flesh. Anthocyanins are potent antioxidants compared to other phenolic classes in apple peel (Tsao *et al.,* 2005) and have been shown to provide protection against a range of human diseases (Lila, 2004). Hence, the presence of red pigmentation in the flesh is associated with potential health benefits (Boyer and Liu, 2004). Macheix *et al.* (1990) indicated that the intensely pigmented deep purple and black *Rubus, Ribes, Vaccinium, Empetrum, Vitis* and *Sambucus* berries are the richest sources of anthocyanins (>200 μg $g^{-1}$ FW). Anthocyanin levels in cultivated apples are much lower. Wojdyło *et al.* (2008) reported that the contents of cyanidin 3- galactoside and cyanidin 3-glucoside, the two primary anthocyanins in the peel of cultivars with red or partial red colour, ranged from 10 to 550 μg $g^{-1}$ DW (≈2.3 to 126.5 μg $g^{-1}$ FW) . Anthocyanins were generally a minor component of the total polyphenol pool of which concentrations in apple peel ranged from 5 to 27 mg $g^{-1}$ DW (≈ 1.15 to 6.21 mg $g^{-1}$ FW). In some cultivars, e.g., Geneva Early, anthocyanins made up 30% of total phenolics in the peel. Lee *et al.* (2003) and Tsao *et al.* (2005) found that quercetin (40%), epicatechin (23%) and procyanidin B2 (22%) are the major contributors to apple total antioxidant activity while vitamin C (13%), phloretin (9%) and chlorogenic acid (9%) are of lesser importance. Anthocyanins in the skin of red cultivars contributed on average about 1% to the total antioxidant activity.

Apple flesh typically contains much lower levels of antioxidants and phenolics than the peel (Abrosca *et al.,* 2007; Drougoudi *et al.,* 2008; Wolfe *et al.,* 2003). Antioxidant activity concentrations and the total phenolic concentration of apple peel are respectively, about 1.5 to 9.2 times and 1.2 to 3.3 times greater than in the flesh (Drogoudi *et al.,* 2008). Hence, a positive correlation is observed between the antioxidant capacity of the whole fruit and that of the peel (Lata and Tomala, 2007). Sadilova *et al.* (2006) indicated that the average anthocyanin concentrations in the peel and flesh of red-fleshed 'Weirouge' apple were 20.6 μg $g^{-1}$ FW and 8.1 μg $g^{-1}$ FW, and phenolic concentrations were about 1684 mg $kg^{-1}$ FW (≈ 168.4 μg $g^{-1}$ FW galic acid equivalents) in the peel and 379 mg $kg^{-1}$ FW (≈ 37.9 μg $g^{-1}$ FW galic acid equivalents) in the flesh. The apple cultivar, Fyriki, with its bright red flesh had the highest antioxidant activity (11.9 mg $g^{-1}$ DW ascorbic acid equivalents) and total phenolics (9.8 mg $g^{-1}$ DW gallic acid equivalents) of a range of cultivars. 'Fyriki' was the only red-fleshed cultivar assessed. The green-fleshed cultivar, Mutsu, also had a high antioxidant capacity and a high level of total phenolics (5.6 mg $g^{-1}$ ascorbic acid and 5.4 mg $g^{-1}$ gallic acid equivalents, respectively) indicating that the presence of anthocyanins is not necessarily indicative of high levels of phenolics. Drougoudi *et al.* (2008) concluded that the most nutritious apple fruit appear to possess a dark red peel colour, but a lighter flesh colour with lower soluble solid contents.

The antioxidant capacity in a range of highly pigmented berries correlated better to their level of total phenolics than to anthocyanin levels (Moyer *et al.,* 2002) even though anthocyanin levels did correlate strongly to the levels of total phenolics. Whereas the antioxidant capacity of apple peel correlated positively with levels of total phenolics (Wojdyło *et al.,* 2008; Tsao *et al.,* 2005) and apples with a high phenolic content had a higher antioxidant capacity (Boyer and Liu, 2004), a high anthocyanin content does not necessarily imply that peel also has a high total phenolic and, therefore, high antioxidant capacity (Drougoudi *et al.,* 2008; Wojdyło *et al.,* 2008; Lata and Tomala, 2007). If the lack of a correlation between anthocyanin and total phenolic levels in the peel also applies to the flesh, red-fleshed apples may not necessarily be any healthier than white-fleshed apples.

The potentially higher phenolic levels in the peel of red-fleshed apples may make these fruit more prone to browning upon exposure to oxygen during eating. Browning is a process during which phenolics are oxidised by polyphenol oxidase (PPO) into quinone, which forms melanin upon non-enzymatic polymerization (Awad and Jager, 2000). Browning in apple is not a desirable trait in the fresh market industry. According to Martinez and Whitaker (1995), concentrations of phenolic compounds, PPO activity, pH, temperature and oxygen availability in the tissue are factors that determine the rate of enzymatic browning. Drougoudi *et al.* (2008) found that red-fleshed 'Fyriki' had a high phenolic content and browned faster when cut into slices when compared to the white-fleshed cultivars Starkrimson, Jonagored, Mutsu, Granny Smith and Fuji.

The aim of this study was to assess internal flesh and peel colour variability of progeny derived from a cross between the red-fleshed apple selection, 'KAZ91' (*M. niedzwetzkyana* Dieck.), and the white-fleshed cultivar Meran (*M. domestica* Borkh.) in order to determine the genotypic variability of the red flesh trait. Pigmentation patterns within the flesh of red-fleshed progeny were assessed to investigate variation within and between seedlings of the same family. Genetic determination of external colour and other quality traits were also investigated in progenies derived from crosses between the *M. domestica* Borkh. cultivars Reinette Burchard x Treco Red Gala and Meran x Treco Red Gala. Anthocyanin concentrations and total phenolics in the peel of red- and white-fleshed progeny as well as in the flesh of red-fleshed progeny were correlated to test possible associations. This was done to assess whether apples with red flesh will necessarily also be dark red in skin colour and also to determine whether high anthocyanin levels are indicative of high levels of total phenolics. The rate of flesh browning was assessed to investigate whether red-fleshed apples are more sensitive to browning compared to white-fleshed apples due to their supposedly high phenolic content.

# MATERIALS AND METHODS

*Plant material and planting design*: Fruit were collected from seedling trees of three apple breeding families planted at the Drostersnes experimental farm of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij in the Western Cape Province of South Africa (Mediterranean-type climate, latitude: 33.33'S, longitude: 18.63'E). The trees on M793 rootstock where planted in November 2003 at a spacing of 0.75 m x 4 m (1.5 ha and 4725 trees) in rows facing East - West. The families were: 'KAZ91' x 'Meran' (Family 1) (Fig. 1 A1-A2), 'Reinette Burchard' x 'Treco Red Gala' (Family 2) (Fig. 1 B1-B2) and 'Meran' x 'Treco Red Gala' (Family 3) (Fig. 1 C1-C2). Bud wood of 'KAZ91' was collected in 1996 in Kazakhstan as a selection of the red-fleshed species *M. niedzwetzkyana* Dieck.. 'KAZ91' has a dull red over colour that can vary from fully red to weak red (personal communication, I. Labuschagné). Fruits are astringent and acidic with a firm but not crispy texture. Material of 'Reinette Burchard' was also collected in 1996 in Kazakhstan. Fruit of this genotype has a yellow skin. 'Meran' is a brightly-striped cultivar with a green-yellow background colour and it is classified as a striped bi-colour (personal communication, I. Labuschagné). 'Meran' fruit show poor colouration in the Western Cape of South Africa. 'Treco Red Gala' is a red-striped apple with good eating quality. The objective of crossing 'KAZ91' and 'Meran' was to transfer some of the positive eating quality traits from 'Meran' and the red–flesh colour from 'KAZ91' to their progeny. The breeder included 'Reinette Burcard' in the breeding programme as another Kazakhstan selection. 'Treco Red Gala' was included in the programme for its good skin colour.

*Fruit collection*: Fruit were harvested from 23 February until 31 March in 2007 and from 11 February until 24 April in 2008. Not all seedlings were bearing fruits. In 2007, about 184 trees with the required two to five fruit were available for Family 1, 147 trees for Family 2 and 145 trees for family 3. In 2008, about 206 trees were available for Family 1, 192 trees for family 2 and 173 trees for family 3. Individual fruits from one tree were used.

*External fruit colour*: The external fruit colour of three fruit per seedling was measured on the most coloured side (highest pigment concentration or blush

coverage) and on the side with lowest pigment concentration (ground colour), halfway between the calyx and stem end using a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan). Lightness (L), chroma (C), and hue angle (H º) were recorded. Colour coverage was measured using a high resolution Nikon DXM1200 digital camera with a 0.63x relay lens (Innovative solutions (IMP) Scientific and Petitions Pty Ltd, Johannesburg). Images were taken from the most and least coloured sides of fruit and optimal lighting was achieved by using a microlite fluorescent ring light for epi-illumination (Innovative solutions (IMP) Scientific and Petitions Pty limited Johannesburg). The proportion of the fruit area with red pigmentation was calculated using Image Pro-Plus 4.5 Software for image analysis (Innovative solutions (IMP) Scientific and Petitions Pty limited Johannesburg).

*Flesh colour and browning:* Fruit from all three families were halved and flesh colour measured immediately after cutting and again after 20 min with a chromameter to assess flesh colour and to determine the extent of colour change due to oxidative browning. Lightness (L) and chroma (C) values as well as hue angle (Hº) were recorded. The extent of flesh red colour coverage was assessed in fruit of Family 1 using the above mentioned high resolution Nikon DXM1200 camera. One photo of each cut half of the fruit was taken and the percentage of the flesh with red colour calculated with Image Pro-Plus 4.5 software. The distribution of anthocyanin pigmentation in the flesh was recorded.

*Maturity indices:* Firmness was measured on peeled opposite pared sides of fruit using a fruit texture analyser with an auto penetrometer (GUSS FTAWin Version 4.51). Total soluble solids (TSS) content was determined using a refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Average TSS levels were determined per seedling. For measurement of titratable acidity (TA), halved fruit from three or two apples per seedling were blended together and 5 ml juice titrated with 0.1 N NaOH to a pH of 8.2 using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland). Titratable acidity was expressed in percentage malic, ascorbic and tartaric acid.

*Anthocyanin and phenolic assessment:* In 2007, phenolic concentrations were assessed for Family 1 and Family 2 peel, and Family 1 flesh. Fruit were peeled and

the peel and slices of flesh were placed in small plastic bags on ice in a cooler, for transport to the laboratory at Stellenbosch University where it was frozen in liquid nitrogen and stored at -80 ºC until analysis was performed. The peel and flesh were milled in liquid nitrogen. Pigments were extracted from 2 g fresh sample with 5 ml cold methanol and 3 M HCl (95:5 v/v). The mixture was stirred in a fridge for 1 h at 4 °C. Samples were centrifuged for 10 min at 10000 x 10*g* at 4 °C. The supernatant was vortexed after adding 5 ml solvent, and centrifuged again for 10 min where after the two supernatants were added together and filtered using 0.45 μm filters (Millex-HV, Millipore Corporation, Milford, MA, USA). Total anthocyanins were assessed by measuring absorbance at 530 nm and 653 nm (CARY 50 Bio, Varian Australia (PTY) Ltd., Melbourne, Australia). Absorbance at 530 nm was adjusted for chlorophyll by subtracting 24% of absorbance at 653 nm (Mancinelli *et al.,* 1975; Murray and Hackett, 1991). Idaein (cyanidin-3-galactoside) (Carl Roth, Karlsruhe, Germany) was used to obtain a standard curve from which anthocyanin concentrations were calculated. Total phenolics were measured at 280 nm and quantified from a gallic acid (SIGMA® Chemical Co, Stenholm, Switzerland) standard curve.

The same procedure as in 2007 was used in 2008 for the analysis of anthocyanins and phenolics in the peel and flesh of all three families except that samples were freeze dried. Samples were stored in the dark until extraction of 0.5 g dry sample with 10 ml methanol and 3 M HCl (95:5 v/v). The results obtained were converted to FW for comparison with the 2007 results with a formula derived by calculating the DW to FW ratio in 'Golden Delicious', 'Granny Smith' and 'Royal Gala' apples. The following formulas were used to convert concentrations on DW basis to FW basis:

Peel x μg $g^{-1}$ FW = (y μg $g^{-1}$ DW*23)/100

Flesh x μg $g^{-1}$ FW = (y μg $g^{-1}$ DW*15)/100

*Statistical analysis:* Analysis of variance (ANOVA) was carried out separately for 2007 and 2008 and a joint analysis was performed to evaluate the family variation (F), variation between years (Y) and year x family (Y X F) interaction. Data were analysed using SAS General Linear Model procedures (SAS Institute, Cary, N.C.). ANOVA and correlation analysis were performed after testing data for normality (Shapiro and Wilk, 1965). Multiple comparisons were performed using Duncan's multiple range tests. The variance structure in the seedling families were broken down

to estimate the underlying causal components of variance from the observed traits by applying standard quantitative genetic principles (Falconer and Mackay, 1996). Intra-class correlation coefficients were calculated as described by Labuschagné *et al.* (2002) using variance components (SAS Variance Component Estimation procedure). Variance components were also used to calculate a repeatability estimate according to Falconer & Mackay (1996), for red flesh coverage. Correlation analysis in Family 1 was performed on data from two seasons, whereas data from one season was used in Family 2 and Family 3.

## Results and Discussion

*External fruit colour:* Family 1 fruit were generally redder (lower hue angle), darker (lower L value) and duller (lower C value) in colour on both sides of the fruit compared to fruit of Family 2 but not 3 (Table 1). Family 1 fruit also had larger blush coverage than fruit of Families 2 and 3 (Table 2). Family 2 fruit had the least blush coverage (Table 2). Significant year x family interaction in fruit colour was observed (Table 1). Fruit of Family 2 developed less red colour in 2008 compared to 2007, while fruit of Families 1 and 3 developed redder colour although not significantly so in Family 3. Family 1 and Family 3 had higher mean values for blush coverage in 2008 than in 2007 (Table 2). In 2007, L values for family 2 and family 3 showed a more normal distribution on the sun exposed side of fruit (Fig. 2A). In 2008, L values of Family 1 and Family 3 were skewed towards lower values indicating that more fruit accumulated high levels of anthocyanins (Fig 3A). Hue angles of Family 2 showed a flatter distribution and L values also tended to be higher (Fig 3A,B) due to poor red colour development in fruit of this family in 2008 (Table 1). The distribution curves for hue angle and lightness values in Family 1 were skewed towards a low mean value and showed an extension towards higher values indicating a predominance of dark red fruit in the progeny of this Family in 2008 (Fig. 3A ,B). Lata and Tomala (2007) also reported considerable annual fluctuation in colour and anthocyanin levels in the skin of 19 apple cultivars with Fuji and Pinova, respectively accumulating 5 times more and 2.5 times less anthocyanin in 2004 than in 2005. These differences can most likely be attributed to the prevailing climatic conditions at

the time of maximal anthocyanin accumulation just prior to harvesting. Anthocyanin synthesis in apple peel is stimulated by low night temperatures (Curry, 1997).

Background lightness and hue of all three families were skewed towards and peaked at high values (Fig 4A, B and 5A, B) indicating that less anthocyanin accumulated on the shaded sides of more fruit. However, background hue angles of Family 1 also showed a minor peak at about 35 º indicating segregation between full red and blushed or green fruit in this family.

*Anthocyanin and phenolic concentrations in peel:* Table 3 and Fig 6A & B indicate that Family 1 and 3 seedlings had on average high anthocyanin concentrations in their peel when compared to Family 2 seedlings. Highest mean anthocyanin concentrations were found in Family 1 (0.26 mg $g^{-1}$ FW) and Family 3 (0.22 mg $g^{-1}$ FW) with the lowest mean anthocyanin concentrations in Family 2 (0.08 mg $g^{-1}$ FW). Mean anthocyanin concentrations in both Families 1 and 2 were slightly higher in 2007 compared to 2008, but the difference was not significant (Table 3). Unlike for skin colour, no year by family interaction in anthocyanin levels was observed (Table 3). Anthocyanin concentrations ranged from 0.01 to 2.83 mg $g^{-1}$ FW in 2007 and 0.00 to 1.18 mg $g^{-1}$ FW in 2008 (Table 4 & 5). Wojdyło *et al.* (2008) in a survey of 67 apple cultivars found anthocyanin concentrations to range from 0 to 4 mg $g^{-1}$ DW (≈0.92 mg $g^{-1}$ FW).

Mean levels of total phenolics (gallic acid equivalents) in the peel of both Family 1 and Family 2 were considerably higher in 2008 than in 2007 (Table 3). High mean phenolic concentrations were found in Family 1 (2.82 mg $g^{-1}$ FW) and Family 3 (2.43 mg $g^{-1}$ FW) whereas the mean phenolic concentration in Family 2 was much lower (1.08 mg $g^{-1}$ FW) (Table 3). Families 1 and 3 also had higher maximum levels of total phenolics in peel compared to Family 2 (Table 3-5). Sadilova *et al.* (2006) found high average phenolic concentration in the peel of red-fleshed 'Weirouge' apple (1.68 mg $g^{-1}$ FW) galic acid equivalent. Phenolic levels varied more between seedlings in 2007 compared to 2008 with both higher maximum and lower minimum levels found (Table 4 & 5). Total phenolics in the peel ranged from 0.11 to 20.12 mg $g^{-1}$ FW (Table 4 & 5). Wojdyło *et al.* (2008) found total phenolic levels in 67 apple cultivars

to vary between 5.2 and 27.2 mg $g^{-1}$ DW (≈1.2-6.27 mg $g^{-1}$ FW). It appears that 'KAZ91' imparts high levels of anthocyanins and total phenolics to its progeny. Kondo *et al.* (2002) found that 'Redfield', derived from a cross between 'Wolf River' (white-fleshed) and '*M. niedzwetzkyana'* (red-fleshed), had higher total phenolics in its peel and flesh compared to 'Fuji' (red skin) and 'Oorin' (yellow skin).

*Colour coverage of cut fruit:* The number of seedlings in Family 1 that had some red pigmentation in their flesh were about 25% and 35% in 2007 and 2008, respectively (Table 6). The increase in the proportion of red-fleshed seedlings from 2007 to 2008 highlights the apparent reduced precocity of red-fleshed genotypes (Volz *et al.,* 2006). The mean percentage red-flesh coverage of red-fleshed seedlings was rather low at 30.635 and 31.206 in 2007 and 2008, respectively (Table 2). This corresponds with the confined distribution of red pigmentation reported in most red-fleshed progenies (Chagne *et al.,* 2007; Volz *et al.,* 2006).

Red-fleshed seedlings showed considerable variation with regard to anthocyanin concentration and percentage colour coverage in their flesh (Fig 8). Anthocyanin distribution patterns observed in halved red-fleshed fruit of Family 1 in 2008 included, i.e. red cortex and core (≈1% of red-fleshed seedlings) and white core and red cortex (70%). The remaining 29% of the red-fleshed seedlings displayed within tree variation in anthocyanin pattern, i.e., fruits with red core and cortex, red core and white cortex or white cortex and core were found on the same tree (see Fig 9 for an example). These results are consistent with the patterns of anthocyanin distribution reported by Chagne *et al.* (2007) and Volz *et al.* (2006).

*Anthocyanin and phenolic assessment of the flesh:* The mean anthocyanin concentration in Family 1 flesh was 0.065 mg $g^{-1}$ FW in 2007 and 0.050 mg $g^{-1}$ FW in 2008 (Table 3; Fig 10). The highest anthocyanin concentration measured was 0.14 mg $g^{-1}$ FW (Table 4 & 5). Sadilova *et al.* (2006) found high average anthocyanin concentrations in the flesh of red-fleshed 'Weirouge' (0.081 mg $g^{-1}$ FW). To put these concentrations in perspective; the average anthocyanin concentration in the peel of Family 1 seedlings was 0.25 and 0.24 mg $g^{-1}$ FW in 2007 and 2008, thus on average, 43 times greater than in the flesh. Moyer *et al.* (2002) found that anthocyanin

concentrations in various *Rubus*, *Vaccinium* and *Ribes* species and cultivars ranged from 0.14 to 6.27 mg $g^{-1}$ FW. Hence, it terms of potential health benefits, anthocyanin levels in red apple flesh is not nearly comparable to levels in apple peel or in anthocyanin-rich berries. Yet, red-fleshed apples may still possess significant health benefits if anthocyanin levels in the flesh are accompanied by high levels of total phenolics.

In 2008, high mean total phenolics were found in the flesh of Family 1 seedlings (0.54 mg $g^{-1}$ gallic acid equivalents) followed by Family 3 (0.40 mg $g^{-1}$ gallic acid) and Family 2 (0.33 mg $g^{-1}$ FW gallic acid) in 2008 (Table 3; Fig 11A & B). Phenolic concentrations in the three families ranged from 0.02 to 0.99 mg $g^{-1}$ FW gallic acid in 2007 and from 0.07 to 2.66 mg $g^{-1}$ FW in 2008 (Table 3, 4 & 5). However Sadilova *et al.* (2006) found that the average phenolic concentration in the flesh of red-fleshed 'Weirouge' apple was 0.376 mg $g^{-1}$ FW galic acid equivalent. The phenolic concentrations in Family 1 peel was on average 7 and 5 times greater than in the flesh in Family 1 and Family 2, respectively. This is considerably higher than Drogoudi *et al.* (2008) who found 1.5 to 3.3 times higher levels of phenolics in apple peel than in the flesh, but comparable to Tsao *et al.* (2003) (2.5 to 5.7 times higher in peel).

*Rate of browning:* Flesh colour generally increased in chroma, but decreased in hue angle and lightness during the 20 min after fruit were cut. Family 1 fruit increased more in chroma compared to Family 2 and Family 3 while Family 2 fruit showed a greater decrease in hue angle compared to the other Families (Table 7). Families showed a comparable reduction in lightness values (Table 7). Chroma values decrease with decreasing colour saturation (Maskan, 2001). Significant differences were observed for the change in lightness (ΔL), chroma (ΔC) and hue (ΔH º) between the parents and controls (Table 7). 'Golden Delicious' and 'Royal Gala' showed a far greater decrease in hue angle compared to parents while 'KAZ91' decreased the least in hue angle. However, 'KAZ91' fruit decreased significantly more in chroma while 'Reinnette Burchard' and 'Meran' showed the smallest decrease. 'Golden Delicious', 'Royal Gala' and 'KAZ91' showed a significantly greater decrease in lightness value. The different patterns observed in the changes of hue angle, lightness value and chroma among parents and the two reference cultivars cast doubt on the usefulness of

numeric colour measurements to assess the extent of browning. This may be due to the red pigmentation in the flesh of 'KAZ91' (and its progeny). A visual assessment of browning, in addition to objective colour measurement, may have addressed this problem. Sadilova *et al.* (2006) found that red-fleshed 'Weirouge' apple were not easily prone to oxidative browning and concluded that it may be due to high acid content that stabilizes red colour.

