

FRUIT QUALITY ASSESSMENT IN PIGMENTED GRAPEFRUIT (*Citrus paradisi* Macf.) FOR VARIETAL DIVERSIFICATION

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Citrus industry of Pakistan is dominated by Kinnow mandarin while grapefruit cultivation, despite having enormous nutraceutical properties, is negligible and concentrated by pigmented cv. Shamber only. Hence, fruit of six elite pigmented grapefruit cultivars viz. Flame 'F', Pink Ruby 'PR', Red Blush 'RB', Rio Red 'RR', Star Ruby 'SR' and Shamber 'S' were evaluated for physico-chemical quantitative and morphological qualitative traits. Fruit of all genotypes had similar spheroid shape, with convex base and truncate apex. Fruit size and weight were higher in 'F' (455 g) and 'RR' (366 g) while ratio FL:FD was more in 'S', 'SR' and 'PR'. Peel was markedly thin in both 'SR' and 'PR' varieties. Developed seeds were minimum (2.2) in 'PR'. Among quantitative traits, total soluble solids (TSS) were higher in 'SR' (8.51 °Brix) followed by 'PR' (8.87 °Brix). Ratio TSS:TA (6-7.8) and ascorbic acid contents (61-73 mg/100g) were higher in 'RB' and 'PR'. Anthocyanins were remarkably higher 0.82 mg/100g in 'RB' followed by 'PR'. Total sugars were much higher (5.3%-5.6%) in 'FS', 'PR' and 'SR' as compared with 'S'. Pearson's correlation analysis revealed strong positive correlations among fruit diameter and fruit weight, fruit weight and acidity, peel thickness and sugars while acidity and anthocyanins were negatively correlated. These findings indicate good potential of 'PR', 'RB' and 'SR' varieties compared with 'S' which is no more a commercial variety in the leading grapefruit producing countries. Further studies are suggested for comparative analysis of pigmented and non-pigmented varieties for genotypic diversification and breeding programs.

Keywords: Bud sport, Mutants, Pink flesh, Pomelo, Quantitative traits.

INTRODUCTION

Grapefruit (*Citrus paradisi* Macf.) is an evergreen subtropical Citrus cultivar formerly mentioned in literature as 'The forbidden fruit' by Hughes (1750). Grapefruit was considered as a bud sport however, now it is known as a spontaneous hybrid of *Citrus grandis* L. and *C. sinensis* L. Osbeck (Scora *et al.*, 1982; Oueslati *et al.*, 2017). Production of high-quality grapefruit is restricted to tropical and sub-tropical areas having hot and humid climatic conditions hence it may not be cultivated as widely as other citrus species. Amongst commercial citrus cultivars, Grapefruit has the largest fruit size excluding Chakotra. Its fruit harvesting can be delayed, and it could be left on the trees for longer times however, fruit fly may damage the crop. In Punjab-Pakistan, grapefruit harvesting starts in September-October when fruit is still green in color and flesh color is not fully developed. Grapefruit varieties offer diversity in fruit morphology, flesh color and seediness. Fruit having pigments like red and pink, are rich in vitamin A content and lycopene, have higher phytonutrient and antioxidant properties. Cultivation and consumption of pigmented grapefruit varieties is rapidly rising due to its higher nutraceutical and anticancer properties.

In global citrus industry, share of grapefruit production is less than 5% and its cultivation is intensified in countries like China, South Africa, USA, Turkey and Mexico. Amongst the largest exporters of fresh fruit, South Africa exports 0.23 million tons followed by China, USA and Turkey. In Texas, Rio Red is the main cultivar being cultivated on about 24% acreage of grapefruit production in USA (USDA-NASS, 2014). In USA, pigmented grapefruit is liked more and 75% of the grapefruit sold in USA are cultivars having pink to red pigmented flesh (Kesinger, 2002). In Pakistan, citrus fresh fruit production is 2.27 million tons from an area of 0.19 million hectares. About 95% of the citrus production of the country is shared by Punjab mainly comprising of cultivar Kinnow mandarin. Grapefruit is cultivated on five thousand hectares and its production share is merely 0.3% of citrus production in Pakistan (www.faostat.org). It is mainly cultivated in subtropics of central Punjab as small orchards. Shamber (seedless) and Foster Pink (seedy) are main cultivars commercially grown in Punjab and other parts of the country while the latter has little commercial significance. Morpho-chemical and genetic diversity assessment is useful for cultivar characterization and screening (Jaskani *et al.*, 2006). In grapefruit, spontaneous bud sport variation has been