However, Drogoudi *et al.* (2008) found that the rate of browning was faster in red-fleshed 'Fyriki' and concluded that this may be due to the high levels of phenolics found in this cultivar. However, we did not find a correlation between the change in lightness, chroma and hue angle of the flesh and total phenolics in the flesh (data not presented). The level of total phenolics is not the only factor that determines the browning potential of apple flesh. According to Martinez and Whitaker (1995), concentrations of phenolic compounds, PPO activity, pH, temperature and oxygen availability in the tissue are factors that determine the rate of enzymatic browning. Amiot *et al.* (1992) found no correlation between phenolic content and degree of browning measured by reflectance and absorbance in eight apple cultivars. However, browning susceptibility did correlate with levels of chlorogenic acid. Further research on Family 1 could entail a detailed qualitative assessment of the phenolic profile in the flesh and peel.

*Correlation analysis - Family 1 red-fleshed seedlings:* A weak positive correlation was observed for anthocyanin levels in the peel and flesh of red-fleshed seedlings ($R = 0.26$; $P < 0.0034$). However, the percentage red-flesh coverage did not correlate with anthocyanin levels in the peel. These results indicate that peel colour is not necessarily indicative of the intensity and extent of red colour in the flesh.

A positive correlation was observed between the percentage red-flesh coverage and anthocyanin levels in the flesh (0.51; $P = 0.0001$). The weakness of the correlation can possibly be attributed to the variation in anthocyanin levels independent of flesh coverage. Anthocyanin levels did not correlate with levels of total phenolics in either the peel or flesh. This indicates that peel colour and flesh colour is not an indicator of phenolics level in the fruit. Hence, red-fleshed apples may not necessarily have a higher antioxidant capacity and be healthier than fruit with a white to yellow flesh

colour. Yellow and green fruits may also contain high levels of phenolics in the peel or flesh. Moyer *et al.* (2002) found that some *Viccinium* and *Ribes* fruits with red-flesh had low anthocyanin and total phenolics concentration when compared to berries with no pigment in their flesh. Despite having the highest anthocyanin concentration in its peel (≈20% of total phenolic content), 'Empire' had the lowest level of total phenolic content and the lowest antioxidant capacity of eight apple cultivars studied by Tsao *et al.* (2005).

However, when comparing average anthocyanin and phenolic levels in red- and white-fleshed progeny, the picture looks somewhat different. Higher anthocyanin levels were found in the peel of red-fleshed seedlings compared to white fleshed seedlings in 2007 while there was no difference in 2008 (Table 8). This seasonal difference may be due to the time of harvesting and changes in environmental conditions. Fruits were harvested earlier in 2007 than in 2008. Total phenolic levels in the peel of red and white fleshed seedling did not differ in either season. However, total phenolics were considerably higher in red-fleshed seedlings compared to white-fleshed seedlings in both 2007 and 2008. This indicates that although a high level of anthocyanins in the flesh does not necessarily indicative of high levels of total phenolics, red-fleshed progeny, on average, do contain more total phenolics in their flesh compared to white-fleshed progeny. Hence, it appears that the phenylpropanoid pathway is up regulated in red-fleshed seedlings, but that the level of flux into the pathway does not necessarily correlate with levels of anthocyanins, as one of the final products.

*Correlation analysis in Family 2 and Family 3 seedlings:* In Family 2, peel phenolics and flesh phenolics were positively correlated ($R = 0.41$; $P = 0.0001$). However, no correlation was observed for peel phenolics with peel anthocyanin ($R = 0.195$; $P = 0.0008$) and flesh phenolics with peel anthocyanin levels ($R = 0.032$; $P = 0.6738$) in Family 2. A positive correlation was found between peel phenolics and peel anthocyanin levels ($R = 0.44$; $P = 0.0001$) in Family 3 but no correlation was observed for flesh phenolics with peel anthocyanin levels ($R = 0.124$; $P = 0.124$) in Family 3.

*Fruit maturity:* Family 3 fruit were on average firmer and had higher starch levels compared to fruit of Families 1 and 2 in both 2007 and 2008 (Table 9), suggesting that these fruit may have been harvested too early. Family 3 fruit on average had higher TSS levels compared to the other families and this may have been the reason why this family was harvested at lower maturity. Family 1 fruits were on average more acidic (0.63 % malic acid) than fruit of the other families and were higher in all of malic acid, citric acid and tartaric acid when compared to Family 2 and Family 3 (Table 10). Skendrovic Babojelic *et al.* (2007) found that 'Granny Smith' had the highest total acids of 0.69 % expressed as malic acid compared to 'Pink Lady' (0.54%) and 'Idared' (0.45%). The acidity in Family 1 ranged from 0.10 to 1.34 % malic acid. *M. niedzwetzkyana* Dieck. tends to be very acidic and seems to transfer this trait to some of its progeny.

*Variance components:* Variance components where calculated on 2007 and 2008 data (Table 11). When compared to other components contributing to the total variance, low or high variance could be attributed to blocking effects in all traits. Variance contributed to year effects was relatively small when expressed as percentage of total variance. Corresponding values due to Year x Family interaction was small except for percentage over colour (27 %). Low between family variation for all traits was also found. Significant variation between seedlings could be shown, reflecting the high genotypic variation present within families and indicating heterogenic variability between parents for the traits assessed. This was specifically found for red flesh coverage when calculated across families (67%), and around 50% to the total variance for other traits, but less for over colour C-values (35%) and background colour C-values (35%). The residual values calculated as error values between individual fruit per seedling, and expressed as a percentage of total variance were found to be around 30% in most traits, except over colour C (49%), background colour C (56%) and percentage starch breakdown (45%). Relatively high values for between fruit variance suggest that differences attributable to endogenous physiological factors can have a considerable influence on the phenotypical expression of traits within seedling trees. Corresponding values for red flesh coverage calculated across families was however, relatively low (16%).

*Degree of genetic determination:* Intraclass correlation coefficients were calculated from within and between family estimates of variance components and residual variance components. Intraclass correlation coefficients relevant to selection between families (*t*1) appear to be consistently small (ranging from 0 to 0.16) (Table 11). By comparison, the intraclass correlation coefficients relevant to selection between seedlings within crosses (*t*2) show medium to high values. Lowest *t*2 values were found for over and background color C values (0.41 and 0.38, respectively) and values between 0.52 and 0.64 were found for the other traits. For red flesh coverage a high *t*2 value was calculated (0.80) when all seedlings in the families were added in the calculation. The repeatability estimate for red flesh coverage in red fleshed seedling only, was 0.54.

## CONCLUSION

Crossing white-fleshed 'Meran' with *M. niedzwetzkyana* Dieck. selection 'KAZ91' yielded ~25% to 35% red-fleshed seedlings over a period of two seasons. Skin and flesh colour intensity as well as the pattern of anthocyanin accumulation in the flesh varied considerably within the *M. niedzwetzkyana* progeny. It would be prudent to assess consumer preference for the different intensities and distribution patterns of flesh pigmentation. Fruit from the same tree also varied with regard to the intensity of red flesh colour and red-flesh coverage also differed from one season to the next. The significant implication of this for production and marketing of red-fleshed apples is that it may be difficult to set and comply with standards for percentage coverage and intensity of red flesh colouring. Quantification of the variance in flesh colour between fruit of the same seedling is of considerable importance due to the above limitation. Small intraclass correlation coefficients *(t1)* were found between families, however medium to high intraclass coefficients (*t2*) were found between seedlings within crosses. High interclass coefficients (*t2*) were found for red-flesh coverage compared to other traits. Seasonal variation in anthocyanin and phenolic levels was observed within all three families. More research is needed to assess the effect of environmental conditions and canopy position on the accumulation of anthocyanin in fruit flesh of

red-fleshed apple. Intermediate to high genetic determination within seedling families suggests that the response to individual selection and intercrossing of superior parentals will be successful and that genetic advances are expected to be relatively rapid.

No correlation was found between anthocyanin and total phenolic levels in the peel and flesh, indicating that red-fleshed fruit do not necessarily have higher antioxidant capacities than white-fleshed fruit. In addition, only a weak correlation was observed for total phenolic levels in skin and flesh of Families 1 and 3. However, red-fleshed progeny of Family 1 did, on average, have higher phenolic levels in their flesh compared to white-fleshed seedlings. The absence of a strong correlation between skin and flesh pigmentation means that high anthocyanin concentrations in the flesh do not imply that skin colour will be dark red, and therefore potentially unattractive to most consumers. Family 1 seedlings had high anthocyanin and phenolic levels in their peel and flesh, and were generally also acidic compared to seedlings of Families 2 and 3, thus displaying their wild *M. niedzwetzkyana* Dieck. heritage. Family 3 seedlings on average had high starch breakdown, firmness and TSS when compared Families 1 and 2 and this may be due to harvest time. The affect of *M. niedzwetzkyana* parentage on taste needs to be assessed. The *M. niedzwetzkyana* Dieck. family appeared more prone to oxidative flesh browning, but the severity of browning did not correlate with total phenolics.

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Table.1 Lightness and chroma values as well as hue angles for blush coverage and ground colour of apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1); 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| | | Sun exposed (best coloured) side | | | Shaded (worst coloured side) | | |
|---|---|---|---|---|---|---|---|
| | | Lightness | Chroma | Hue | Lightness | Chroma | Hue |
| Fam1 | 2007 | 42.61$^{d}$ | 39.51$^{c}$ | 28.75$^{d}$ | 65.63 $^{b}$ | 38.38$^{c}$ | 76.89$^{c}$ |
| | 2008 | 38.26$^{e}$ | 38.71$^{d}$ | 23.44$^{e}$ | 60.21$^{c}$ | 37.29$^{d}$ | 69.77$^{d}$ |
| Fam2 | 2007 | 46.54$^{b}$ | 43.84$^{a}$ | 35.37$^{b}$ | 72.80$^{a}$ | 41.25$^{a}$ | 82.51$^{b}$ |
| | 2008 | 48.90$^{a}$ | 42.05$^{b}$ | 38.51$^{a}$ | 73.31$^{a}$ | 40.38$^{b}$ | 86.40$^{a}$ |
| Fam3 | 2007 | 45.45$^{c}$ | 43.99$^{a}$ | 32.78$^{c}$ | 72.55$^{a}$ | 36.96$^{d}$ | 81.72$^{b}$ |
| | 2008 | 43.16$^{d}$ | 41.63$^{b}$ | 31.74$^{c}$ | 64.72$^{b}$ | 40.69$^{ab}$ | 66.61$^{e}$ |
| Pr>F | | | | | | | |
| Family | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Seedling within family | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Year | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0767 | 0.0001 |
| Year*Family | | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

$^{z}$ Treatment means with the same superscript are significant at $p < 0.05$ according to Duncan's Multiple Range Test

Table 2. Percentage red colour coverage in the peel and flesh of apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1); 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| | | Blush coverage (%) | Red-flesh coverage (%)[x] |
|---|---|---|---|
| Fam1 | 2007 | 76.11[b] | 30.64 [ns] |
| | 2008 | 89.68[a] | 31.21 |
| Fam2 | 2007 | 49.54[d] | - |
| | 2008 | 48.94[d] | - |
| Fam3 | 2007 | 65.03[c] | - |
| | 2008 | 74.71[b] | - |
| Pr>F | | | |
| Family | | 0.0001 | - |
| Seedling within family | | 0.0001 | - |
| Year | | 0.0001 | 0.8996 |
| Year*Family | | 0.0001 | - |

[z] Treatment means with the same superscript are significant at $p < 0.05$
[x] Only red-fleshed seedlings were included in the analysis of red-flesh coverage.
[ns] Non-significant

Table 3 Anthocyanin concentration and total phenolic levels in the peel and flesh of apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1); 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| Families | | Anthocyanin (mg $g^{-1}$ FW) | | Total phenolics (mg $g^{-1}$ FW)[y] | |
|---|---|---|---|---|---|
| | | Peel | Flesh (mg $g^{-1}$ FW) | Peel | Flesh |
| Fam1 | 2007 | 0.25 [a] | 0.0067 [ns] | 2.14 [c] | 2.30 [d] |
| Fam1 | 2008 | 0.24 [ab] | 0.0053 | 2.84 [a] | 5.40 [a] |
| Fam2 | 2007 | 0.11 [c] | - | 1.08 [e] | - |
| Fam2 | 2008 | 0.08 [c] | - | 1.77 [d] | 3.30 [c] |
| Fam3 | 2007 | - | - | - | - |
| Fam3 | 2008 | 0.22 [b] | - | 2.43 [b] | 4.00 [b] |
| Pr>F | | | | | |
| Family | | 0.0001 | - | 0.0001 | 0.0001 |
| Seedling within family | | 0.0001 | - | 0.2930 | 0.0006 |
| Year | | 0.2113 | 0.3946 | 0.0001 | 0.0001 |

[z] Treatment means with the same superscript are significant at $p < 0.05$ according to Duncan's Multiple Range Test
[y] Measured at 280 nm and expressed in gallic acid equivalents.
[ns] Non-significant

Table 4. Variation in the anthocyanin and total phenolic level in the peel and flesh of apple fruit from adult seedling trees in three breeding families i.e., 'KAZ91' x 'Meran' (Fam1) and 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) in 2007.

| | Anthocyanin (mg $g^{-1}$ FW)[x] | | | | Total phenolics (mg $g^{-1}$ FW)[y] | | | |
|---|---|---|---|---|---|---|---|---|
| Trait and Family | Min | Max | Mean | Variance | Min | Max | Mean | Variance |
| Peel | | | | | | | | |
| Fam1 | 0.01 | 2.83 | 0.25 ± 0.02 | 0.10 | 0.35 | 20.12 | 2.14 ± 0.17 | 5.98 |
| Fam2 | 0.00 | 0.81 | 0.11 ± 0.01 | 0.02 | 0.11 | 4.63 | 1.08 ± 0.06 | 0.46 |
| Flesh | | | | | | | | |
| Fam1 | 0.00 | 0.14 | 0.01 ± 0.001 | 0.0003 | 0.02 | 0.99 | 0.23 ±0.011 | 0.025 |

[y] Measured at 280 nm and expressed in gallic acid equivalents.

Table 5. Variation in the anthocyanin and total phenolic levels in the peel and flesh of apple fruit from adult seedling trees of three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1), 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2008.

| | Anthocyanin (mg $g^{-1}$ FW) | | | | Total phenolics (mg $g^{-1}$ FW)[y] | | | |
|---|---|---|---|---|---|---|---|---|
| Trait and Family | Min | Max | Mean | Variance | Min | Max | Mean | Variance |
| Peel | | | | | | | | |
| Fam1 | 0.00 | 0.96 | 0.24 ± 0.01 | 0.03 | 0.79 | 5.73 | 2.84± 0.06 | 0.66 |
| Fam2 | 0.00 | 0.63 | 0.08 ± 0.01 | 0.01 | 0.74 | 3.31 | 1.77± 0.04 | 0.27 |
| Fam3 | 0.00 | 1.18 | 0.22 ± 0.01 | 0.04 | 0.87 | 5.89 | 2.43 ± 0.07 | 0.74 |
| Flesh | | | | | | | | |
| Fam1 | 0.00 | 0.09 | 0.005 ± 0.001 | 0.001 | 0.07 | 2.18 | 0.54± 0.01 | 0.06 |
| Fam2 | - | - | - | - | 0.01 | 0.77 | 0.33± 0.01 | 0.01 |
| Fam3 | - | - | - | - | 0.12 | 2.66 | 0.40 ± 0.02 | 0.05 |

[y] Measured at 280 nm and expressed in gallic acid equivalents.

Table 6. Segregation of red- and white-fleshed seedlings in the progeny of Family 1 ('KAZ91' x 'Meran') in 2007 and 2008.

| Year | Flesh colour | Number of seedlings |
|---|---|---|
| 2007 | White | 138 (75%) |
| | Red | 46 (25%) |
| | Total number of seedlings | 184 (100%) |
| 2008 | White | 134 (65%) |
| | Red | 72 (35%) |
| | Total number of seedlings | 206 (100%) |

Table 7. Change in Lightness (L) and Chroma (C) values as well as in Hue angle (Hº) of the flesh of apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1); 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3), the four breeding parents as well as two control cultivars within 30 min after being cut.

| Progenies | ΔLightness | ΔChroma | ΔHue |
|---|---|---|---|
| Fam1 | 4.37[a] | -6.83[b] | 5.23[b] |
| Fam2 | 3.84[a] | -4.34[a] | 7.16[a] |
| Fam3 | 3.83[a] | -4.25[a] | 5.70[b] |
| Pr>F | | | |
| Family | 0.2300 | 0.0001 | 0.0074 |
| Seedling within family | 0.0001 | 0.0001 | 0.0001 |
| Parents and controls | | | |
| Golden Delicious | 8.22[a] | -6.99[b] | 12.62[a] |
| Royal Gala | 5.22[a] | -5.79[b] | 10.04[ab] |
| KAZ91 | 7.34[a] | -13.43[c] | 1.73[c] |
| Reinnette Burchard | 3.36[b] | -2.50[a] | 7.90[b] |
| Meran | 3.43[b] | -3.76[a] | 6.35[b] |
| Treco Red Gala | 5.05[b] | -6.43[b] | 7.63[b] |
| Parents | 0.0006 | 0.0001 | 0.0014 |

[z] Treatment means with the same superscript are significant at $p < 0.05$ according to Duncan's Multiple Range Test

[x] Final value was subtracted from the initial value, therefore a posative change in L, C and H will indicate a decrease and a negative change an increase

Table 8. Average anthocyanin and total phenolic concentrations (mg $g^{-1}$ FW) within the peel and flesh of red- and white-fleshed progeny of 'KAZ91' x 'Meran' in 2007 and 2008.

| | | 2007 | | 2008 | |
|---|---|---|---|---|---|
| | | Red-fleshed | White-fleshed | Red-fleshed | White-fleshed |
| Peel | Anthocyanin | 0.44 ± 0.04 | 0.18 ± 0.02 | 0.27 ± 0.10 | 0.22 ± 0.01 |
| | Phenolics | 2.39 ± 0.39 | 2.03 ± 0.19 | 2.83 ± 0.02 | 2.84 ± 0.09 |
| Flesh | Phenolics | 0.32 ± 0.02 | 0.20 ± 0.01 | 0.64 ± 0.03 | 0.48 ± 0.02 |

Table 9. Firmness and starch levels of apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1); 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| | | Starch remaining (%) | Firmness |
|---|---|---|---|
| Fam1 | 2007 | $44^{c} \pm 1.5$ | $8.5^{f} \pm 0.09$ |
| | 2008 | $48^{b} \pm 1.6$ | $9.6^{c} \pm 0.08$ |
| Fam2 | 2007 | $38^{d} \pm 1.4$ | $8.8^{e} \pm 0.09$ |
| | 2008 | $49^{b} \pm 1.5$ | $9.4^{d} \pm 0.10$ |
| Fam3 | 2007 | $62^{a} \pm 1.5$ | $10.0^{b} \pm 0.08$ |
| | 2008 | $48^{b} \pm 1.5$ | $10.3^{a} \pm 0.08$ |
| Pr>F | | | |
| Family | | 0.0001 | 0.0001 |
| Seedling within family | | 0.0001 | 0.0001 |
| Year | | 0.3670 | 0.0001 |
| Year*Family | | 0.0001 | 0.0001 |

[z] Treatment means with the same superscript are significant at $p < 0.05$ according to Duncan's Multiple Range Test

Table 10. Total soluble solids (TSS) as well as the percentage malic, citric and tartaric acid measured in apple fruit from adult seedling trees in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1), 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| Families | TSS(ºBrix) | Malic acid (%) | Citric acid (%) | Tartaric acid (%) |
|---|---|---|---|---|
| Fam1 | $15.4^{b} \pm 0.12$ | $0.63^{a} \pm 0.017$ | $0.59^{a} \pm 0.016$ | $0.67^{a} \pm 0.018$ |
| Fam2 | $15.7^{b} \pm 0.12$ | $0.47^{b} \pm 0.015$ | $0.45^{b} \pm 0.015$ | $0.51^{b} \pm 0.017$ |
| Fam3 | $16.2^{a} \pm 0.24$ | $0.46^{b} \pm 0.016$ | $0.44^{b} \pm 0.016$ | $0.49^{b} \pm 0.018$ |
| Pr>F | 0.0017 | 0.0001 | 0.0001 | 0.0002 |

[z] Treatment means with the same superscript(s) are significant at $p < 0.05$ according to Duncan's Multiple Range Test.

Table 11 Variance components and intra-class correlation coefficients for characters associated with apple colour development in three breeding families, i.e., 'KAZ91' x 'Meran' (Fam1), 'Reinnette Burchard' x 'Treco Red Gala' (Fam2) and 'Meran' x 'Treco Red Gala' (Fam3) in 2007 and 2008.

| | Source of variation | | | | | | | Intraclass correlation | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | Variance total (Vt) | Year (Y) | Family (F) | Y x F | Seedling (Within F) | Block | Residual | $t_1^z$ | $t_2^y$ |
| Over colour | | | | | | | | | |
| L | 105.6 | 0.46 | 13.92 | 2.83 | 56.35 | 0.00 | 32.04 | 0.13 | 0.64 |
| C | 40.45 | 1.45 | 0.11 | 0.45 | 14.03 | 0.11 | 19.76 | 0.11 | 0.41 |
| H | 243.61 | 0.00 | 32.70 | 4.52 | 132.64 | 0.78 | 73.75 | 0.13 | 0.64 |
| Background | | | | | | | | | |
| L | 167.44 | 7.73 | 24.44 | 8.46 | 70.10 | 0.00 | 56.71 | 0.14 | 0.55 |
| C | 44.96 | 0.00 | 0.83 | 3.46 | 15.57 | 0.00 | 25.10 | 0.018 | 0.38 |
| H | 670.77 | 6.68 | 23.26 | 44.27 | 351.41 | 0.06 | 245.15 | 0.034 | 0.58 |
| % over colour | 1097.5 | 38.48 | 299.61 | 11.04 | 470.33 | 1.04 | 278.04 | 0.27 | 0.62 |
| % Starch breakdown | 1251.95 | 0.00 | 0.00 | 76.71 | 617.46 | 4.90 | 557.78 | 0.00 | 0.52 |
| Firmness | 5.08 | 0.13 | 0.43 | 0.14 | 2.66 | 0.0044 | 1.72 | 0.084 | 0.60 |
| Red flesh coverage (%) | | | | | | | | | |
| Across families | 212.84 | 0.00 | 35.29 | 0.00 | 142.17 | 0.00 | 35.38 | 0.16 | 0.80 |

| Red flesh coverage (%) | Variance total (Vt) | Year (Y) | Seedling (S) | Y x S | Seedling (Within Reps) | | Residual | | $r^x$ |
|---|---|---|---|---|---|---|---|---|---|
| Within family 1 | 784.96 | 0.00 | 425.16 | 53.56 | 21.36 | | - | 284.88 | 0.54 |

[z] Intra-class correlation coefficient for between-family variation: $t_1 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_W^2}$

[y] Intra-class correlation coefficient for within-family variation: $t_2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$

[x] Repeatability estimate: $r = \frac{V_G + V_{Eg}}{V_P}$

Fig. 1 Variation in fruit colour among the progeny of three apple breeding families, i.e., 'KAZ91' x 'Meran' (A1-2), 'Reinnette Burchard' x 'Treco Red Gala' (B1-2) and 'Meran' x 'Treco Red Gala' (C1-2) in 2007 (top row) and 2008 (bottom row).