very extensively utilized and several new commercially known cultivars have been available, however, little is known about their bearing behavior, fruit quality and production under local agroclimatic conditions. Diversity assessment initiates with morphological characterization (Huh *et al.*, 2008). Same cultivars may produce differently under different agro-climatic conditions; hence screening plays an important role in selection of varieties for a certain environment. Most of the available commercial cultivars have originated through classical breeding methods like selections, natural bud sports, interploidy crossing and mutations (Anderson, 2000; Hao and Deng, 2002; Usman *et al.*, 2012, 2018). Citrus breeders are interested to develop seedless varieties having resistance against canker. Seedlessness has been the most desired trait due to higher consumer acceptance and demand for seedless varieties is rising (Ye *et al.*, 2009).

Since 1990, citrus researchers have been interested in selection of better varieties with more red flesh and peel color due to increasing market demand and higher prices of red varieties. Star Ruby is one of the leading global red flesh cultivars and is extensively cultivated in South Africa, Turkey, Spain and Australia. Shamber is grown widely in Argentina and Pakistan. Ruby Red has been source of more deep colored cultivars including Rio Red and Ray Ruby. Ruby Red has been main cultivar in Turkey, India and China (Shen, 2000; Singh *et al.*, 2002). Rio Red was selected in 1984 due to its better yield, deep red flesh color and other fruit traits compared with Ruby Red. Cultivar Flame was released in 1987 by USDA, Florida (Rouse *et al.*, 2001). Its flesh color variation is dependent upon climatic conditions of that area. Flame has replaced Ruby Red in Argentina and has large plantations in Cyprus, Australia and South Africa (Da Graca *et al.*, 2004). Among Texas cultivars, lycopene level was the highest in Star Ruby and Rio Red.

Despite availability of other leading grapefruit varieties, there prevails cultivar Shamber based monoculture and no information was available about bearing behavior, fruit production and quality of other elite varieties. Hence, current study was aimed to characterize available grapefruit varieties for morphological qualitative and physico-chemical quantitative fruit traits and select superior genotypes for general cultivation and molecular breeding applications.

MATERIALS AND METHODS

Plant material: Plant material including shoots and ten mature fruit samples of elite grapefruit varieties including Flame 'F', Pink Ruby 'PR', Red Blush 'RB', Rio Red 'RR', Star Ruby 'SR' and Shamber 'S' were harvested at physiological maturity from Citrus Research Institute (CRI), Sargodha situated at 32.07°N, 72.68°E. Physical fruit analysis was performed in Pomology lab of Institute of Horticultural Sciences and biochemical analysis was carried out in

Biochemistry Lab, University of Agriculture, Faisalabad, Pakistan for chemical properties.

Morphological qualitative traits: Fruit morphological qualitative parameters were determined following standard descriptors (IPGRI, 1999) include shape (base and apex), skin (epicarp) color, surface texture, albedo color, adherence of albedo to pulp, nature (conspicuousness) of oil glands, segment shape uniformity, fruit axis, cross-section shape of axis, pulp color, its uniformity and firmness.