Fig. 2 Frequency distribution for lightness values (A) and hue angle (B) measured on the exposed sides of apple fruit of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3) in 2007. Means of parents are indicated with arrows.

Fig. 3 Frequency distribution for lightness values (A) and hue angle (B) measured on the exposed sides of apple fruit of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard'(RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3) in 2008. Means of parents are indicated with arrows.

Fig. 4 Frequency distribution for lightness values (A) and hue angle (B) measured on the shaded sides of apple fruit of of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3) in 2007. Means of parents are indicated with arrows.

Fig. 5 Frequency distribution for lightness values (A) and hue angle (B) measured on the shaded sides of apple fruit of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3) in 2008. Means of parents are indicated with arrows.

Fig.6 Frequency distribution for anthocyanin concentration (mg $g^{-1}$ FW) in the peel of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3). Only Families 1 and Family 2 were assessed in

2007 (A) while all three families were assessed in 2008 (B). Means of parents are indicated with arrows, the parents were only assessed in 2008.

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Fig 7 Frequency distribution for total phenolic concentration (mg gallic acid equivalents $g^{-1}$ FW) in the peel of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3). Only Families 1 and Family 2 were assessed in 2007 (A) while all three families were assessed in 2008 (B). Means of parents are indicated with arrows, the parents were only assessed in 2008.

Fig.8 Examples of different patterns of anthocyanin distribution in the flesh of 'KAZ91' x 'Meran' seedlings.

Fig.9 Example of variation in anthocyanin distribution between fruits picked from the same seedling tree of 'KAZ91' x 'Meran'.

Fig. 10 Frequency distribution for anthocyanin concentration (mg $g^{-1}$ FW) in the flesh of apple breeding family 'KAZ91' (KZ) x 'Meran' (MR) in 2007 and 2008 .

Fig.11 Frequency distribution for total phenolic concentration (mg gallic acid equivalents $g^{-1}$ FW) in the flesh of three apple breeding families, i.e., 'KAZ91' (KZ) x 'Meran' (MR) (Family 1), 'Reinnette Burchard' (RB) x 'Treco Red Gala' (TG) (Family 2) and 'Meran' x 'Treco Red Gala' (Family 3). Only Family 1 was assessed in 2007 (A) while all three families were assessed in 2008 (B). Means of parents are indicated with arrows, the parents were only assessed in 2008.

A1

B1

C1

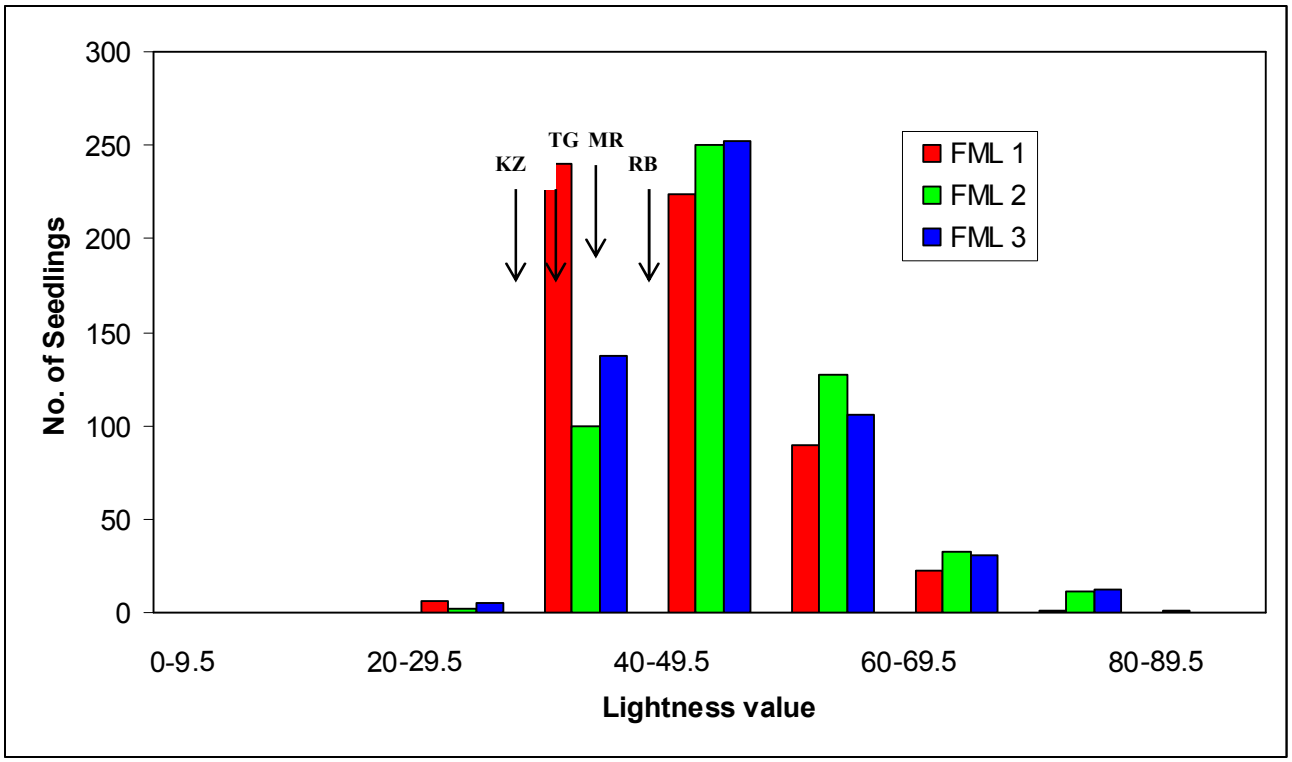
A 2

B2

C2

Fig 1 Paper 1

**(A)**

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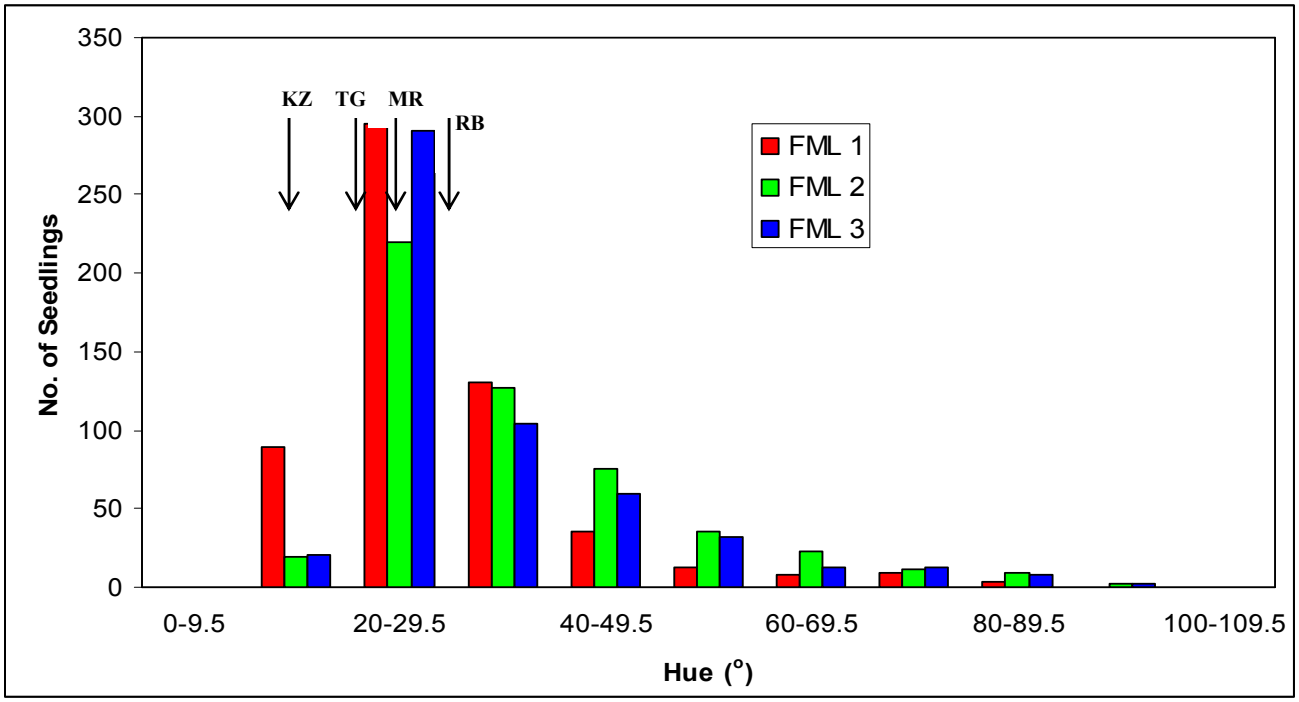


Fig 2 Paper 1

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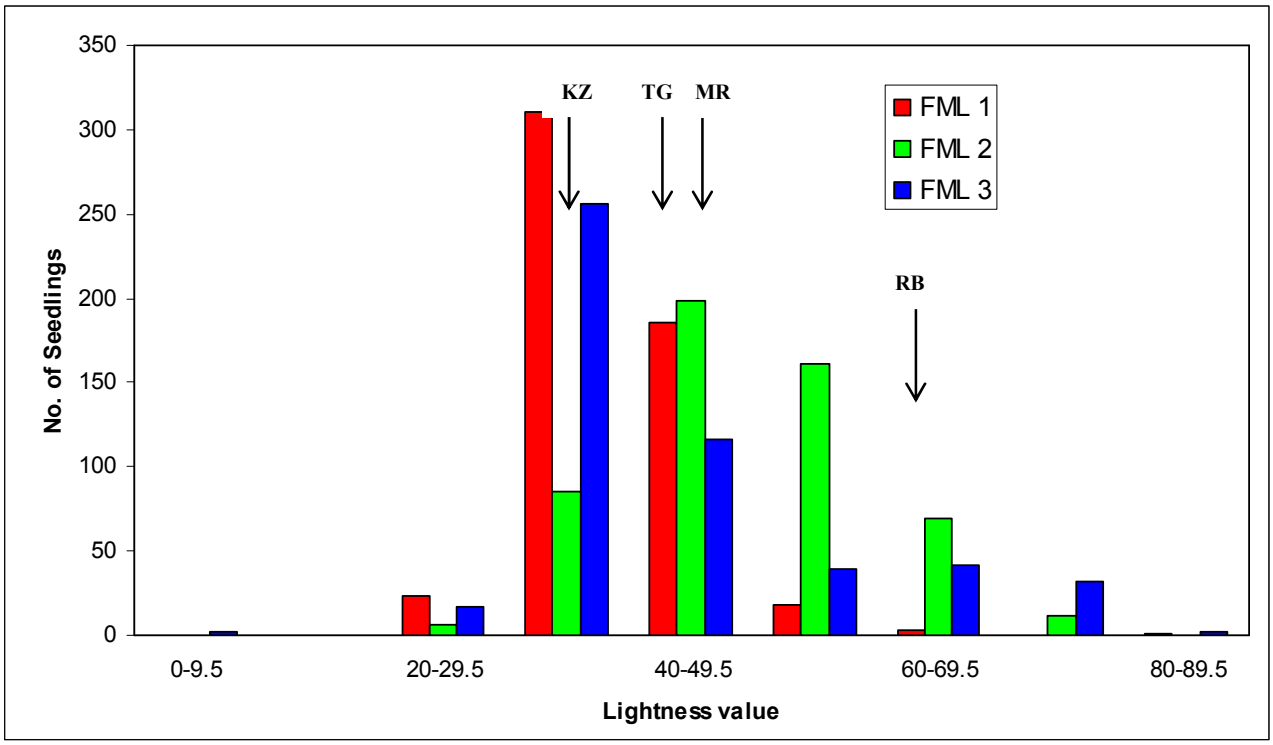


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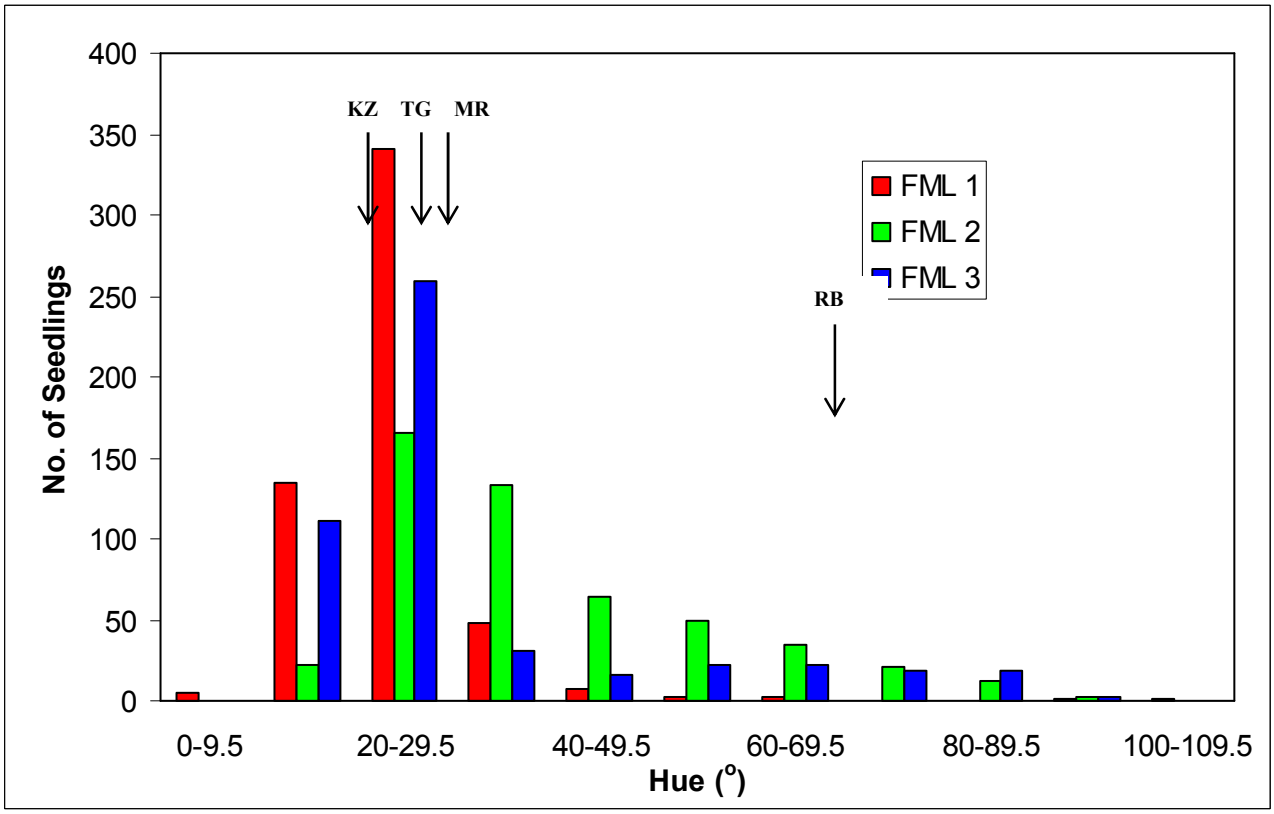


Fig.3. Paper 1

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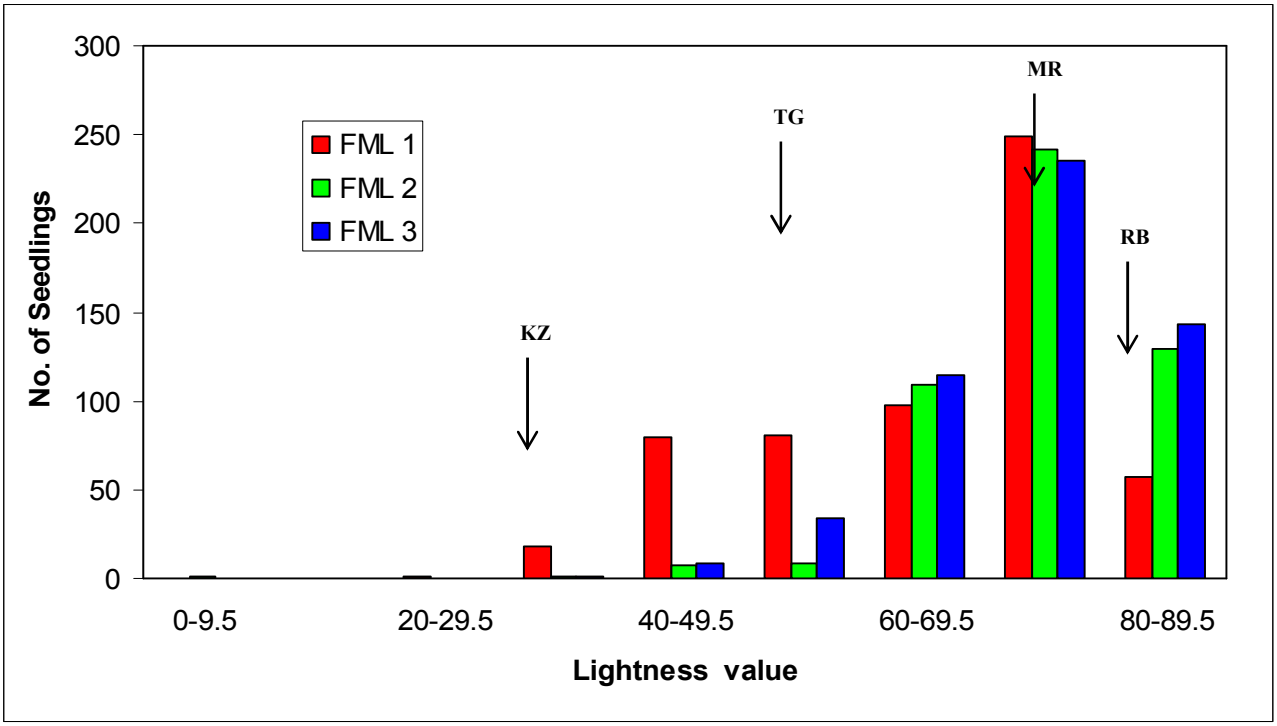


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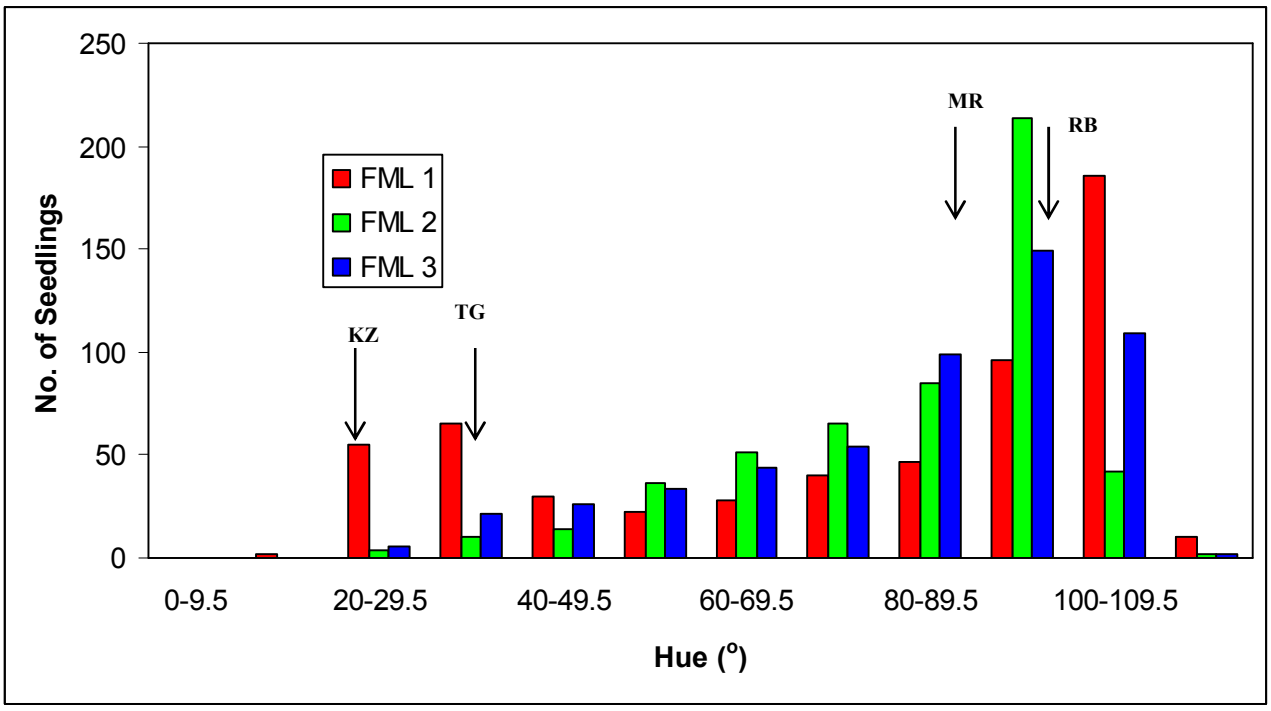


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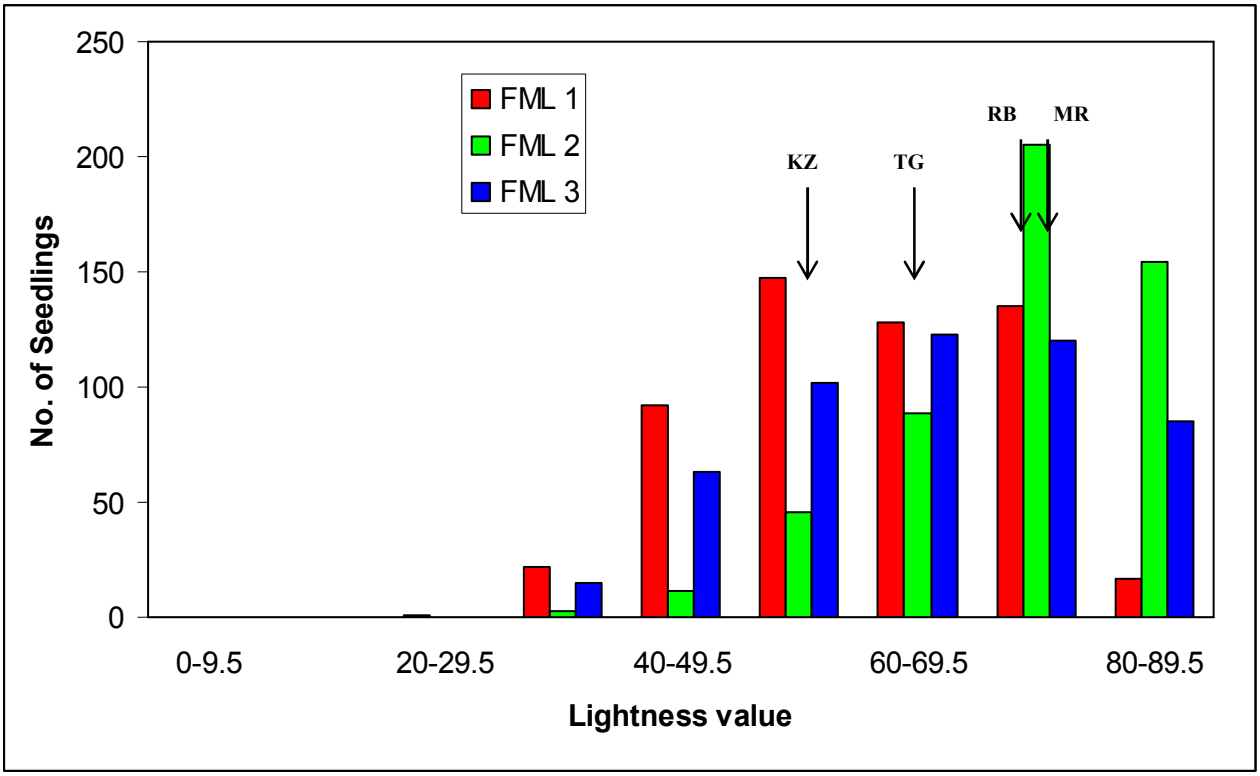


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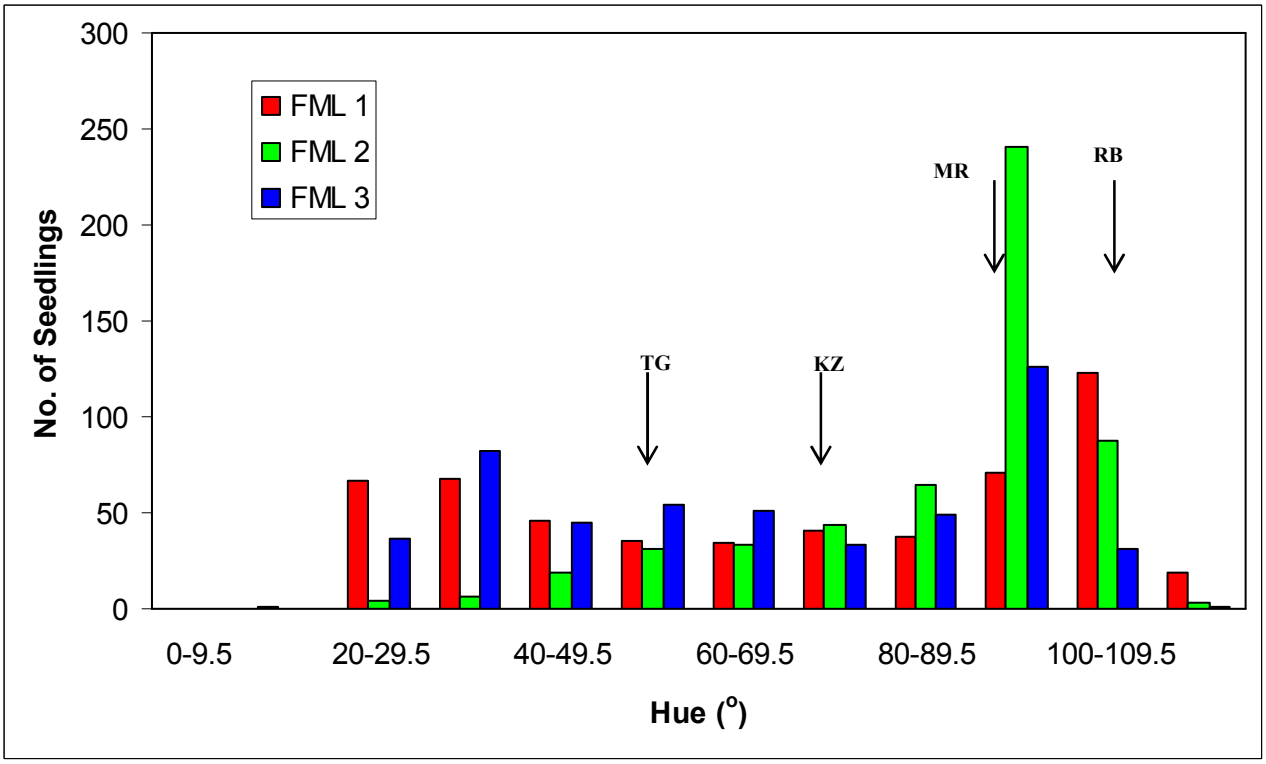


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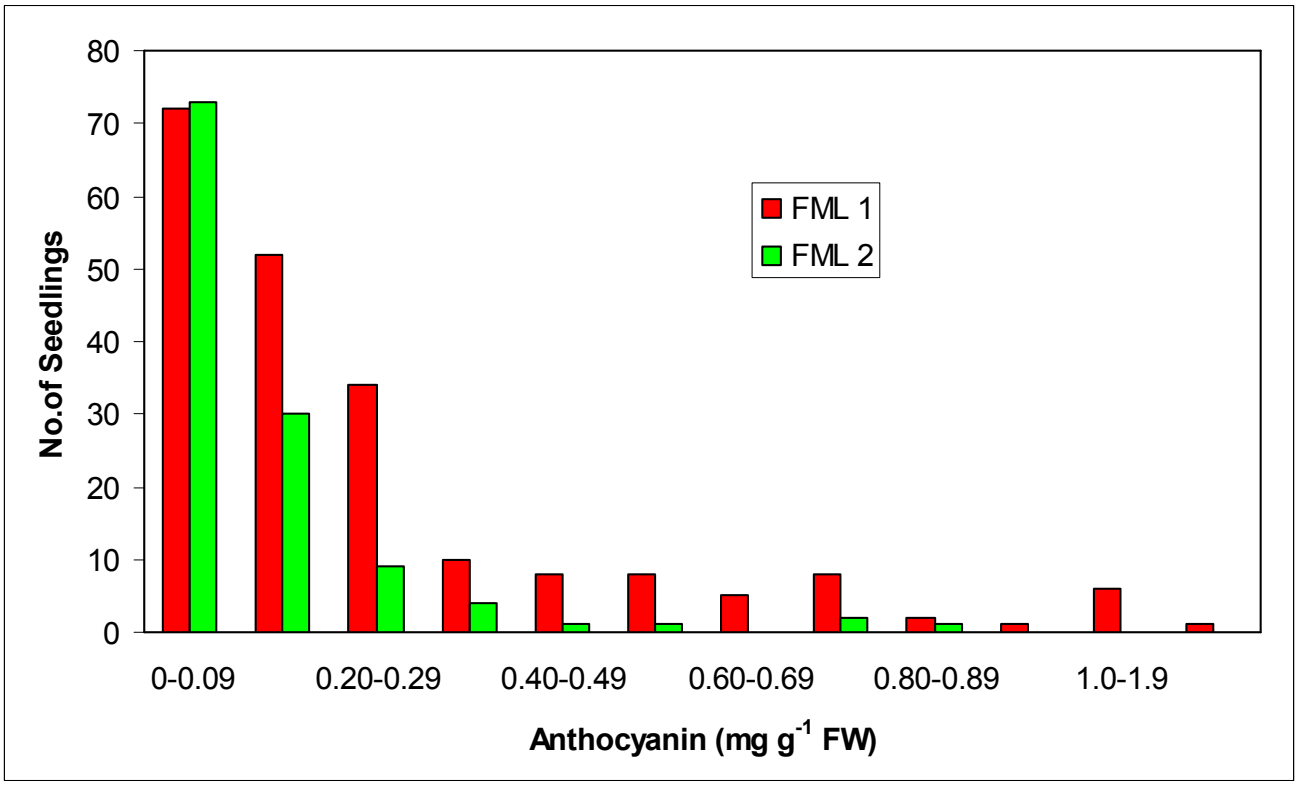


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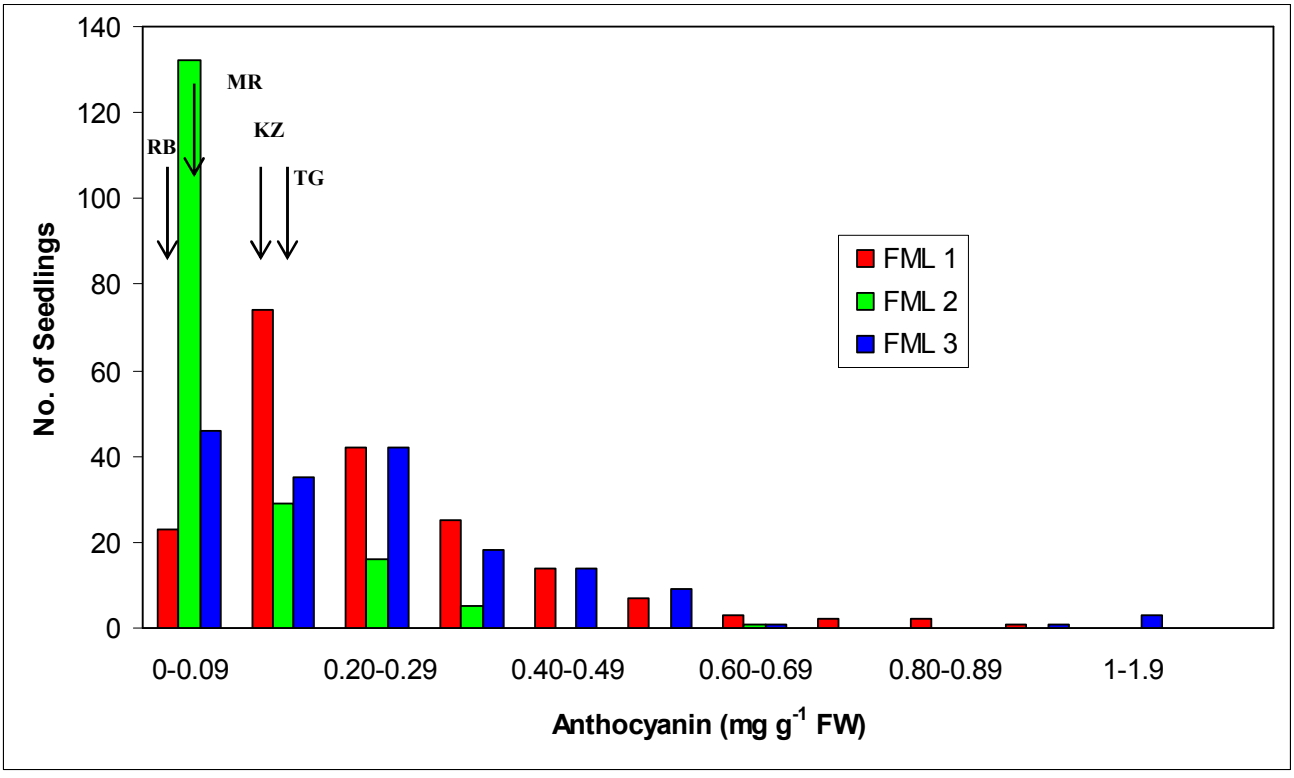


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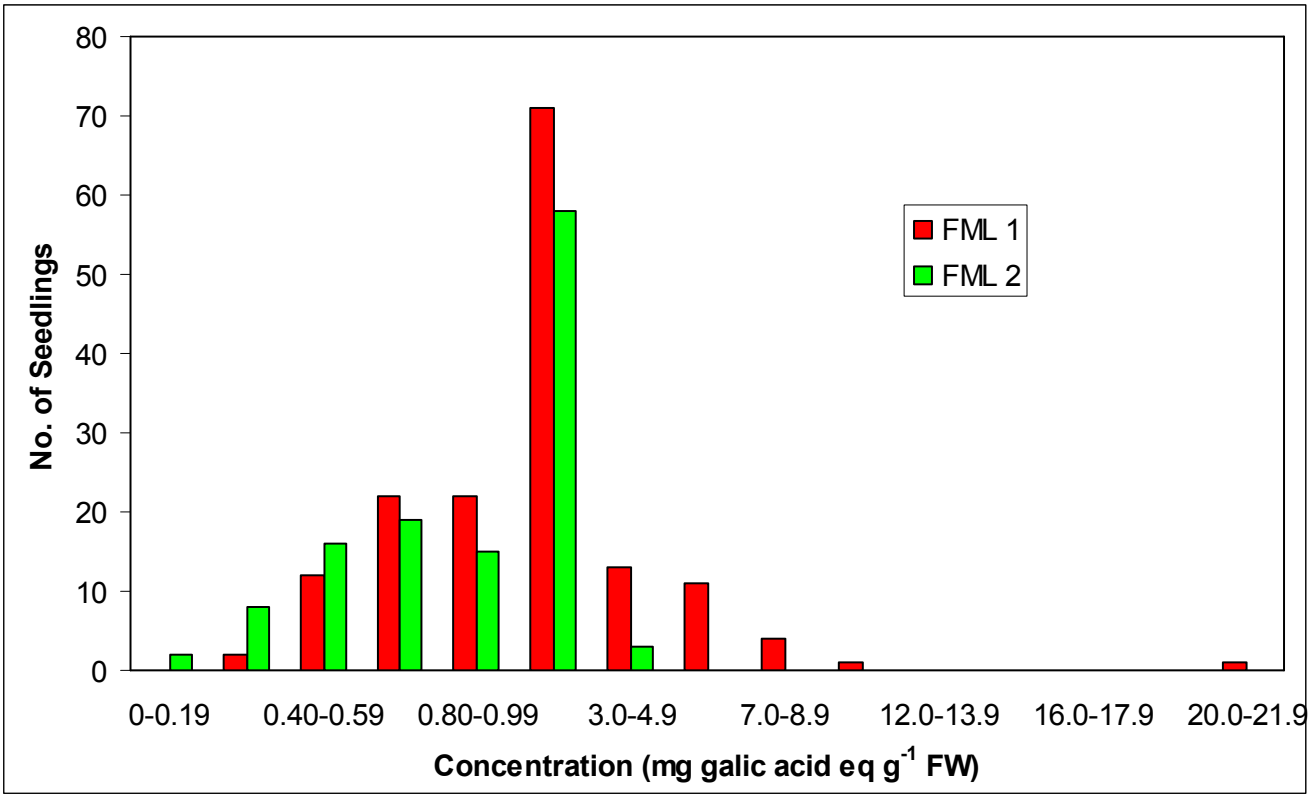


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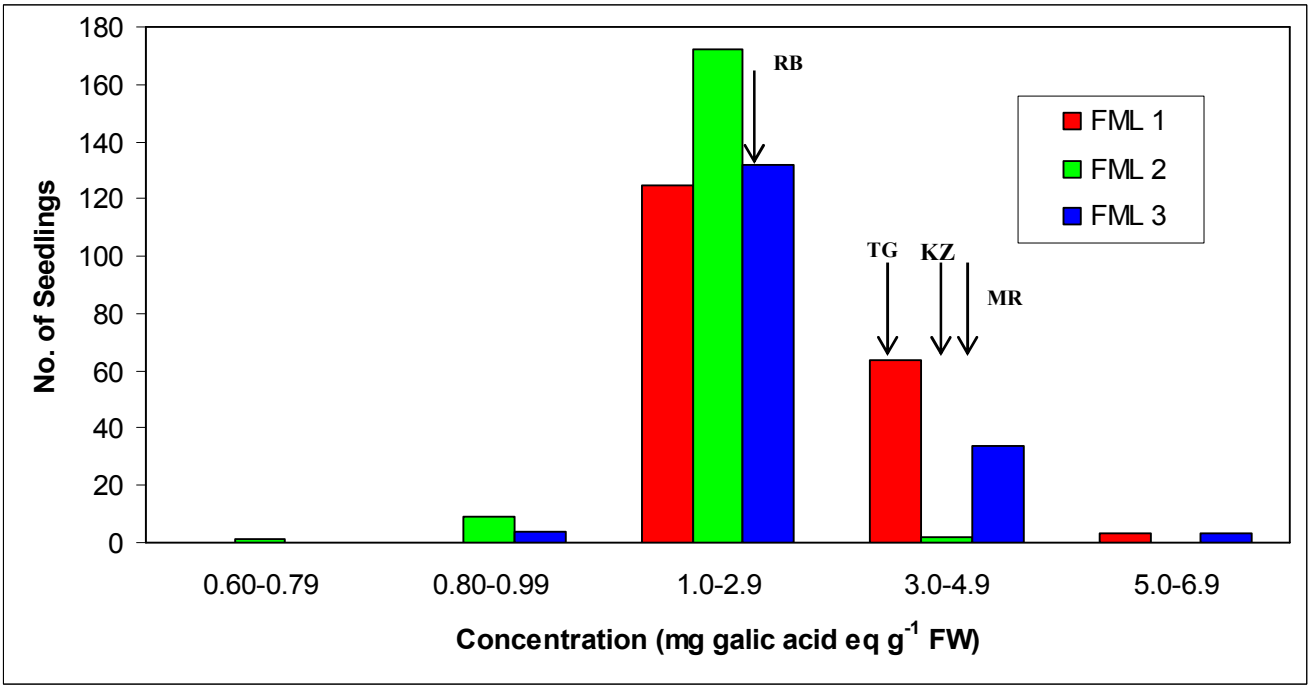


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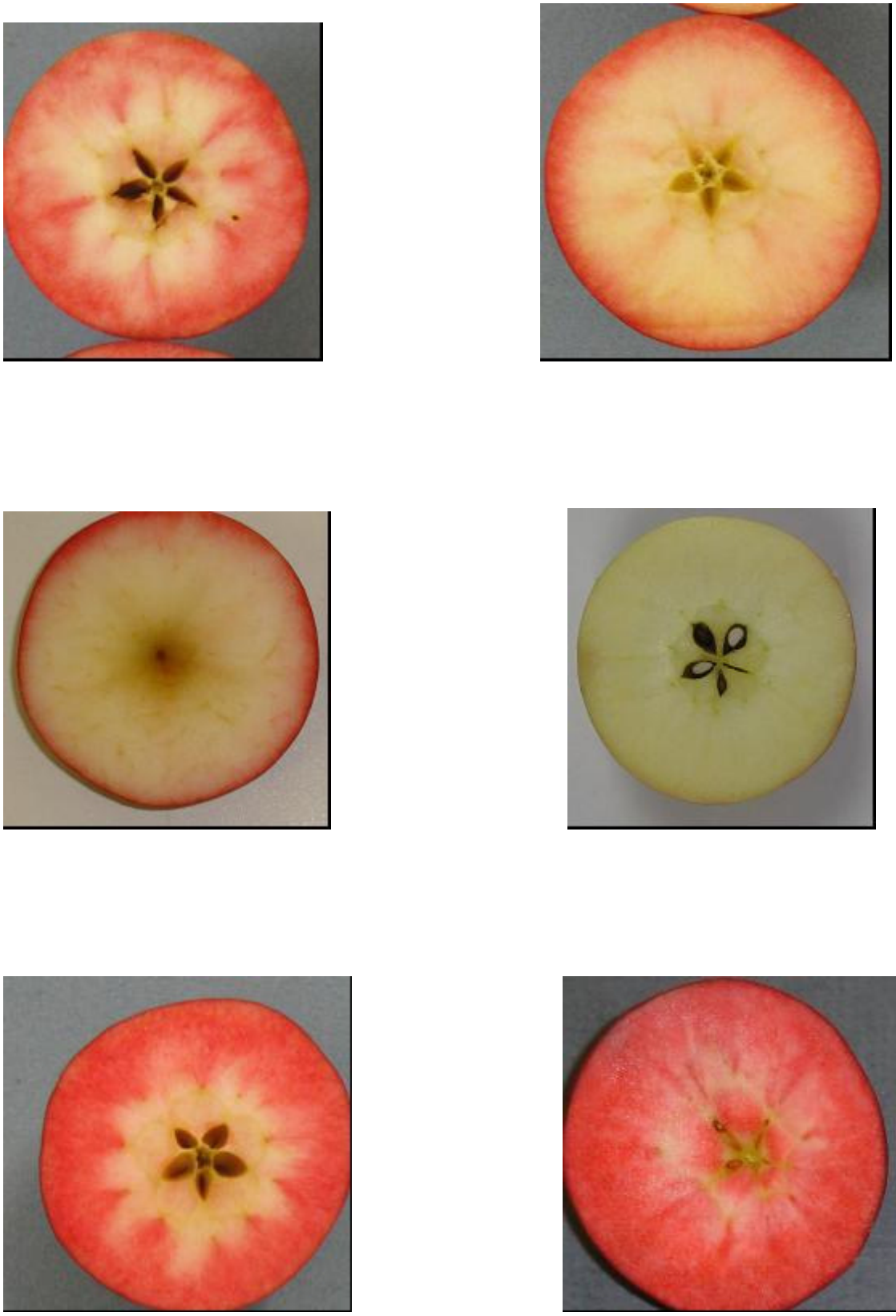