Physico-chemical quantitative traits: Quantitative traits included both physical and biochemical parameters (IPGRI, 1999). Physical properties of the collected ten fruit samples of each variety were evaluated using standard procedures and means were calculated. Data were collected for parameters including fruit weight (FW)(g), fruit size (mm) including length (FL), diameter (FD) and ratio FL:FD, number of segments and number of developed seeds. Following biochemical parameters were also assessed using standard procedures.

a. Total Soluble Solids (Brix) and Titratable Acidity (%): Total soluble solid contents of juice samples were estimated immediately after juice extraction using benchtop refractometer (RX 5000, ATAGO, Japan). A juice drop was put on the refractometer prism and TSS was measured at room temperature in digital scale. Titratable acidity (TA) was measured by standard titration method against alkali (NaOH) following Hortwitz (1960). The ratio TSS:TA was calculated by dividing TSS values by TA values.

b. Ascorbic Acid (mg 100 g⁻¹) and Anthocyanins (mg 100 g⁻¹): The ascorbic acid contents were determined following Ruck (1961). Total anthocyanins were determined following standard pH-differential method (Giusti and Wrolstad, 2001). Absorbance was measured in a UV-1601 spectrophotometer (Shimadzu) at 510 nm and 700 nm and calculated following standard procedures.

c. Total sugars (%): Total soluble sugar and reducing sugar contents were measured following modified method of Sadasivam and Manickam (1992). Non-reducing sugars were determined using following equation.

Non reducing sugar = Total soluble sugar – Reducing sugar

d. Experimental layout and data analysis: The experiment was laid out under Completely Randomized Design (CRD) and data were analyzed using Statistic 8.1 software. Analysis of variance (ANOVA) was used to test significance of the dataset. Least Significant Difference (LSD) test ($p \leq 0.05$) was used to compare means (Steel *et al.*, 1997).

RESULTS

Morphological qualitative traits

a. Fruit shape, skin color and surface texture: Fruit shape is one of the most important traits for marketing, attraction of the consumer and aesthetics. All grapefruit varieties had

Table 1. Genotypic diversity for morphological qualitative traits in grapefruit varieties

Variety	Fruit shape			Skin color	Surface texture	Albedo color	Adherence		Nature of oil glands	Segment shape uniformity	Fruit axis			Pulp	
	Whole fruit	Base	Apex				Albedo to pulp	Segment walls			Whole fruit	Cross section	Color	Color uniformity	Firmness
Flame	Spheroid	Convex	Truncate	PY	Smooth	Orange	Medium	Weak	I	Yes	Semi solid	Regular	Creamy orange	Yes	Intermediate
Pink Ruby	Spheroid	Convex	Truncate	PY	Smooth	Orange	Medium	Strong	I	Yes	Hollow	Regular	Pink	No	Intermediate
Red Blush	Spheroid	Convex	Truncate	GY	Smooth	Yellow	Weak	Medium	I	Yes	Hollow	Irregular	Red	Yes	Intermediate
Rio Red	Spheroid	Convex	Truncate	GY	Smooth	Orange	Medium	Medium	I	Yes	Hollow	Irregular	Red	Yes	Intermediate
Shamber	Spheroid	Convex	Truncate	GY	Smooth	Light Yellow	Weak	Strong	I	Yes	Semi solid	Irregular	Pink	Yes	Soft
Star Ruby	Spheroid	Convex	Truncate	GY	Smooth	Yellow	Strong	Medium	I	Yes	Hollow	Regular	Pink	Yes	Intermediate

Abbreviation used: Pale yellow (PY), Green yellow (GY), Inconspicuous (I)

spheroid shape fruit with convex base and truncate apex. Fruit skin color of mature fruit ranged from pale yellow to green yellow. Surface texture was smooth in all the varieties (Fig. 1, Table 1).



Figure 1. Representation of whole fruit and cross section of pigmented grapefruit varieties including A) Flame, B) Shamber, C) Star Ruby, D) Pink Ruby, E) Red Blush and F) Rio Red.

b. Albedo color, albedo adherence, oil glands and segment shape: Fruit albedo color ranged from light yellow to orange color while its adherence to pulp was weak in 'RB' and 'S' and strong in 'SR'. Oil glands were inconspicuous in all varieties and segment shape was uniform (Table 1).

c. Fruit axis, pulp color and firmness: Fruit axis was hollow in most of the varieties excluding F and S which had semi solid axis. Cross section of fruit axis was regular in 'F', 'PR' and 'SR' while it was irregular in other varieties. Pulp color was pink in 'PR', 'S' and 'SR', red in 'RB' and 'RR', and creamy orange in 'F'. Pulp was soft in 'S' and intermediate in other varieties (Fig. 1, Table 1).