Fig.8. Paper 1

Fig. 9 Paper 1

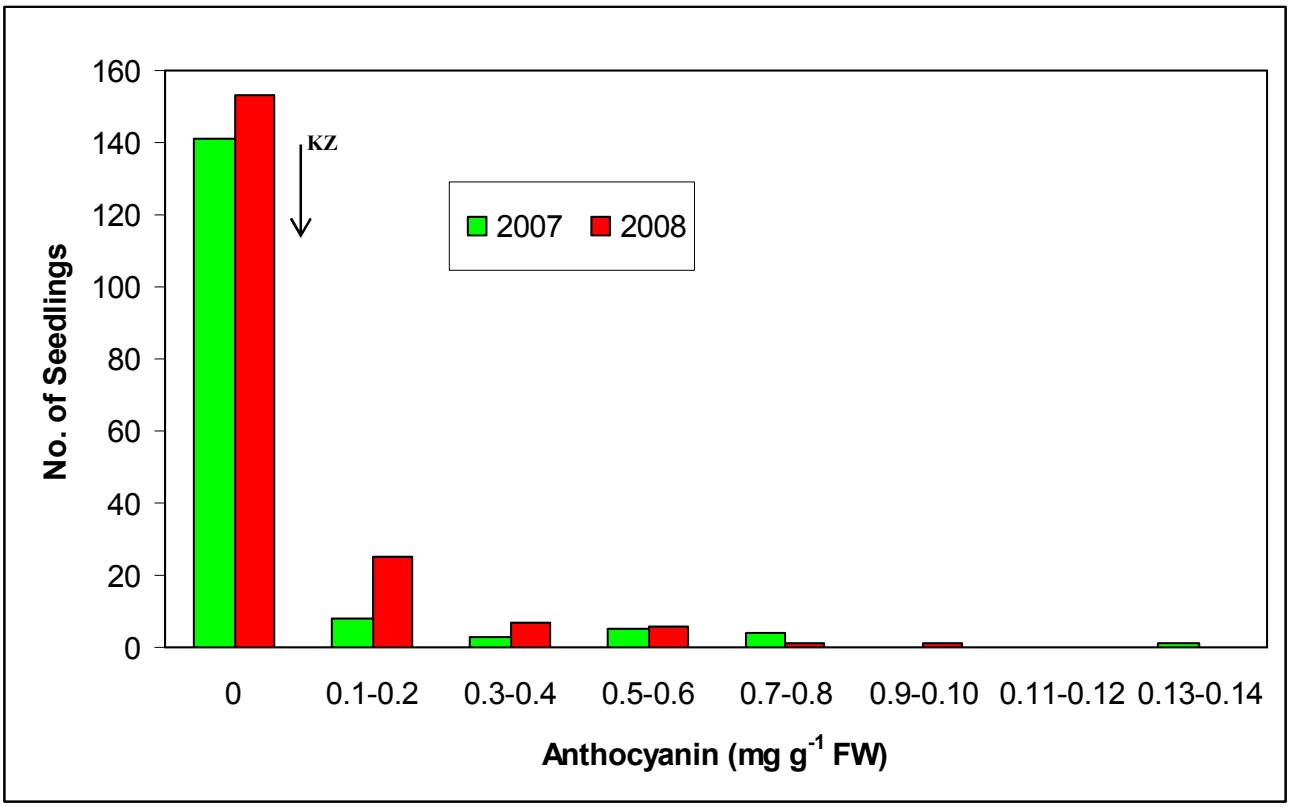
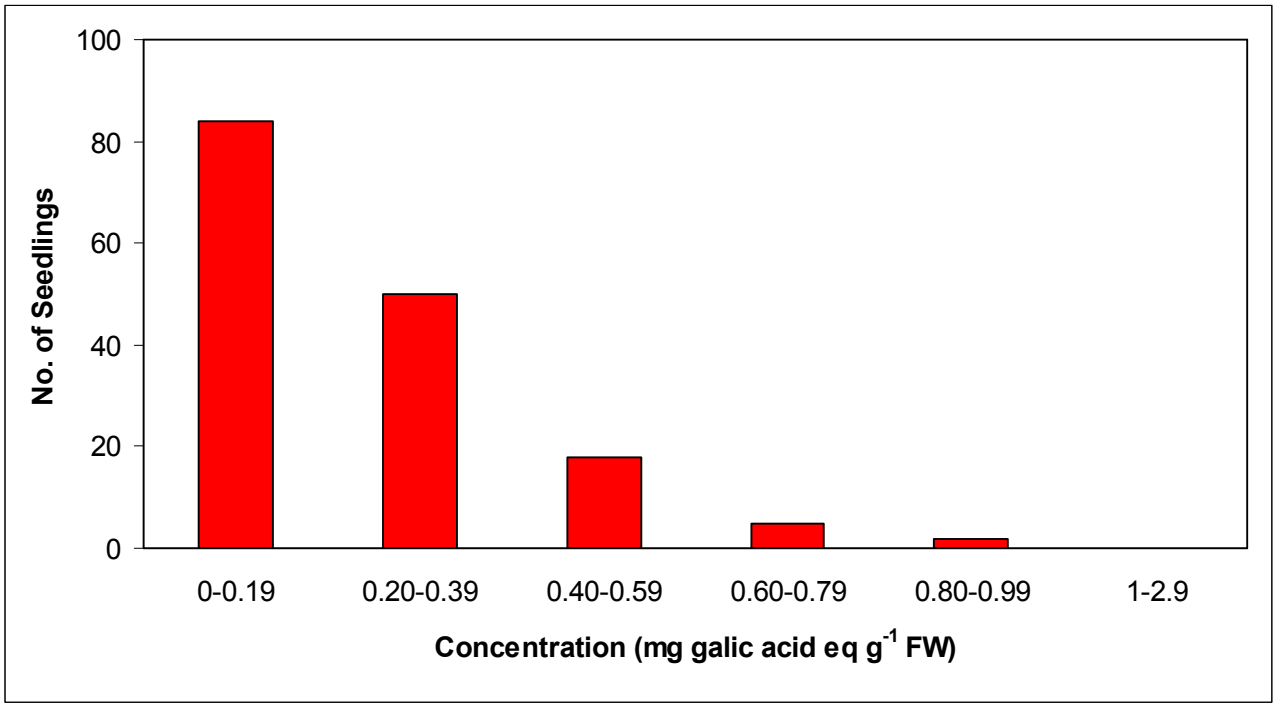



Fig. 10 Paper 1

A




B

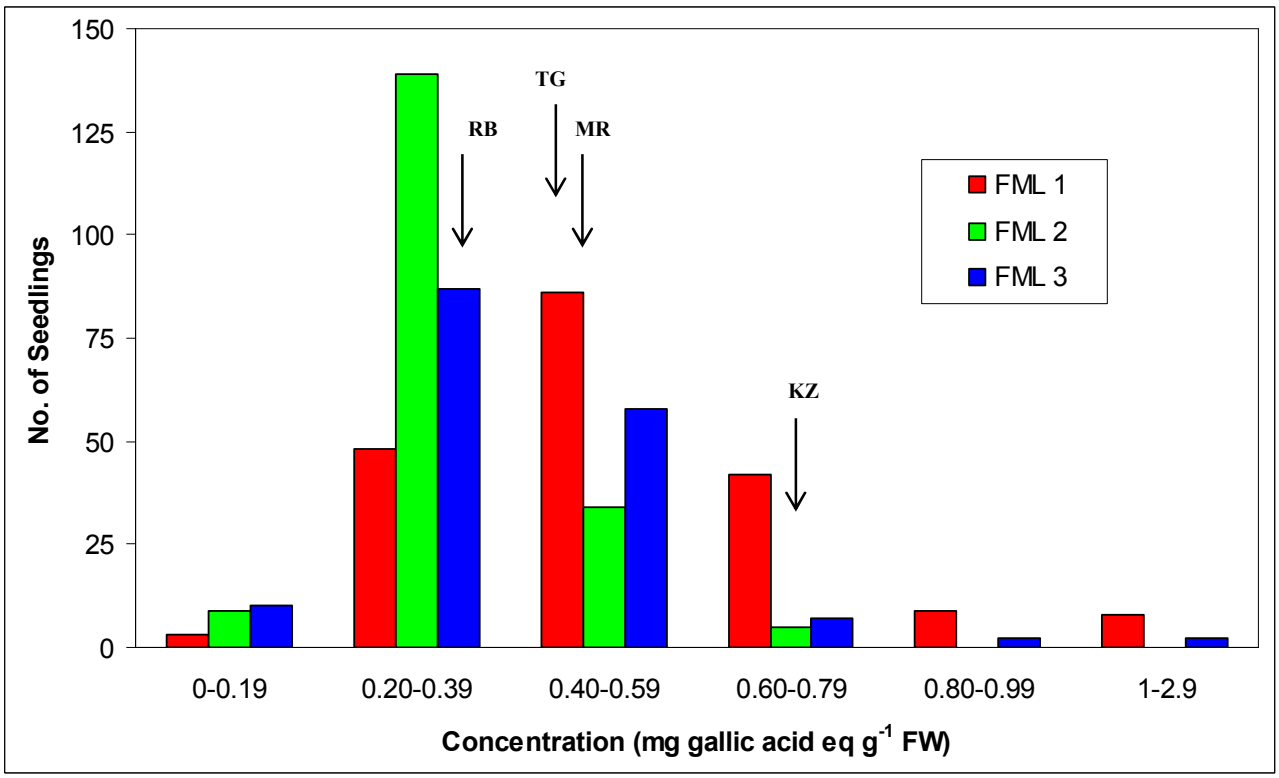



Fig. 11 Paper 1

# PAPER 2: CONSUMER PREFERENCE FOR COLOUR AND TASTE OF RED-FLESHED APPLE GENOTYPES.

*Abstract:* **The main objective of this study was to investigate consumer preference for taste and flesh colour of red-flesh apple seedling genotypes derived from a cross between red-fleshed selection 'KAZ91' (*Malus niedzwetzkyana* Dieck.) and white-fleshed 'Meran' (*M. domestica* Borkh.). White- fleshed 'Cripp's Pink' was used as a reference starndard. The majority of consumers (≈74%) preferred the white-fleshed, commercial cultivar Cripps' Pink while ≈64% of consumers preferred the appearance of the fruit with a red cortex and white core with red pigmentation extending into the pith between carpals, giving rise to an attractive "floral" pattern. With regards to taste, consumers preferred genotypes that were juicy, flavoursome, crispy and crunchy. Preference of taste had strong positive correlations with juiciness, apple flavour, crispness and crunchiness. However, no correlation was found for preference of taste and TSS, TA, TSS:TA ratio, percentage red-flesh coverage and firmness. A weak correlation was found between preference for taste and sweetness and no correlation was found between preference for taste and sourness. Mealiness was found to affect preference for taste negatively. Few seedling genotypes had a comparable preference to Cripps' Pink and Meran, both commercial cultivars. These genotypes invariably had a good texture and flavour. A subset of preferred seedling genotypes had high red flesh coverage and high acidity, suggesting that some consumers may prefer this combination. The presence of peel affected all the sensory attributes that were assessed and, in a few genotypes, gave rise to a slight bitter taste and astringent mouthfeel. However, the presence of peel did not have a significant effect on consumer preference of taste. Astringency, percentage red-flesh coverage, TA and sour taste grouped together in the preference map suggesting that these traits may be associated.**

## INTRODUCTION

Internal and external fruit quality of apples has been enhanced through conventional breeding practices by selection of progeny with good eating quality and appearance

(Kellerhals *et al.,* 1999). Quality is a combination of all attributes that makes a product acceptable to consumers (Harker *et al.,* 2003). Consumer preference for apples is usually driven by appearance and/or taste (P'eneau *et al.,* 2006; Pre-Aymard *et al.,* 2005). Appearance includes the external fruit colour, shape and cosmetic appeal as well as flesh colour, whereas taste includes flavour, as well as textural and mouth-feel attributes (Harker *et al.,* 2008).

Recent breeding efforts of various apple breeding programs have focused on the development of red-fleshed cultivars utilising the red-fleshed species *M. niedzwetzkyana* (Chagne *et al.,* 2007; Volz *et al.,* 2006). The presumption is that red-fleshed apples will find a market due to the novel appearance of the fruit and the supposedly high antioxidant capacity of the red flesh (Freshplaza, 2008). The majority of apple cultivars have white, greenish or yellowish flesh (Janick *et al.*, 1996). Quite a few cultivars with red flesh exist, with the apple germplasm collection at Geneva containing thirty-three *M. domestica* accessions with pink or red flesh (USDA, ARS, National Genetic Resources Program, 2009). In 2008, the innovation company, Next Fruit Generation, introduced a red-fleshed cultivar after 12 years of breeding with commercial production expected to commence in the 2009/2010 season (Freshplaza, 2008). A few existing red-fleshed cultivars are sold under the trade name Rosetta™ apples, e.g., Pink Pearmain®, Blush Rosetta™, Christmas Pink™ and Pink Parfait™ (Greenmantle Nursery, 2009).

Previous studies have shown that consumers usually prefer skin colours and cultivars that they are familiar with. For example, Gamble *et al.* (2006) found that consumers did not prefer red-skinned pears. However, they considered that this lack of preference stems from unfamiliarity of consumers with red-skinned pears. When considering apple, it is not known whether consumers will find the unfamiliar red colour of the flesh acceptable and preferable compared to the traditional flesh colours. However, Jaeger and Harker (2005) found that consumers were willing to exchange the normal green-fleshed kiwi fruit for novel red/yellow-fleshed kiwi fruit. Red pigmentation within red-fleshed progeny varies in intensity and may be confined to the core or cortex, or may occur throughout the fruit (Chagne *et al.,* 2007; Volz *et al.,* 2006). Knowledge on the intensity of red flesh colour and the distribution of red

pigmentation in the flesh that consumers prefer will be most helpful in the selection of new red-fleshed cultivars.

Various consumer groups may differ in their preference for fruit appearance. Cliff *et al.* (2002), using images to assess consumer preference for apple appearance (colour and shape), found that New Zealand consumers preferred apples that were round and striped whereas Nova Scotia consumers preferred blushed apples of any shape and background colour. New Zealand consumers preferred red and green apples over yellow apples whereas British Columbia and Nova Scotia consumers preferred red apples. A consumer preference study by McCracken *et al.* (1994) showed that consumers base their decision to purchase an apple on its colour and price, so called pre-purchase factors. Flavour and texture were the most important attributes in determining the after-purchase assessment (McCracken *et al.,* 1994). Red-flesh colour will obviously not play a primary role in the initial purchase decision. Hence, the skin colour and general appearance of these apples should also be kept in mind. It is furthermore important that red-fleshed fruit also taste satisfactory.

Sensory attributes of importance in apples include texture traits (crispness, hardness, firmness, crunchiness, juiciness, mealiness and skin toughness) and flavour traits (apple flavour, sweetness, sourness and astringency) (Kuhn and Thybo, 2001; Mehinagic *et al.,* 2004). Texture is based on the changes with regards to crispness, hardness, crunchiness and juiciness during maturation (Watkins, 2003). The rearrangement of the primary wall and middle lamella is the primary cause of loss in texture, although a decrease in starch content and turgor may also result in texture loss (Watkins, 2003). Flavour is a combination of taste and aroma. Taste is characterized by the ability of the taste buds to sense sweetness, sourness, bitterness, and saltiness in the mouth and aroma can be described as a combination of volatile odours detected by the nose. Both these quality attributes comprise of a mixture of complex characteristics including concentrations of sugars, phenolic compounds, organic acids, etc. (Watkins, 2003).

Flesh texture is the major factor that determines consumer preference in apples. Consumer preference and willingness to purchase apples generally increase with an

increase in firmness (Harker *et al.,* 2008; McCracken *et al.,* 1994). However, Daillant-Spinnler *et al.* (1996) and Pre-Aymard *et al.* (2005) found that the interaction between texture and flavour of apples also play an important role in consumer preference. Furthermore, consumers also have different and distinct expectations of specific cultivars. In 'Gala', for example, consumer preference related to high firmness and a high sugar level whereas high sugar and acidity increased consumer acceptability for 'Braeburn' (Harker *et al.,* 2008). Attributes that affect consumer's preference for apples negatively include mealiness, a spongy texture and off-flavours (Jaeger *et al.,* 1998), high acidity together with low sugar levels (Janick *et al.,* 1996), and astringency (Guinard and Mazzucchelli, 1996). Jaeger *et al.* (1998) indicated that consumers associated mealiness in apples with a fluffy appearance of the flesh, stale flavour and granular texture. Limited research has been conducted on the effect of skin toughness on consumer acceptability. Daillant-Spinnler *et al.* (1996) found that apple fruit skin does not affect the sensory properties of the flesh and also does not influence acceptability of different cultivars. However, Amos (2007) found a significant increase in the amount of force required to chew an apple with peel in comparison with the apple without peel. Kuhn and Thybo (2001) observed a negative correlation between skin toughness and consumer preference for taste.

In addition to red flesh colour, wild apple species may also impart negative eating characteristics to their progeny. The tannin levels in wild apples are about seven times higher and acidity is about two times higher than in cultivated apples (Dzhangaliev, 2003). Consequently, wild apples, such as *M. niedzwetzkyana* Dieck., often tend to be rather astringent and acidic. Some wild apples also have low sugar levels. Taste in wild apples ranges from sweet, sweetish-sour, sour, sweetish-bitter, bitter to sourish–bitter (Dzhangaliev, 2003). Although various consumer studies have assessed consumer preference for taste of different apple cultivars, we are not aware of any study that has included red-fleshed apples.

The aim of this study was to analyse the preference of South African consumers for the internal appearance and taste of red-fleshed apples derived from a cross between a red-fleshed selection (KAZ91) of *M. niedzwetzkyana* Dieck. and the white-fleshed *M. domestica* Borkh. cultivar, Meran. No information is available as to how the red-flesh

trait influences the taste perception of apples. Consumer preference for the distribution pattern and intensity of red pigmentation in the flesh was also investigated using photographs of representative fruit.

## MATERIAL AND METHODS

### Breeding parents

Apples used in this study originated from a cross between a red-fleshed *M. niedzwetzkyana* selection, KAZ91 and the white-fleshed *M. domestica* cultivar, Meran (Fig 1A and B). Bud wood of 'KAZ 91' was collected in 1996 from wild *M. niedzwetzkyana* trees in Kazakhstan. Fruit from this genotype are normally harvested around mid February in the Western Cape, South Africa. 'KAZ91' has a dull red over colour that can be fully red to weak red. It is normally a large-sized fruit of about 65-75 mm in diameter. Fruits of 'KAZ 91' are astringent and acidic with a firm but not crispy texture (personal communication, I. Labuschagné). Meran is a brightly-striped red apple with a green-yellow background colour and it is classified as a striped bi-colour. It has a good texture, sweet taste, is crispy and juicy, but it lacks flavour. 'Meran' fruit are large in size, about 70 mm in diameter and show poor colouration in the Western Cape of South Africa. 'Meran' is harvested from early to mid-April.

### Fruit collection

Fruit were collected from Drostersnes experimental farm of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij in the Western Cape Province of South Africa (Mediterranean-type climate, latitude: 33.33'S, longitude: 18.63'E). Seedling trees, grafted on M793 rootstock, were planted in November 2003 in an East West row direction and a spacing of 0.75 m x 4 m on M793 rootstock. Fruits were harvested from 206 trees replicated in four blocks from 11 February 2008 until 24 April 2008 and were stored in a cold room at -0.5ºC. Of the 206 trees evaluated, about 35% of the seedlings were red-fleshed and 65% white-fleshed. Twenty-two genotypes were

randomly selected from the 35% red-fleshed seedlings and two white fleshed genotypes were also selected by mistake. These selections were coded depending on the percentage red-flesh coverage ranging between 0.33 (R1) and 44.7 (R22). The two white fleshed seedlings were abbreviated as W1 and W2.

**Descriptive sensory analysis**

Eight judges were trained in descriptive sensory analysis according to the procedure described by Lawless and Heymann (1998) and tested for consistency. An unstructured 100 mm line scale was used for analysis with the left side of the scale representing low values and the right side of the scale representing higher values of the respective attributes. For training of judges, five seedlings where used with 'Cripps' Pink' as a control or reference standard.

The twenty-four seedlings were tasted in six sessions from 5 to 6 August 2008, including one peeled fruit per seedling (Assessment 1). Sixteen of these seedlings were reassessed in four sessions on 12 August 2008 using one unpeeled fruit per seedling (Assessment 2). The excluded eight genotypes did not have enough fruit for the second assessment. One of the parents, 'Meran', was also included in every session, as well as 'Cripps' Pink' as a reference standard, which was always served in the last position on the evaluation tray. 'KAZ91' fruit were not assessed due to poor eating quality resulting from prolonged storage. Samples were presented in a complete randomized order on Petri dishes (Kimix, South Africa), each sample marked with a three digit codes. Each judge tested six samples per session. The sample size was 1/8 apple for each of the eight judges. Tasting was conducted in sensory booths with artificial light and a controlled room temperature of 21 ºC. For Assessment 1, judges were provided with knives to remove the peel and were instructed to only taste the flesh. Eight sensory attributes were assessed for apples without peel (Table 1). Bitterness and skin toughness were also assessed when tasting with peel during Assessment 2. Biscuits (Woolworth, South Africa) and water were used as pallet cleaners between the tastings.

**Consumer sensory analysis**

Ninety-six consumers between the ages of 18 and 41 that consume apples regularly participated in the consumer sensory analysis on 8 August 2008. Consumers were asked to complete four questionnaires. Questionnaire 1 and 2 were used to assess degree of liking of internal and external colour, respectively, making use of photographs of representative samples. Consumers were asked to indicate their preference for internal and external appearance making use of a nine point hedonic scale ranging from 9 (Like extremely) to 1 (Dislike extremely) (Lawless and Heymann, 1998). Questionnaire 3 and 4 assessed preference for taste without peel (peeled) and with peel (unpeeled), respectively. Consumers were asked to taste apples with or without peel and indicate their degree of liking on the nine point hedonic scale as described above. Each consumer tasted six samples without and six samples with peel. The sample size was ¼ apple per consumer, further subdivided into a peeled and an unpeeled half. Each genotype was assessed by 16 consumers. Each samples was coded with a three-digit random code and served in a Petri dish (Kimix, South Africa) in a randomized order at room temperature (21°C). In the case of peeled samples, consumers were provided with knives to remove the peel and instructed to only taste the flesh. Consumers used water as pallet cleaner between tasting.

**Physical measurements**

Physical measurements were done on three fruit per seedling and parental genotype. External fruit colour was measured on the most and least pigmented sides of the fruit, midway between the calyx and stem using a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan). Lightness (L), chroma (C), and hue angle (H º) were recorded. Colour coverage was measured using a high resolution Nikon DXM1200 digital camera with a 0.63x relay lens (Innovative solutions (IMP) Scientific and Petitions Pty Ltd, Johannesburg, South Africa). Images were taken of the most and least coloured sides of fruit and the proportion of the fruit area with red pigmentation was calculated using Image Pro-Plus 4.5 Software for image analysis (Innovative solutions (IMP) Scientific and Petitions Pty Ltd, Johannesburg, South Africa).

Optimal lighting was achieved by using a microlite fluorescent ring light for epi-illumination (Innovative solutions (IMP) Scientific and Petitions Pty, Johannesburg, South Africa).

The extent of red flesh colour coverage was assessed using the above mentioned high resolution Nikon DXM1200 camera. One photo of each cut half of the fruit was taken and the percentage of the flesh with red colour calculated with Image Pro-Plus 4.5 software. The distribution of anthocyanin pigmentation in the flesh was recorded and seedling selections ranked and coded according to the percentage of their flesh covered by red pigmentation (Table 2).

Firmness was measured using a fruit texture analyzer with an auto penetrometer (GUSS FTAWin Version 4.51, Innovative solutions (IMP), Scientific and Petitions Pty Ltd, Johannesburg, South Africa), with 11 mm plunger size on two opposite sides of the fruit. Flesh slices of the three apples per seedling were blended together and used to assess total soluble solids (TSS) with a refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan) and titratable acidity (TA) by titrating 5 g of juice from each sample with 0.1 N NaOH to a pH of 8.2 using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland).

**Statistical analyses**

*Descriptive sensory analysis*

Eight judges were trained for descriptive sensory analysis. A randomized block design was used for descriptive sensory analysis with six treatments per judge. All data were subjected to test-retest analysis of variance (ANOVA) using SAS® software (Version 9; SAS® Institute Inc, Cary, USA) to test for reliability, i.e., temporal stability (Judge*Replication interaction) and internal consistency (Judge*Product interaction) (SAS®, 2002). The Shapiro-Wilk test was used to test for non-normality of residuals (Shapiro and Wilk, 1965). If non-normality was significant ($P \leq 0.05$) and caused by skewness, the outliers were identified and removed until the data were normal or symmetrically distributed (Glass *et al.,* 1972). Using line plots indicating temporal

stability and internal consistency, single odd judges were identified and removed. Panel reliability was also assessed using PanelCheck software (Version 1.3.1, Nofima, AS, Norway). After the data were subjected to all the procedures mentioned above, a final analysis of variance (ANOVA) was performed. Multiple comparisons were performed using Student's t least significant test at the 5% significance level.

*Consumer sensory analysis*

Ninety-six consumers were sourced. A randomized block design with twenty-four treatments was used for consumer sensory analysis with six treatments per consumer for both peeled and unpeeled assessments. ANOVA was performed on the consumer data using SAS® software (Version 9; SAS® Institute Inc, Cary, USA). The Shapiro-Wilk test was again used to test for non-normality of residuals (Shapiro and Wilk, 1965). If non-normality was significant ($P \leq 0.05$) and caused by skewness, the outliers were identified and removed until the data were normal or symmetrically distributed (Glass *et al.,* 1972).

*Multivariate statistical techniques*

XLSTAT software 2007 (Version, 7.5.2, Addinsoft, New York, USA) was used to conduct multivariate statistical analysis. The consumer preference data (y-space), and trained panel and physical data (x-space), were subjected to an external preference mapping using Partial Least Square (PLS) to establish the relationship between physical and sensory attributes and consumer degree of liking.

## RESULTS AND DISCUSSION

### Consumer socio- demographics

The group consisted of ninety-six consumers, of which 79% were female and 21% male. Ninety percent of the consumers were between the ages of 18 and 30. With regard to consumption of apples by consumers, 15% of the consumers consume apples 5 to 7 times per week, 33% approximately 3 to 4 times per week, 23% once a week and 27% at least twice a month.

**Preference of appearance**

All the genotypes used in this study were red in skin colour and were equally preferred by consumers on appearance (data not presented). Since there was no significant difference between female and male consumers in liking of flesh colour for the seven genotypes tested, their data were pooled. Consumers showed a significant preference for white flesh colour (Fig. 3) with 74% of consumers indicating a positive degree of liking (i.e., scores ≥6) (Fig. 4). With regard to red flesh colour, consumers preferred fruit with a red cortex and white core with red pigmentation extending into the pith between carpals giving rise to an attractive floral pattern (Fig. 3). Sixty- four percent (64%) of the consumers indicated that they liked the appearance of the latter pattern (Fig. 4). Most consumers recorded a slight dislike for the other distribution patterns, i.e., light red cortex and core, and light pink cortex and core (Fig. 3 and 4). Consumers generally buy fruits that they are familiar with (Harker *et al.,* 2003). The higher preference for white flesh shown in this study does not necessarily indicate that consumers would not consider buying red-fleshed fruit. Jaeger and Harker (2005) found that consumers were willing to exchange the familiar green-fleshed kiwi fruit for a novel red/yellow-fleshed kiwi fruit. It is interesting to note that despite the availability of red-fleshed *M. domestica* genotypes in germplasm collections (USDA, ARS, National Genetic Resources Program, 2009), as well as other red-fleshed cultivars, e.g. Rosetta apples (Greenmantle Nursery, 2009), none of these apples has been developed into major cultivars. Maybe this is due to the negative texture and flavour characteristics associated with wild apples (Dzhangaliev, 2003). Most of the Rosetta apples have good flavour and texture, but some have negative traits such as sourness and poor texture (Greenmantle Nursery, 2009).

**Sensory characteristics**

Different sensory attributes in apples were assessed by the trained panel with and without peel (Table 1). Significant interaction between genotype and the presence or absence of peel was found for most of the sensory attributes assessed (sour taste,

sweet taste, apple flavour, crispness, crunchiness and mealiness). The presence of skin decreased acidity and juiciness in some genotypes and increased the crispness, crunchiness, sweetness and flavour of others (Fig. 5, 6, 7 and 8). However, in agreement with the results of Daillant–Spinnler *et al.* (1996), the effect of peel on sensory attributes was generally minor. The presence of peel seemed to decrease the range of sensory scores for crispness, crunchiness, juiciness, sourness and apple flavour by increasing scores at the low end of the spectrum. This effect of peel has not been reported before in previous studies that assessed the effect of peel on apple sensory characteristics (Daillant–Spinnler *et al.,* 1996; Kuhn and Thybo, 2001)

With regard to texture attributes, mean values for crispness ranged from 18 (W2) to 49 (R18) without peel (Fig. 5) and from 29 (R14) to 52 (W1) with peel (Fig. 6). Hence, the presence of peel seemed to increase the experience / perception of crispness at the lower range of the spectrum. 'Cripps' Pink' crispness was higher than that of 'Meran' and almost all the selections, except for R2 and R18, when tasted without peel, and W1 when tasted with peel (Fig. 5 & 6). Mean values for crunchiness ranged from 17 (R10) to 47 (R7) without peel (Fig. 5) and from 27 (R8) to 44 (W1) with peel (Fig. 6). As for crispness, the presence of peel had a greater effect on the crunchiness of genotypes that had low crunchiness when tasted without peel. Quite a number of genotypes, i.e., R1, R2, R6, R7 and R18, had comparable or higher crunchiness than 'Cripps' Pink', particularly when tasted without peel. Mean values for juiciness ranged from 23 (W2) to 60 ('Cripps' Pink') (Fig. 5 & 6) and was seemingly not affected by the presence or absence of peel. Most selections were less juicy than 'Cripps' Pink', with the exception of W1, R15 and R6. Few of the genotypes were perceived as being mealy. Without peel, mean values for mealiness ranged from 0 to 22 (R11) while with peel, perceived mealiness was much lower varying between 0 and 8 (Fig. 7 & 8). Skin toughness varied little between selections from 50 ('Meran' and R17) to 67 (R10) (Fig. 5).

Apple flavour was generally low with mean values ranging from 19 (R10) to 51 ('Cripps' Pink') without peel and 30 (R10) to 51 ('Cripps' Pink') with peel (Fig. 7 and Fig. 8, respectively). The presence of peel seemed to increase the apple flavour of

some genotypes. Tasted with peel, 'Meran', W1, R15 and R19 had a comparable apple flavour to that of 'Cripps' Pink'.

Most of the selections were perceived to be low in sweet taste (below 40). Mean values for sweetness ranged from 19 (R10) to 52 (R13) when tasted without peel (Fig. 7) and from 27 (R10) to 51 (R5) with peel (Fig. 8). 'Cripps' Pink' and 'Meran' were in the group of genotypes with a relatively high sweet taste (> 40) when tested with and without peel. R20 was sweeter in taste compared to other genotypes when tasted without peel. The biggest variance between genotypes with regard to sensory characteristics was observed for sour taste, where mean values varied from 24 (W2) to 85 (R15) without peel (Fig. 7) and from 34 (R16) to 80 (R19) with peel (Fig. 8). While high sour taste (> 70) were found in six of the selections, i.e., R7, R14, R15, R18 and R21, another 6 genotypes had a comparatively low sour taste (<40). 'Cripps' Pink' and 'Meran' had a moderate sour taste (45-55). R21, R10 and R9 were perceived to have a slight bitter taste (10-18) (Fig. 8). R21 (11) and R10 (11) were perceived to have a slight degree of astringency when tested with the peel (Fig. 8). No astringency was detected in peeled samples (data not shown).

**Physiochemical measurements**

TSS, TA, firmness and percentage red-flesh coverage was recorded and the TSS/TA ratio calculated. Red flesh coverage in seedling selections varied from 0.3 to 45% (Table 2). Interestingly, 'KAZ91' had only 5.7% red-flesh coverage.

TSS levels varied considerably ranging between 12 (R10 and R17) and 19 ºBrix (R18) (Table 2). Eleven seedling selections as well as 'Meran' had TSS levels of 15 ºBrix. TSS above 15 ºBrix were found in six seedling selections while eight seedling selections and 'KAZ91' had TSS below 15 ºBrix. High TSS was found for 'GoldRush' (17.4 ºBrix) followed by 'Fuji' (16.0 ºBrix) and 'Granny Smith' (15.3 ºBrix), with low TSS found in 'Golden Delicious' (13.5 ºBrix) (Abbott *et al.,* 2004).

Corrigan *et al.* (1997) found high TSS in 'Red Doughherty (15.7 ºBrix) followed by 'Cripps' Pink' (13.9 ºBrix), 'Fuji' (12.7 ºBrix) and low TSS in 'Braeburn' (11.2 ºBrix) and 'Granny Smith' (11.0 ºBrix). In agreement with sensory results, measured acidity varied considerably between genotypes ranging from 0.10% to 1.26% expressed as malic acid (Table 2). Skendrovic Babojelic *et al.* (2007) reported TA levels of 0.69% and 0.54% for 'Granny Smith' and 'Cripps' Pink', respectively. These levels are comparable to that of KAZ91 (0.56%), but thirteen of the seedling selections had higher acidity in comparison to 'Granny Smith' (Table 2). Corrigan *et al.* (1997) reported much higher TA levels for 'Granny Smith' (0.95%) and 'Cripps' Pink' (0.90%). Only 3 genotypes had higher TA than this. Eight of the genotypes had TA below 0.30%.

Theoretically, sensory analysis and physiochemical measurements should correlate (Hampson *et al.,* 2000). The instrumental assessment of acidity correlated with the sensory assessment of sour taste (0.83; $P < 0.001$). This is in agreement with Harker *et al.* (2002a) who found TA to be a good indicator of sour taste, but a negative correlation was observed between TA and sweet taste (-0.58; $P = 0.0022$). However, Echeverria *et al.* (2005) and Plotto *et al.* (1997) found no correlation between sweet taste and TA. No correlation was found between TSS and sweetness (0.200; $P = 0.3364$), this is in agreement with Watada *et al.* (1985). However, the TSS/TA ratio correlated positively with sweet taste (0.54, $P = 0.0051$). This indicates that the ratio between TSS and TA has an overriding effect on the perception of sweetness. A higher TSS:TA ratio is required to get the right balance between sweetness and sour taste (Kuhn and Thybo, 2001) and this determines the acceptability of apples (Janick *et al.,* 1996). A high acid and low sugar content result in acidic fruit (Abbot *et al.,* 2004; Janick *et al.,* 1996). Abbott *et al.,* (2004) found that a high TSS:TA ratio between 45.5 and 69.3 resulted in sweet tasting apples whereas a low TSS:TA ratio between 19.1 and 24.5 resulted in sour apples. In pears, a high TSS:TA ratio between 40 and 60 is required for a good balance between sweetness and sourness (Chen *et al.,* 2007). 'Gala' was found to have a TSS:TA ratio of 41 and was perceived to have a sweet taste in comparison to 'Mutsu' (19) which had low sweet taste. 'Mutsu' had high acid content (0.57%) and low TSS (10.7 ºBrix) in comparison to 'Gala' with low acid content (0.32%) and high TSS (13.2 ºBrix).

TSS:TA ratios varied considerably between genotypes ranging from 10 (R15) to 150 (R1) (Table 2). W1 and R5, which were preferred by consumers, had TSS:TA ratios between 60 and 70. R15 and R21 had TSS:TA ratios of 10 to 13, were perceived to be very sour and were disliked by consumers (Tables 2, Fig. 9). However, some seedling selections that were also preferred by consumers, i.e., R2, R3, R6, R18, R19, R20 and R22 (Table 2), had only slightly higher ratios (13 to 26). W2 and R1 had TSS:TA ratios >100 due to very low TA levels. These genotypes were perceived as being low in acidity, but they were not rated any sweeter than 'Meran' and received low scores for liking (Fig. 9). Apples with high sugar and low acidity may taste too sweet and flat (Janick *et al.,* 1996). It seems that the balance between TSS and TA may be more important for good taste than the absolute levels of these parameters. For example, despite its high TSS (19 ºBrix), R18 was perceived to have a low sweet taste and very high acidity.

Flesh firmness ranged between 6 and 11 kg. Seven of the seedling selections had firmness >10 kg (Table 2) suggesting that these fruit may not have been at optimum harvest maturity. However, except for R6 (3%) and R21 (29%), these seedlings had high starch levels (>70%) (Table 2), indicating that they have high innate firmness. No correlation was found between firmness and crunchiness (0.25, P = 0.2183) and also between firmness and crispness (0.24, P = 0.2427). Thybo *et al.* (2003) found that instrumental measurements of firmness with a penetrometer did not correlate with sensory texture attributes. Although Harker *et al.* (2002b) did find a correlation between instrumental firmness measurements and sensory texture attributes, they indicated that differences in texture between cultivars may be difficult to adequately predict with instrumental measurement.

**Preference for taste**

Consumers tasted 22 red-fleshed and two white-fleshed genotypes randomly selected from the progeny of the cross between *M. niedzwetzkyana* and *M. domestica* ('Meran'). Two commercial cultivars with white flesh, i.e., 'Cripps' Pink' and

'Meran', were included in the Assessment as references or control samples. The genotypes were tested with and without peel. There were no significant differences (P>0.05) when the different genotypes were tested with peel and without peel (results not shown). Skin therefore did not seem to play a major role in influencing consumer preference even though it affected the perception of sensory traits (Fig. 5, 6, 7 & 8). Daillant Spinnler *et al.* (1996) found that peeling increased the liking for 'Top Red', decreased the liking for 'Splendour' and 'Fiesta', but had no affect on the liking of 'Granny Smith', 'Fuji', 'Aurora', 'Braeburn' and 'GS33O'.

Genotypes differed considerably with regard to preference of taste. The most liked genotypes, including 'Cripps' Pink' (7.1) and 'Meran' (6.8), were flavoursome, juicy, crunchy and crispy. This seems to be in agreement with previous studies where sweetness and apple flavour (Kuhn and Thybo, 2001), apple firmness (Harker *et al.,* 2008), and crispness and juiciness (P'eneau *et al.,* 2006) were found to be the sensory attributes that affect consumer preference positively. Selections that were mealy, bitter, less crunchy, less crispy, less flavoursome with low sweet and high sour taste were generally liked the least (Fig. 10). This was also in agreement with previous studies (Bignami *et al.,* 2003; Drewnowski and Gomez-Carneros, 2000; Janick *et al.,* 1996; Jaeger *et al.,* 1998; Kuhn and Thybo, 2001).

Most seedling selections were preferred less than 'Cripps' Pink' and 'Meran' with the notable exception of W1 (7.8), which was preferred more, and R2, R3, R5, R6, R9, R18, R19, R20 and R22, and which received a comparable liking score ranging from 5.8 to 6.5. W1 was juicy, slightly less acidic, sweet in taste and displayed a moderate apple flavour and crisp texture when compared to 'Cripps' Pink'. R2, R3, R5, R6, R9, R18, R19, R20 and R22 were perceived by the sensory panel as being slightly less flavoursome, crispy, crunchy and juicy than 'Cripps' Pink'. Despite their high acidity and low sweetness, R18, R19, R20, and R22 were liked by the consumers. These four genotypes had relatively high apple flavour. Their positive liking may also be due to their high red flesh coverage. R21 also had high acidity and high red flesh coverage, but this was offset by low flavour and the highest score for bitterness and astringency. The occurrence of off-flavours and off-tastes could also have contributed to a low

consumer preference for some of the genotypes, but taints were unfortunately not assessed. The preference for the two cultivars showed less variance around the mean degree of liking compared to seedling selections, indicating that consumers were more consistent regarding their preference for the cultivars. Hampson *et al.* (2000) indicated that liking of varieties by consumers may be influenced by their familiarity with the varieties.

**Drivers of degree of liking and correlations**

Daillant-Spinnler *et al.* (1996) and Pre-Aymard *et al.* (2005) found that interaction between texture and flavour of apples plays an important role in consumer preference for apples. Associations between sensory analysis, physical measurements and consumer preference for taste were assessed by means of preference mapping by combining trained panel data and physical measurements data (x-space) with preference data for taste (y-space) (Fig. 10). Since the number of male consumers was low in comparison to the female consumers, the preference results of the different genders might be skewed towards the female preference. Hence, the association between the total group of consumers, biased towards female preference, and the quality attributes will thus be discussed. Strong positive correlations were found for consumer preference of taste with apple flavour (0.77, $P < 0.001$), juiciness (0.77; $P < 0.001$), crispness (0.76, $P < 0.001$) and crunchiness (0.63, $P = 0.0006$). Therefore, apples that were juicy, flavoursome, crispy and crunchy, e.g. W1, R2, R6, R22, 'Cripps' Pink' and 'Meran', were preferred the most by consumers (Fig. 10). A week correlation between sweet taste and preference of taste was found (0.40, $P = 0.0269$). Previously it was found that preference for taste of Danish children was correlated with sweetness (Kuhn and Thybo, 2001).

Red-flesh coverage, astringency, sour taste and TA lie close together on the preference map (Fig. 10), suggesting that these traits may be associated. A positive correlation was observed between red-flesh coverage and TA (0.51, $P = 0.0081$) and sour taste (0.50, $P= 0.0112$). No correlation was found for preference of taste with sourness (-0.02, $P = 0.9138$) and TA (0.008, $P= 0.9683$). High TA's were observed

for R19, R20, and R21 (Fig 10). These three genotypes were also perceived as being highly acidic by the trained panel. R19 was preferred by consumers even though it was perceived to be very sour, had a very low TSS/TA ratio of 13.0 and was less sweet, flavoursome, crispy, crunchy and juicy than 'Cripps' Pink'. Daillant Spinnler *et al.* (1996) found three consumer segments for apple liking, i.e., consumers who liked sweeter and crispy apples (e.g. 'Fuji' and 'Aurora'), those who liked sweet acidic apples (e.g. 'Granny Smith' and 'Braeburn), and a third group who liked apples that are even more acidic than those currently available on the market. No correlation was found between preference of taste and percentage red flesh coverage, anthocyanin concentration in the flesh, astringency and firmness.

As could be expected, a negative correlation was found between mealiness and crispness (-0.70, $P < 0.001$), crunchiness (-0.67, $P = 0.0002$) and juiciness (-0.68, $P = 0.001$). Mealiness has a negative effect on consumer preference for taste (Jaeger *et al.,* 1998) and mealy genotypes, e.g. W2, R9 R11, R12 and R16, were generally disliked (Fig. 10). Mealiness is associated with loss of juice and occurs in apples that are overmature (Vincent, 1989). Towards the end of the sensory analyses, the panel members noted that some of the samples had a spongy texture. This attribute was not experienced during the training phase of the latter panel. One assumes that this phenomenon was also present in the samples analysed by the consumer panel, this could also have had an effect on the final degree of liking results (Jaeger *et al.,* 1998).

## CONCLUSION

Our study indicates that local consumers seem to prefer white flesh colour in apple over most patterns and intensities of red flesh colour. The preference for white flesh is probably due to unfamiliarity with the red flesh colour. Consumers did find "floral" patterns in red pigmentation attractive but, generally indicated less preference for light pink flesh colour, unsymmetrical, blotchy or hazy red pigmentation, as well as for red pigmentation restricted to the flesh immediately underneath the skin. With regard to taste, most of the 24 progeny that were assessed were preferred less by consumers than 'Meran' and 'Cripps' Pink'. However, not all the genotypes were disliked and

one or two were liked as much as the commercial cultivars. These genotypes invariably had a good texture and negative traits such as mealiness were absent. Hence, it does seem possible to select for red-fleshed progeny with good taste.

Since the percentage red flesh coverage correlated positively with acidity and astringency, it may prove difficult to combine an attractive pigmentation pattern with good taste in the same apple. However, some of the very sour apples with high red flesh coverage and good texture and flavour received a positive score for liking, comparable to that of 'Cripps' Pink' and 'Meran'. It seems that at least some consumers have a preference for this combination of traits.

Future research on consumer preference for red-flesh apples should focus more on the segregation of consumer acidic, sweet and sweet-sour apples, and also use a cultivar with high acid content such as 'Granny Smith' as a control cultivar, since most of the seedlings were highly acidic.

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Table 1. Definitions of sensory attributes used in the profiling of seedling selections of 'KAZ91' X 'Meran' as well as 'Cripps' Pink' and 'Meran' as references. Adapted from Haker *et al.* (2002), Mehinagic *et al.* (2004) and Kuhn and Thybo (2001).

| Sensory attributes | Definitions | Scale |
|---|---|---|
| Sour taste | The intensity of sour taste | 0 = None; 100 = Prominent sour taste |
| Sweet taste | The intensity of sweet taste (sucrose) | 0 = None; 100 = Prominent sweet taste |
| Flavour | Intensity of perfumed or aromatic apple flavour | 0 = None; 100 = Prominent general apple flavour |
| Astringency | The intensity of a dry sensation in the mouth | 0 = None; 100 = Prominent astringency |
| Bitterness | Bitter intensity, usually detected at the back of the tongue after swallowing | 0 = None; 100 = Prominent bitterness |
| Skin toughness | The amount of skin particles in the mouth before swallowing | 0 = None; 100 = Extreme thickness |
| Crispness | Intensity of sound generated with the first bite with the front teeth | 0 = None; 100 = Prominent crispness |
| Crunchiness | Intensity of noise generated when chewing with the molars | 0 = Fast Disintegration; 100 = Slow Disintegration |
| Juiciness | Amount of juice released when chewing | 0 = None; 100 = Extremely juicy |
| Mealiness | Intensity of the flesh to break down into very fine dry particles | 0 = None; 100 = Prominent mealiness |

Table 2. Percentage of flesh covered by red pigmentation and means of physiochemical attributes of 'KAZ91', 'Meran' (MR) and seedling selections of 'KAZ91' X MR. W1 and W2 are white-fleshed selections while the percentage of flesh covered by red pigmentation increases from R1 to R22

| **Genotypes** | **Percentage red-flesh (%)** | **TSS (°Brix)** | **TA (%)** | **TSS/TA (%)** | **Firmness (Kg)** | **Starch (%)** |
|---|---|---|---|---|---|---|
| KAZ91 | 5.69 | 13.0 | 0.56 | 23.76 | 8.02 | 54 |
| MR | 0.00 | 15.0 | 0.64 | 23.39 | 10.25 | 16 |
| W1 | 0.00 | 15.0 | 0.24 | 59.79 | 10.50 | 45 |
| W2 | 0.00 | 15.0 | 0.14 | 107.14 | 8.33 | 59 |
| R1 | 0.33 | 15.0 | 0.10 | 150.00 | 11.00 | 100 |
| R2 | 0.33 | 15.0 | 0.70 | 21.43 | 10.00 | 26 |
| R3 | 1.33 | 16.0 | 0.82 | 19.51 | 9.67 | 28 |
| R4 | 9.33 | 17.0 | 0.84 | 20.24 | 10.00 | 5 |
| R5 | 10.00 | 16.0 | 0.23 | 69.57 | 9.33 | 58 |
| R6 | 11.67 | 15.0 | 0.74 | 20.27 | 11.33 | 3 |
| R7 | 12.33 | 14.0 | 0.28 | 50.00 | 11.00 | 88 |
| R8 | 16.00 | 13.0 | 0.23 | 56.52 | 9.00 | 34 |
| R9 | 16.00 | 15.0 | 0.58 | 25.86 | 9.33 | 100 |
| R10 | 19.00 | 12.0 | 0.76 | 15.79 | 11.00 | 65 |
| R11 | 20.33 | 17.0 | 0.81 | 20.99 | 11.00 | 78 |
| R12 | 20.33 | 15.0 | 0.71 | 21.13 | 10.00 | 54 |
| R13 | 23.00 | 15.0 | 0.28 | 53.57 | 9.33 | 92 |
| R14 | 25.33 | 14.0 | 0.88 | 15.91 | 6.33 | 3 |
| R15 | 28.67 | 13.0 | 1.26 | 10.32 | 9.00 | 44 |
| R16 | 31.67 | 15.0 | 0.28 | 53.57 | 9.33 | 87 |
| R17 | 35.00 | 12.0 | 0.65 | 18.46 | 8.33 | 8 |
| R18 | 35.00 | 19.0 | 0.82 | 23.17 | 8.67 | 52 |
| R19 | 38.67 | 15.0 | 1.12 | 13.39 | 10.00 | 100 |
| R20 | 39.67 | 13.0 | 0.68 | 19.12 | 8.33 | 49 |
| R21 | 40.67 | 14.0 | 1.09 | 12.84 | 11.33 | 29 |
| R22 | 44.67 | 16.0 | 0.89 | 17.98 | 8.33 | 83 |

Fig.1 Representative whole (A) and halved (B) fruits of *M. niedzwetzkyana* Diek. selection KAZ91 that was crossed with 'Meran' (*M. domestica*) (Picture courtesy of I. Labuschagné, ARC Infruitec-Nietvoorbij. 2008).