Physico-chemical quantitative traits

a. Fruit size (mm), fruit weight (g) and number of seeds: Higher genotypic diversity was noted in fruit size and fruit weight in the varieties. Fruit of varieties 'F' was averagely heavy (455 g) followed by 'RR' (366g) and other varieties. Consistent with FW, fruit size was significantly higher in 'F' and 'RR' varieties. Fruit diameter was markedly higher in 'F' (105 mm) while it was minimum in 'S' (84 mm). Fruit rind was thicker in 'F' (10.40 mm) and it was the thinnest in 'PR' and 'SR' (5.09 mm). Number of segments ranged from 12-13 and were higher in 'F' and 'PR' among all studied varieties. Similarly, number of seeds ranged from 2.2 to 4.4 with ascending order 'PR', 'F', 'RR', 'S', 'RB' and 'SR' (Table 2).

b. Total soluble solids (TSS), Titratable acidity (TA), and ratio TSS:TA: Juice TSS and TA values showed great variation across different varieties. Values of TSS ranged from 7.29 °Brix in 'RR' to 8.87 °Brix in 'SR'. The lowest acidity (1.09%) was found in 'RB' while it was the highest (1.81%) in 'F'. Ratio TSS:TA was highest in 'RB' (7.80) followed by that of 'PR' (6.13) (Table 2).

c. Anthocyanin ($\text{mg } 100 \text{ g}^{-1}$) and Ascorbic acid content ($\text{mg } 100 \text{ g}^{-1}$): Wide genetic variation was found in anthocyanin and ascorbic acid contents. Anthocyanin content ranged from 0.06 $\text{mg } 100 \text{ g}^{-1}$ in 'F' to 0.82 $\text{mg } 100 \text{ g}^{-1}$ in 'RB'. Anthocyanin content in 'RB' was 13 times higher than 'F' (Fig.1, Table 2). Ascorbic acid content ranged from 52.92 $\text{mg } 100 \text{ g}^{-1}$ in 'SR' to 73.85 $\text{mg } 100 \text{ g}^{-1}$ in 'PR'. Ascorbic acid was 1.4 times higher in 'PR' compared with 'SR'.

d. Total sugars (%): Like other parameters, wide genotypic variation was also observed in total sugars which ranged from 4.63% in 'RR' to 5.67% in 'F' which was 1.23 times higher compared with 'RR' (Table 2). Reducing sugars were significantly higher in 'F' (2.85%), 'PR' (2.80%) and 'SR' (2.69%) while non-reducing sugars were higher in 'RB' (2.90%) and 'S' (2.77%).

Correlation of physico-chemical quantitative traits: Significant correlation was observed in both physical and chemical quantitative traits in grapefruit varieties. Fruit length was positively correlated with FD and FW. Fruit diameter was highly positively related to FW while ratio FL:FD was inversely related to FW. Fruit weight was positively related to TA and negatively related to ratio TSS:TA. Rind thickness

Table 2. Genotypic diversity for physico-chemical quantitative traits in grapefruit varieties

Cultivars	FW (g)	FL (mm)	FD (mm)	FL:FD	RT (mm)	No. of Segm- ents	No. of seeds	TSS (°Brix)	TA (%)	TSS: TA	AA (mg 100 g ⁻¹)	Anthoc y.(mg 100 g ⁻¹)	Total Sugars (%)	RS (%)	NRS (%)
Flame	455.50± 16.33a	87.71± 0.61a	105.17± 1.30a	0.83± 0.00c	10.40± 1.18a	13.60± 0.40a	3.40± 0.51ab	8.09± 0.05c	1.81± 0.01a	4.48± 0.05c	57.85± 4.50b	0.06± 0.03c	5.67± 0.05a	2.85± 0.10a	2.69± 0.06abc
Pink	313.60±	83.76±	90.50±	0.92±	5.09±	13.40±	2.20±	8.51±	1.39±	6.13±	73.85±	0.29±	5.44±	2.80±	2.51±
Ruby	9.88cd	1.22abc	0.68c	0.00ab	0.38d	0.24a	0.20b	0.13b	0.01d	0.13b	1.68a	0.07b	0.01b	0.09a	0.08c
Red	290.08±	79.95±	90.54±	0.88±	9.87±	13.00±	4.40±	8.35±	1.09±	7.80±	61.54±	0.82±	4.98±	1.92±	2.90±
Blush	8.42d	1.13c	1.21c	0.01bc	0.42ab	0.31ab	0.40a	0.16bc	0.07e	0.64a	2.17b	0.12a	0.02e	0.04c	0.02a
Rio Red	366.24± 21.10b	86.52± 1.60ab	97.57± 3.31b	0.89± 0.03bc	8.54± 0.53bc	12.40± 0.24b	3.60± 0.74ab	7.29± 0.03d	1.61± 0.01bc	4.53± 0.05c	53.54± 3.16b	0.13± 0.05bc	4.63± 0.02f	1.97± 0.01bc	2.52± 0.01bc
Shamber	329.90± 11.66bc	81.15± 1.42c	84.18± 1.28d	0.96± 0.02a	7.63± 0.31c	12.40± 0.24b	3.60± 0.51ab	8.11± 0.04c	1.56± 0.01c	5.19± 0.05c	57.85± 4.50b	0.08± 0.02c	5.16± 0.04d	2.23± 0.06b	2.77± 0.07ab
Star	326.60±	83.43±	91.94±	0.90±0.	5.24±0.	12.20±0	4.40±0.	8.87±0.	1.72±0.	5.19±0.	52.92±	0.14±	5.32±	2.69±	2.50±
Ruby	0.00cd	2.17bc	1.04c	02ab	30d	.49b	40a	02a	05ab	16c	3.28b	0.04bc	0.05c	0.18a	0.16c

AA = Ascorbic acid; RS = Reducing Sugar (%); NRS = Non-Reducing Sugar (%)

Table 3. Pearson's correlation analysis of fruit physico-chemical quantitative traits of grapefruit varieties

	Segs	FL	FD	FL:FD	FW	RT	TSS	TA	TSS:TA	AA	Antho.	TS	RS
FL	0.017												
FD	0.243	0.516**											
FL:FD	-0.291	0.116	-0.788**										
FW	0.178	0.652**	0.824**	-0.471**									
RT	0.208	0.149	0.330	-0.263	0.353								
TSS	-0.023	-0.389*	-0.280	0.027	-0.358	-0.333							
TA	-0.167	0.305	0.444*	-0.278	0.575**	0.304	-0.164						
TSS:TA	0.153	-0.318	-0.388*	0.200	-0.521**	-0.369*	0.444*	-0.930**					
AA	0.280	-0.321	-0.103	-0.126	-0.266	0.265	0.215	-0.429*	0.395*				
Antho.	0.249	-0.087	-0.207	0.149	-0.341	-0.452*	0.143	-0.713**	0.660**	0.127			
TS	0.261	-0.094	0.163	-0.239	0.301	0.403*	0.440*	0.275	-0.104	0.303	-0.289		
RS	-0.006	-0.098	0.069	-0.116	0.225	0.617**	0.228	0.333	-0.257	0.306	-0.472**	0.822**	
NRS	0.376*	0.041	0.108	-0.131	0.027	-0.518**	0.215	-0.200	0.307	-0.112	0.425*	-0.043	-0.604**

* = Significant (P<0.05); ** = Highly significant (P<0.01)

was directly related to reducing sugars while it was inversely related to non-reducing sugars. Acidity was negatively related to anthocyanins and ascorbic acid. Ratio TSS:TA was positively related to anthocyanins. Total sugars were strongly related to reducing sugars.