Fig.2. Colour range distribution of 'KAZ91' x 'Meran' progeny represented by single fruit collected from individual seedlings (A) and representative halved red-fleshed seedlings (B).

Fig.3. Degree of liking for flesh colour of five red-fleshed 'KAZ91' x 'Meran' genotypes with different intensities and patterns of red pigmentation and one white-fleshed genotype (N = 94).

Fig.4. Distribution of mean hedonic scores for flesh colour of five red-fleshed 'KAZ91' x 'Meran' genotypes with different intensities and patterns of red pigmentation and one white-fleshed genotype (N = 94).

Fig.5. Mean values for sensory attributes crispness, crunchiness, juiciness and mealiness for twenty four seedling selections of 'KAZ91' x 'Meran' as well as for 'Cripps' Pink' and 'Meran' as reference/control samples tasted without peel (peeled).

Fig.6. Mean values for sensory attributes skin toughness, crispness, crunchiness, juiciness and mealiness for twenty four seedling selections of 'KAZ91 'x 'Meran' as well as for 'Cripps' Pink' and 'Meran' as reference/control samples tasted with peel (unpeeled).

Fig.7. Mean values for sensory attributes sweetness, sourness and apple flavour for twenty four seedling selections of 'KAZ91' x 'Meran' as well as for 'Cripps' Pink' and 'Meran' as reference/control sample tasted without peel (peeled).

Fig.8. Mean values for sensory attributes sweetness, sourness, flavour and bitterness for twenty four seedling selections of 'KAZ91' x 'Meran' as well as for 'Cripps' Pink' and 'Meran' as reference/control sample tasted with peel (unpeeled).

Fig. 9 Overall liking of taste of twenty four seedling selections of ‘KAZ91’ x ‘Meran’ as well as for ‘Cripps’ Pink’ and ‘Meran’ as reference/control sample (N=94)

Fig.10. External preference map indicating the position of the judges (consumers) in relation to the twenty four seedling selections of ‘KAZ91’ x ‘Meran’ (capital letters/bold) female consumers (CPF), Male consumers (CPM), total group of consumers (CPtot) and the nine sensory attributes (italics). The map was obtained using a partial least square regression, where the consumer data (y space) was regressed onto the trained panel data and physical data (x space). t1 indicates the first component and t2 the second component. Sensory data is in italics.

A

B

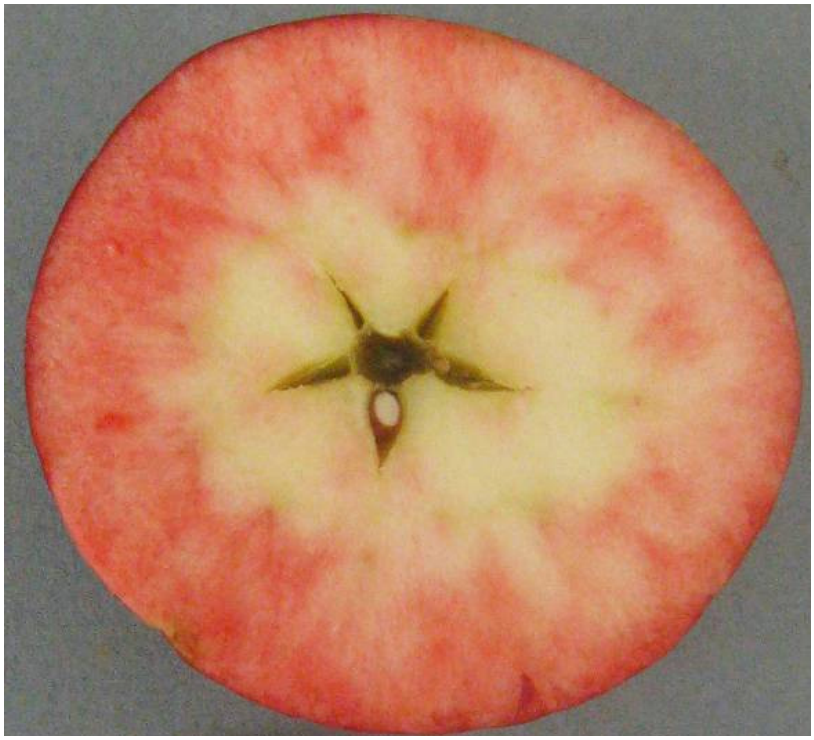
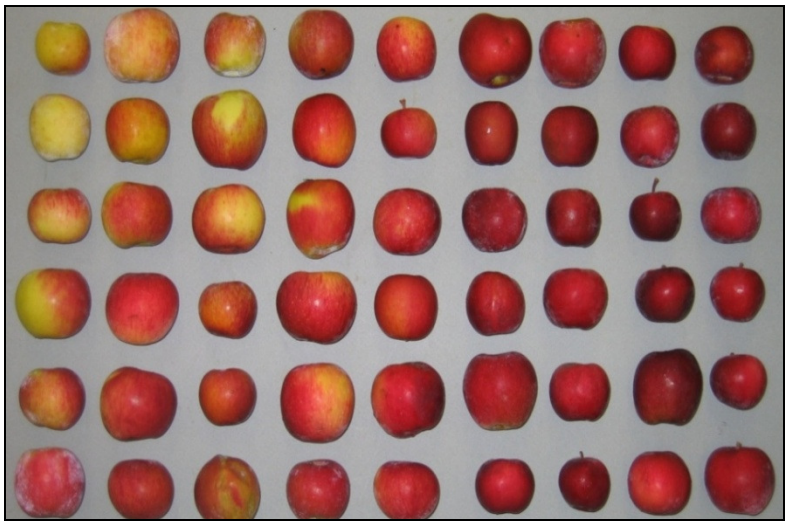


Fig.1. Paper 2

A



B

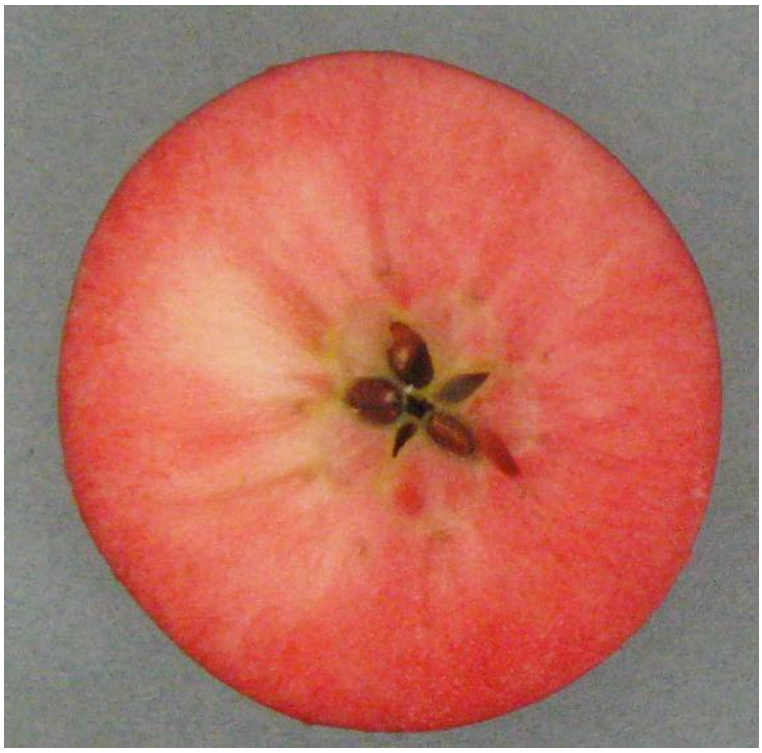


Fig.2. Paper 2

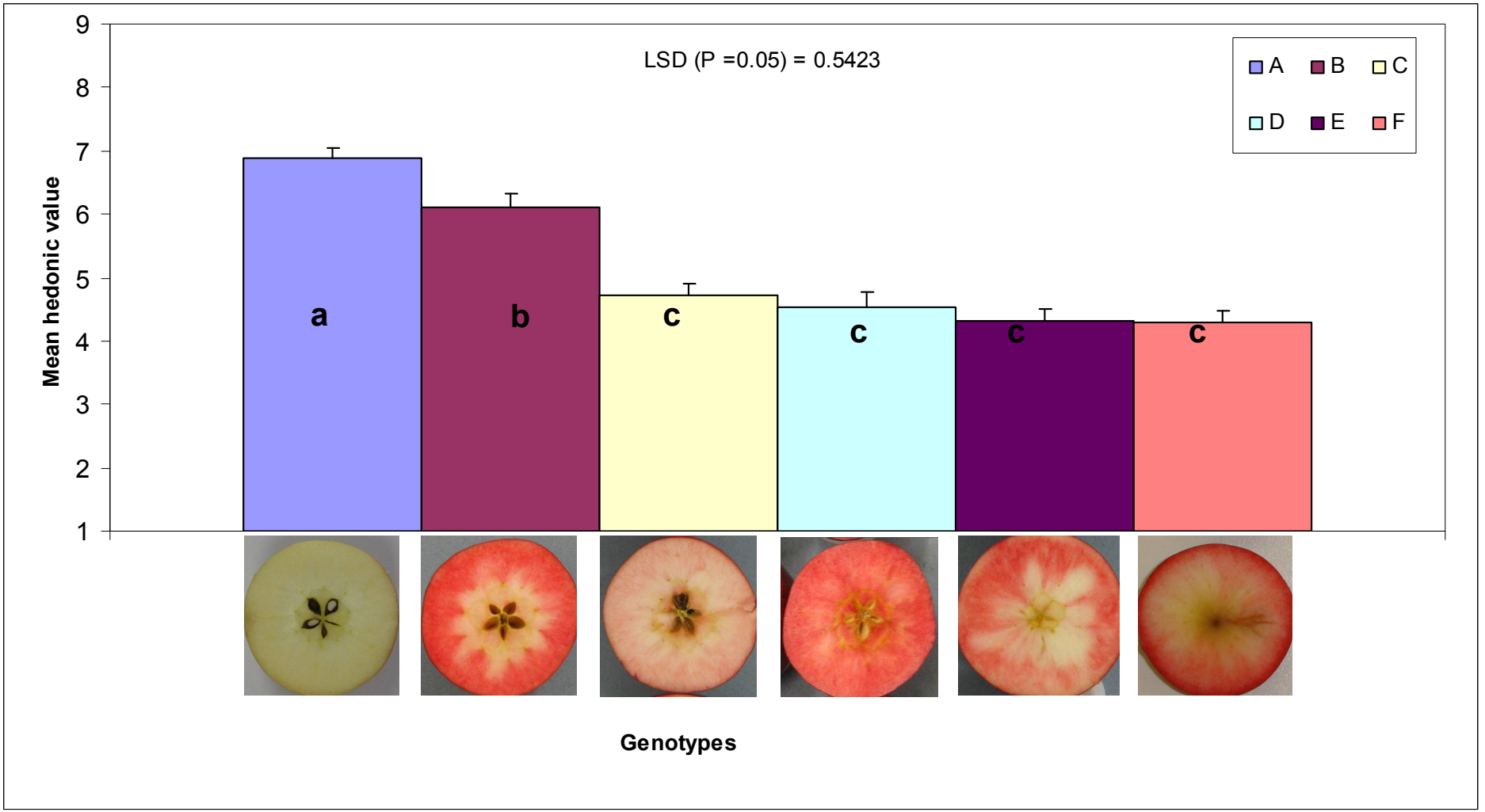

LSD (P =0.05) = 0.5423
A
B
C
D
E
F
Mean hedonic value
9
8
7
6
5
4
3
2
1
a
b
c
c
c
c
Genotypes

Fig.3. Paper 2

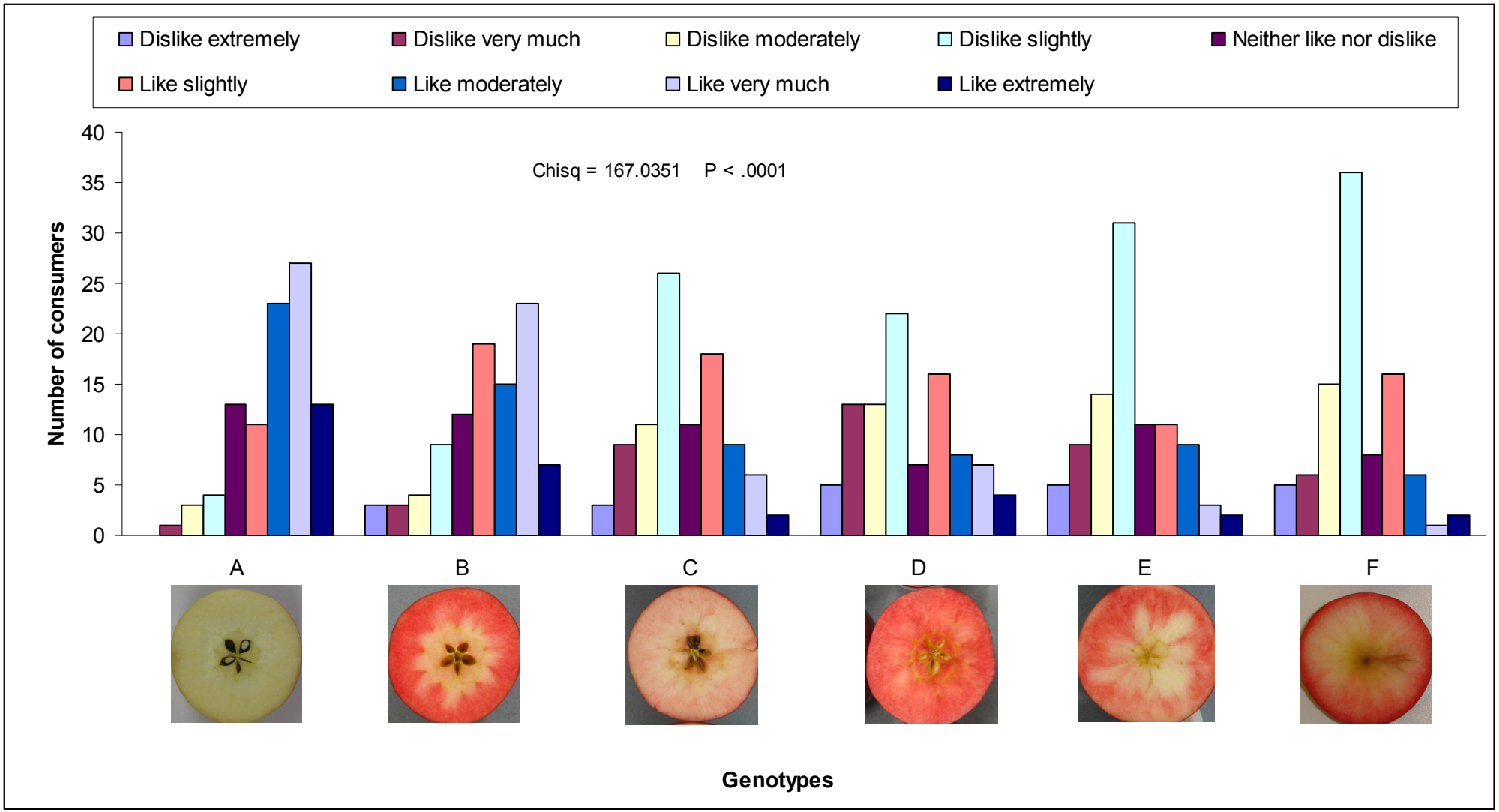

Dislike extremely
Dislike very much
Dislike moderately
Dislike slightly
Neither like nor dislike
Like slightly
Like moderately
Like very much
Like extremely
Chisq = 167.0351 P < .0001
Number of consumers
0
5
10
15
20
25
30
35
40
A
B
C
D
E
F
Genotypes

Fig.4. Paper 2

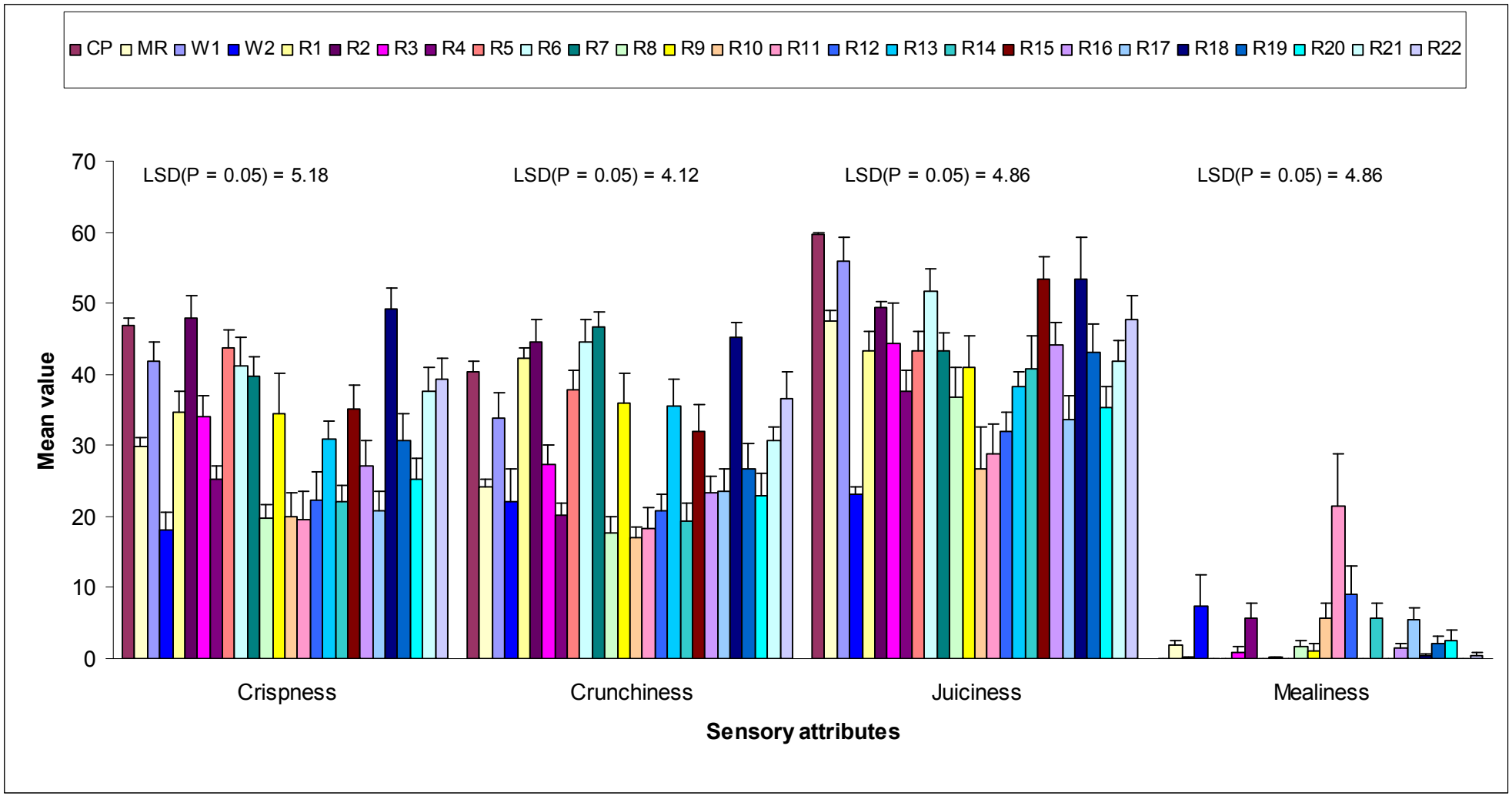

CP MR W1 W2 R1 R2 R3 R4 R5 R6 R7 R8 R9 R10 R11 R12 R13 R14 R15 R16 R17 R18 R19 R20 R21 R22
LSD(P = 0.05) = 5.18
LSD(P = 0.05) = 4.12
LSD(P = 0.05) = 4.86
LSD(P = 0.05) = 4.86
Mean value
70
60
50
40
30
20
10
0
Crispness
Crunchiness
Juiciness
Mealiness
Sensory attributes

Fig.5. Paper 2

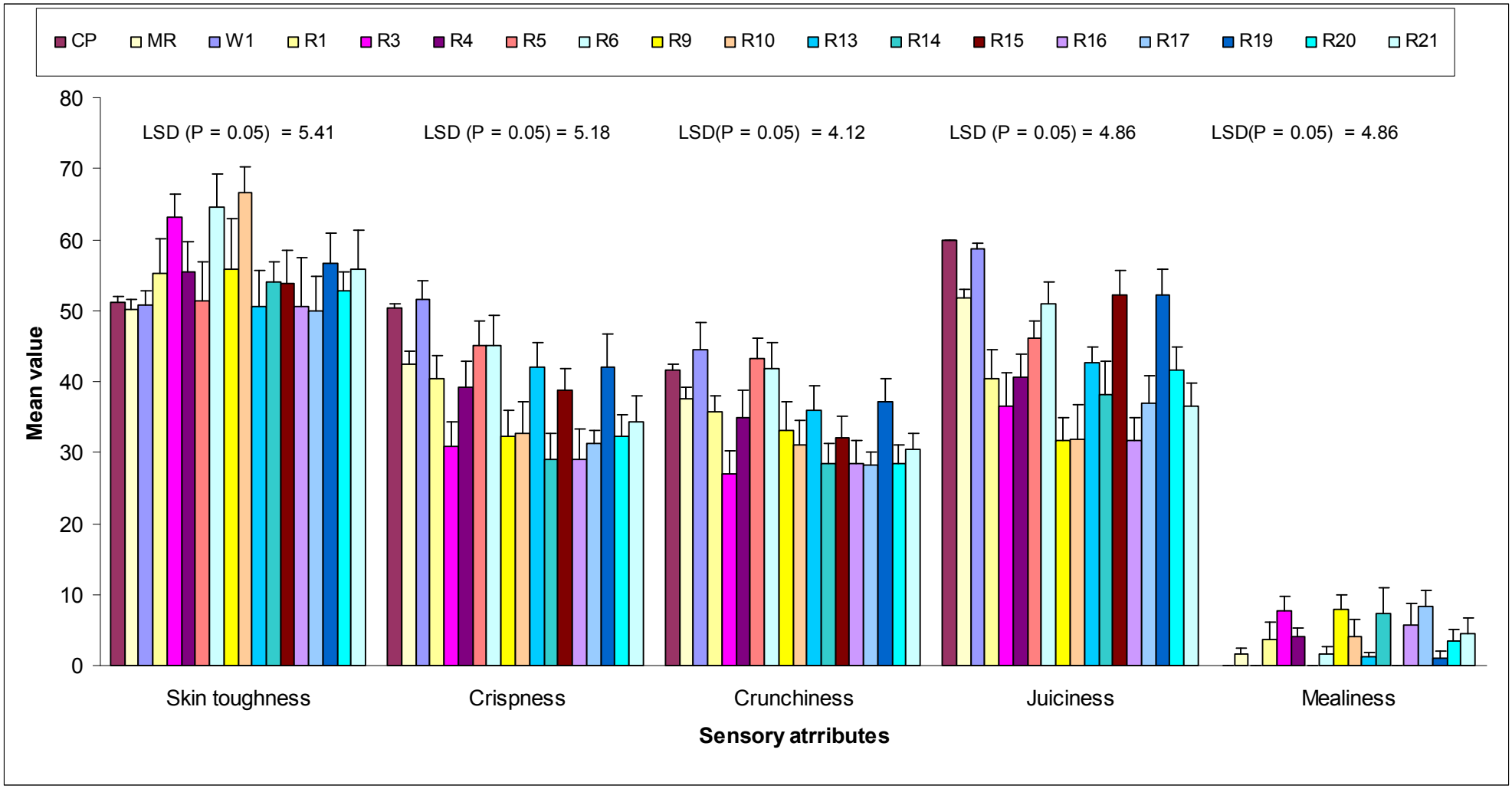

CP
MR
W1
R1
R3
R4
R5
R6
R9
R10
R13
R14
R15
R16
R17
R19
R20
R21
LSD (P = 0.05) = 5.41
LSD (P = 0.05) = 5.18
LSD(P = 0.05) = 4.12
LSD (P = 0.05) = 4.86
LSD(P = 0.05) = 4.86
Mean value
80
70
60
50
40
30
20
10
0
Skin toughness
Crispness
Crunchiness
Juiciness
Mealiness
Sensory atrributes