DISCUSSION

Fruit size and quality estimation is required for varietal assessment, selection and recommendation of better varieties for growers and other stakeholders. In Pakistan, grapefruit cultivation is limited to only one cultivar i.e., Shamber. For long time the potential of other varieties (such as 'F', 'PR', 'RB', 'RR' and 'SR') has not been explored for genotypic diversification despite availability of leading varieties which are known for horticultural traits elsewhere. However, development of fruit size and quality is also affected by agroclimatic conditions of that area. Hence, the study was aimed to assess fruit size and quality in the pigmented varieties for the selection of new candidate genotypes to broaden the varietal base for cultivation, extend the marketing window and consumer choice as well. Fruit morphological parameters (such as size, weight, color, number of seeds) and biochemical profile (such as TSS, acidity, ascorbic acid and anthocyanin) are considered as important quality indicators in

this regard (Zamman *et al.*, 2019). Sugar acid ratio stands as the key commercial indicator in citrus (Lado *et al.*, 2014). Wide variation has been reported in grapefruit varieties for fruit quantitative and qualitative traits (Ozeker, 2000; Farid *et al.*, 2015; Baswal *et al.*, 2016). Fruit weight and size was higher in 'F' and 'RR'; however, peel was thinner in 'SR' and 'PR' with low number of seeds. Higher fruit weight, fruit size and a smaller number of seeds was found in 'Java' and 'Ruby' varieties of grapefruit while peel was thinner in 'Marsh' and 'Mac Carty' (Farid *et al.*, 2015). Peel thickness was reduced, and acidity was decreased with fruit maturity (Sinclair, 1972; Ozeker, 2000). Hence, fruit should be harvested at proper physiological maturity to attain better fruit size and fruit quality. Tree age has significant impact on fruit size, quality and yield. Young and vigorous plants gave heavier fruit and higher yield. In agreement to our studies, fruit of 'Marsh' had 12-13 segments (Ozeker, 2000).

Usually TSS (%) and ratio TSS:TA recommended for commercialization of grapefruit varieties is 6-7% and 5.5-7, respectively (Davies and Albrigo, 1994; Lado *et al.*, 2014). In this study, all varieties showed TSS more than 8 °Brix excluding 'RR' (7.29 °Brix) while TSS:TA was more than 6-7 in 'PR' and 'RB' indicating their potential for commercialization. Ascorbic acid was higher in 'PR', sugar contents were more in 'F' and 'PR' and anthocyanins were

remarkably higher in 'RB'. Cultivars 'Ruby' and 'Thompson' showed higher maturity index while ascorbic acid was higher in 'Natsu Mikan' and 'Foster'. β -carotenoids were higher in 'Mac Carty' and 'Marsh' (Farid *et al.*, 2015). This varietal variation in fruit qualitative and quantitative parameters could be attributed to genotypic and environmental variability in these studies. Increase in TA showed rise in fruit weight, decrease in anthocyanins and ascorbic acid while ratio TSS:TA was related to anthocyanins. This indicates that anthocyanins enrichment is likely to be related to increase in TSS and decrease in TA in the pigmented varieties. These studies highlight the potential of available variability in grapefruit varieties. Though fruit weight and size were relatively low however, fruit quality was better in 'PR', 'RB' and 'SR'. These three genotypes could be taken as the new potential candidates for commercialization and replacement of 'Shamber'. However, per plant production, yield estimation and fruit quality of these varieties would depend upon variable agroclimatic conditions and grafting on different rootstocks.

Conclusion: Area under grapefruit cultivation should be enhanced and early harvesting of semi mature green fruit must be discouraged. Elite varieties like 'PR', 'RB' and 'SR' offer better fruit quality and could be potential candidates for further multiplication and cultivation.

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