Fig.6. Paper 2

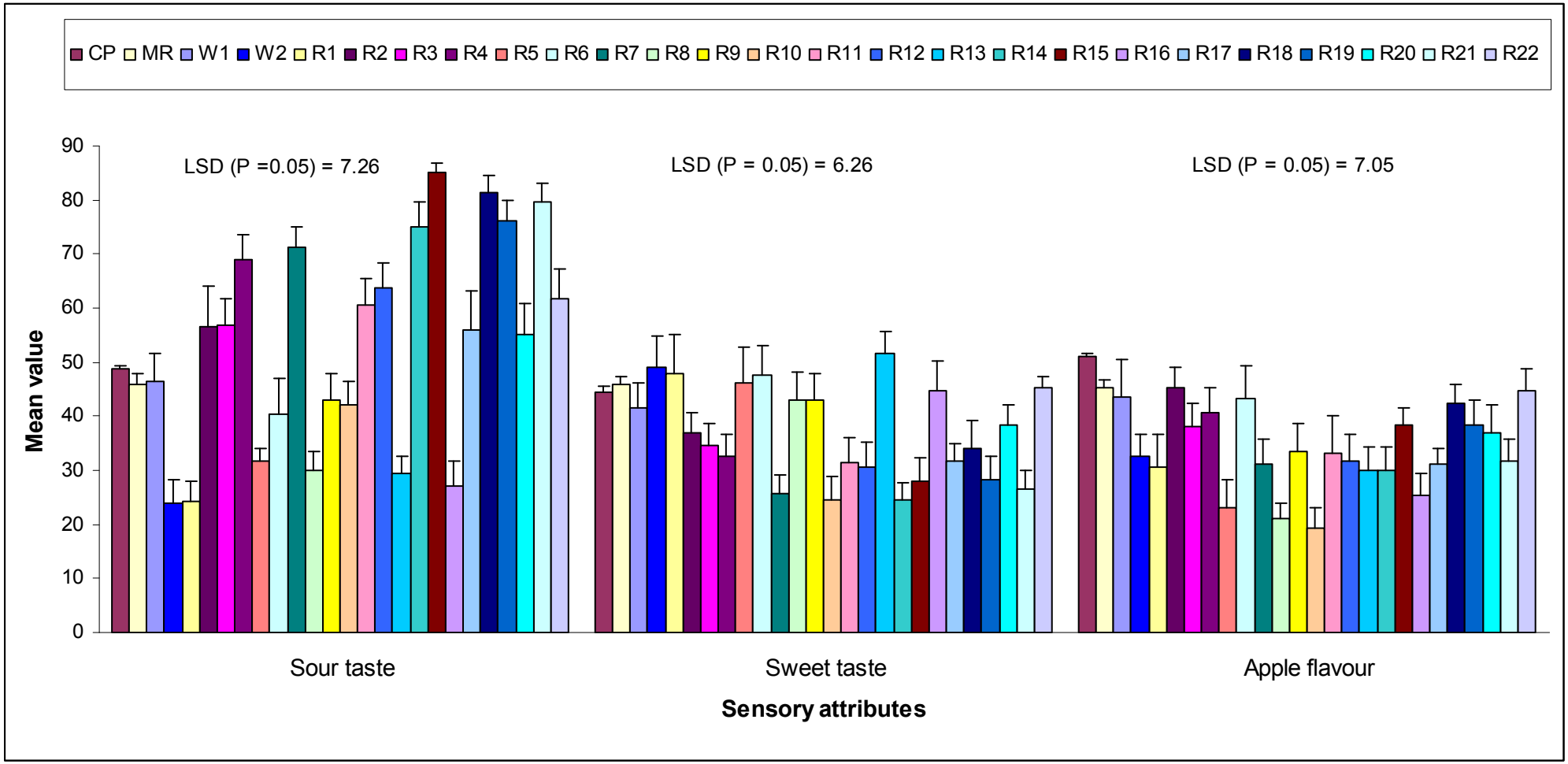

CP MR W1 W2 R1 R2 R3 R4 R5 R6 R7 R8 R9 R10 R11 R12 R13 R14 R15 R16 R17 R18 R19 R20 R21 R22
LSD (P =0.05) = 7.26
LSD (P = 0.05) = 6.26
LSD (P = 0.05) = 7.05
Mean value
90
80
70
60
50
40
30
20
10
0
Sour taste
Sweet taste
Apple flavour
Sensory attributes

Fig.7. Paper 2

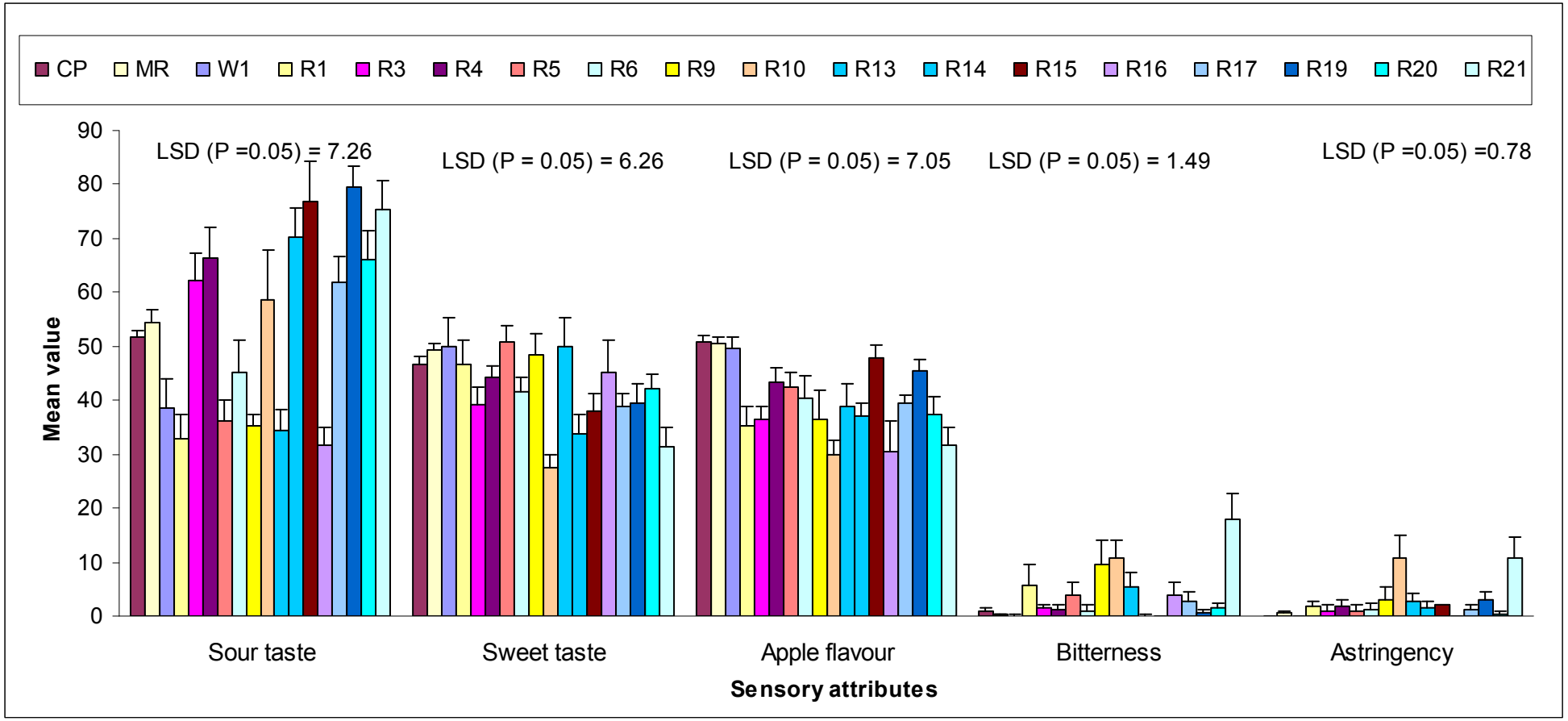

CP
MR
W1
R1
R3
R4
R5
R6
R9
R10
R13
R14
R15
R16
R17
R19
R20
R21
LSD (P =0.05) = 7.26
LSD (P = 0.05) = 6.26
LSD (P = 0.05) = 7.05
LSD (P = 0.05) = 1.49
LSD (P =0.05) =0.78
Mean value
0
10
20
30
40
50
60
70
80
90
Sour taste
Sweet taste
Apple flavour
Bitterness
Astringency
Sensory attributes

Fig.8. Paper 2

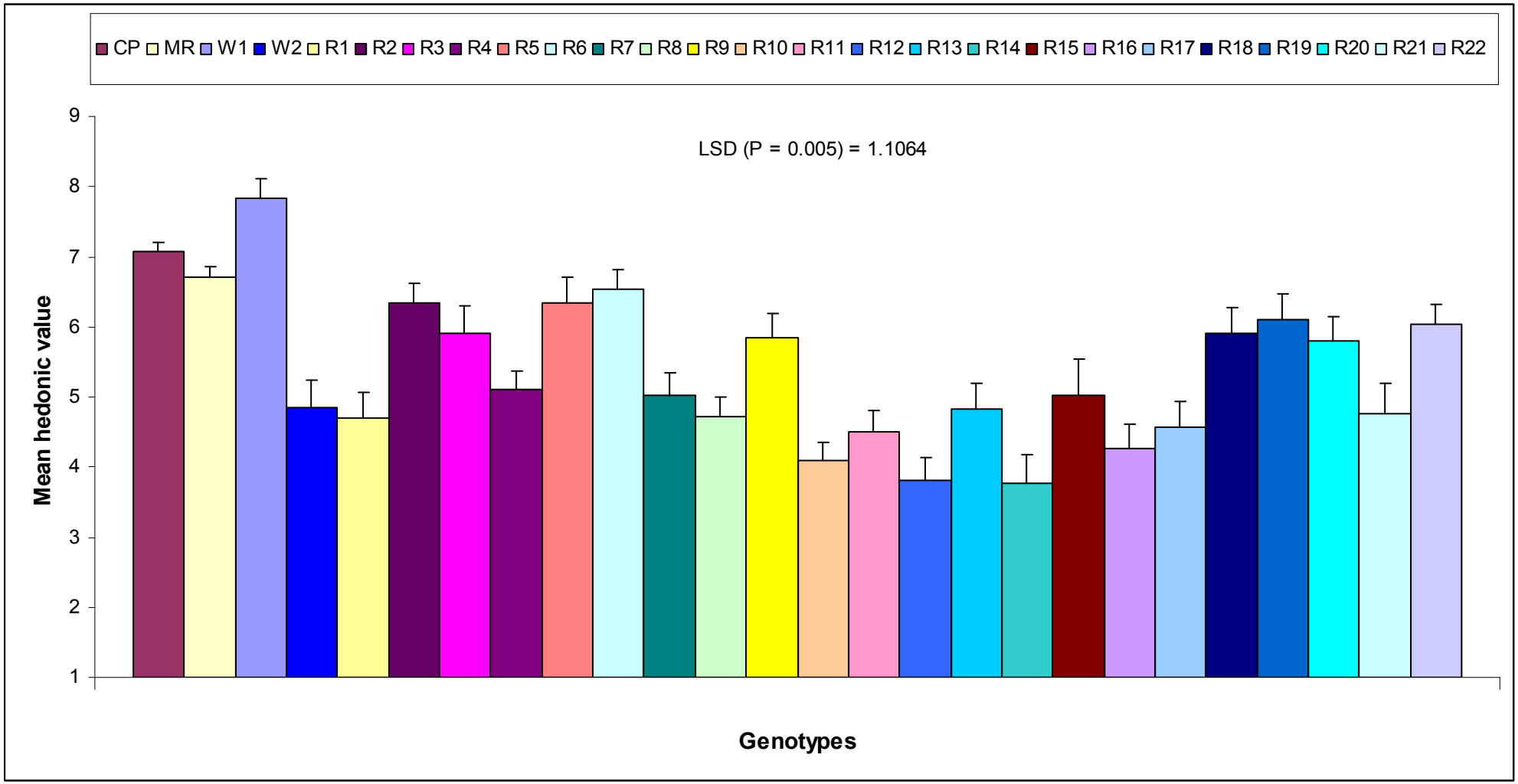

CP MR W1 W2 R1 R2 R3 R4 R5 R6 R7 R8 R9 R10 R11 R12 R13 R14 R15 R16 R17 R18 R19 R20 R21 R22
LSD (P = 0.005) = 1.1064
Mean hedonic value
9
8
7
6
5
4
3
2
1
Genotypes

Fig. 9 Paper 2

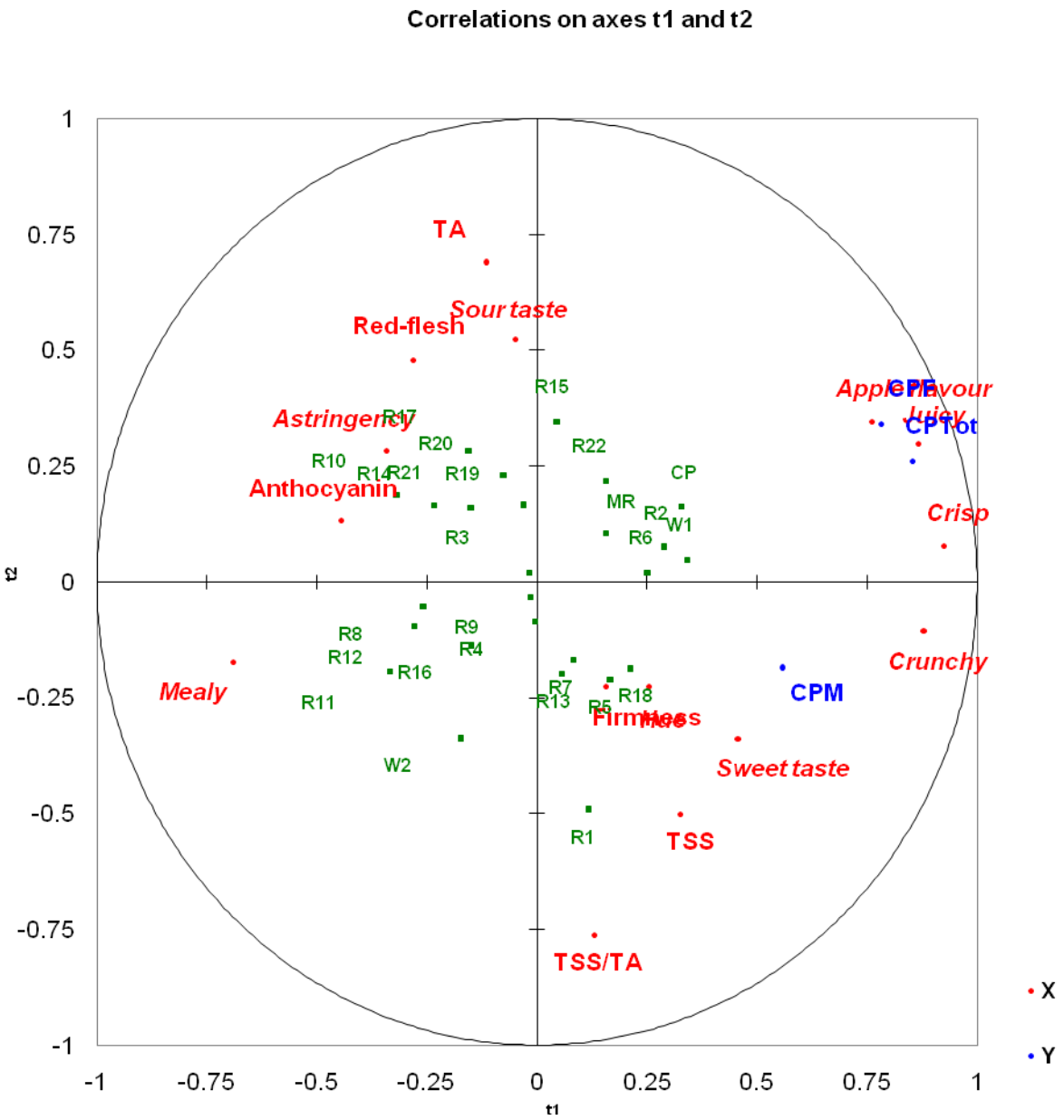



Fig.10. Paper 2

# GENERAL DISCUSSION AND CONCLUSIONS

Knowledge of segregation patterns for appearance and taste of red-fleshed apples, variation in the intensity and extent of red colour in the peel and flesh, the extent of browning in red-fleshed progenies and genetic variation between and within families is needed to identify superior parents and to increase breeding efficiency for red-fleshed apples.

Fruit of Family 1 ('KAZ 91' x 'Meran') were on average dark red with greater blush coverage and higher anthocyanin concentrations compared to fruit of Families 2 ('Reinette Burchard' x 'Treco Red Gala') and 3 ('Meran' x 'Treco Red Gala'). The number of Family 1 seedlings with red-fleshed fruits increased from 25% of bearing trees in 2007 to 35% in 2008. This may be attributed to the lower precocity of red-fleshed seedlings (Volz *et al.,* 2006). No correlation was found between anthocyanin levels in the peel and flesh of red-fleshed seedlings. Since dark red skin colour may not be attractive to consumers, it is a positive finding that skin colour is not indicative of the intensity of flesh colour. Since anthocyanin levels in the peel and flesh did not correlate with phenolic levels, dark red and red-fleshed seedlings do not necessarily have a higher antioxidant capacity than less red and white-fleshed seedlings. Total phenolics is by far the greatest contributor to apple antioxidant capacity (Wodjyło *et al.,* 2008; Wolfe *et al.,* 2003). However, a detailed chemical analysis of red-fleshed apples is required to assess the antioxidant capacity of red- compared to white-fleshed apples. On average, the 'KAZ91' progeny did show higher levels of total phenolics compared to fruit of Families 2 and 3, irrespective of flesh colour.

As found for a red-fleshed family in New Zealand derived from a *Malus niedzwetzkyana* selection (Chagne *et al.,* 2007; Volz *et al.,* 2006), different distribution patterns and intensities of red pigmentation were observed in the flesh of red-fleshed seedlings. These patterns and colour intensities also varied between seasons and between fruit from of the same seedling trees (Volz *et al.,* 2006). Hence, it may prove difficult to comply with colour standards for production. A detailed genetic study is needed to determine how different distribution patterns are inherited in order to select for progenies with uniform colour that will comply with the

production standards. The repeatability estimate for red flesh coverage in red-fleshed seedlings was intermediate, indicating some variance in the extent of red-flesh coverage between seasons. Further research is required on the effect of the environment on anthocyanin synthesis in the flesh.

'KAZ91' progeny were on average more prone to flesh browning than fruit of Families 2 and 3. However, no correlation was found between browning potential and phenolic level. Assessment of browning via colour change was problematic due to the interference of red flesh colour. Hence, future quantification of the browning potential of red-fleshed genotypes will require alternative assessment methods. 'KAZ91' progeny were also found to be high in acidity. This is not an unexpected result since wild apples are known to be acidic (Dzhangaliev, 2003). Evidently, *M. niedzwetzkyana* may transfer some of its negative traits to its progeny.

Variance components of the various traits that were recorded were calculated for the two seasons. A high intraclass correlation coefficient was found for the extent of red-fleshed coverage (0.80) in the 'KAZ91' progeny indicating a high level of genetic determination. High genetic determination suggests that selection will be successful and genetic advancement is expected to be relatively rapid.

Finally we assessed consumer preference for the appearance of the flesh and taste of the 'KAZ91' progeny in comparison to white-fleshed 'Cripps' Pink' that was included in the assessment as a control cultivar. Preference for appearance was highest for white flesh colour and for apples with a red cortex and white core, giving rise to an attractive "floral" pattern. The overall preference for white flesh colour may be attributed to the unfamiliarity of consumers with red-fleshed apples. However, the preference for the "floral" pattern indicates possible commercial viability for red fleshed cultivars. Futher consumer preference studies should be performed with people from various cultural backgrounds and countries of origin.

The presence of peel did not have an influence on consumer preference for taste. The driving force for preference of taste was found to be crunchiness, crispness, juiciness and apple flavour. Apples that met these criteria in the absence of negative traits such as mealiness, astringency and high acidity were generally acceptable to consumers

irrespective of flesh colour. However, relatively few of the red-fleshed progeny met these criteria, generally due to poor texture and very high acidity. Preference mapping indicated that negative traits, i.e., astringency, TA and sour taste, associates with percentage red-flesh coverage. Interestingly, very acidic genotypes that were also high in red colour coverage were preferred by consumers, if they also had an acceptable texture and flavour. The positive association between red colour coverage and acidity should be followed up in a subsequent study.

At this point in time we have a better understanding of the genetic variability of external and internal colour and other quality-related traits within and between three breeding families. Quantification of variation of flesh colour between fruits of the same tree will provide much needed information for horticulturalists and marketers. We also established that consumers do like the taste of some red-fleshed apples regardless of the high acid content in some of the genotypes and the driving force for preference of taste are crispness, crunchiness, juiciness and apple flavour. We also established that consumers prefer the appearance of red-fleshed apples with an attractive floral "pattern", i.e., a white core and red-cortex.

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Wojdyło, A., J. Oszmianski, and P. Laskowski. 2008. Polyphenolic compounds and antioxidant activity of new and old apple varieties. J. Agric. Food Chem. 56: 6520-6530.

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