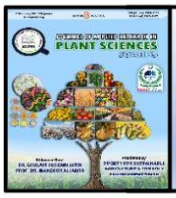
J. Appl. Res. Plant Sci. Vol. 4(2), 687-694, 2023, https://doi.org/10.38211/joarps.2023.04.02.177

Research Article

OPEN ACCESS



Available on https://www.joarps.org
Journal of Applied Research in Plant Sciences
(JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)

Morpho-Biochemical Assessment of Pomegranate Germplasm Under Subtropical Climatic Conditions of Faisalabad, Pakistan

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Article Received 01-02-2023, Article Revised 20-03-2023, Article Accepted 15-04-2023.

Abstract

A field study was conducted for assessment five different varieties of pomegranate (Tarnab gulabi, NARC-1, NARC-2, Kandhari and Sultan) under subtropical agro-climatic conditions. The investigation was conducted under RCBD (randomized complete block design) with five treatments and three replications. Various morphological (plant height, leaf area), fruit physical (fruit size, fruit weight, seed%, juice % and peel %) and biochemical (TSS, pH, titratable acidity, ascorbic acid contents, total sugars, reducing sugars and non-reducing sugars, total antioxidants and total phenols) parameters were studied. Findings noted were subjected to variance analysis at 5% probability. Wide morph-biochemical variation was observed for most of the examined traits. Results showed that NARC-I showed highest value for fruit weight (224.07g), juice % (34.33%), non-reducing sugars (4.53%), titratable acidity (1.67%) and total antioxidant contents (4.53 DPPH inhibition %age) in juice. Kandhari had maximum seed % (42.67%), peel % (39.23%) and vitamin C (12.13 mg/100ml) contents. Highest plant height (11.23 feet), leaf area (1031.4mm), juice pH (3.43), TSS (19.20 ^{o}brix), reducing sugars (11.70%) and total phenols (11.70 µg GAE L^{-1}) was observed in Sultan strain. Based on the outcomes of this investigation, it can be concluded that Sultan performed better in terms of vegetative and fruit physical and bio-chemical attributes under subtropical agro-climatic conditions of Faisalabad.

Keywords: *Subtropical climate, Pomegranate, Varieties, Morpho-biochemical attributes*

Introduction

Cultivated species of pomegranate (*Punica granatum* L. family Punicaceae), fruit tree has rich economical, ecological as well as sociological benefits. It is naturally available biological source of nutrients and bioactive compounds. The plant is a deciduous tree or shrub having an extensive history of use as a food and traditional medicine. Tree is native to regions of Iran and northern India. Major producer of pomegranate includes Afghanistan, Saudi Arabia, Iran, Palestine, India, Africa, China and America (Banihani *et al*., 2013). It can be grown from tropical and sub-tropical regions of world thus having wide arrange of available cultivated varieties *i.e.* more than 760. The tree of pomegranate is grown as evergreen in the tropical regions while deciduous in subtropical climatic regions. Pomegranate has good tolerance against iron chlorosis, active limestone, drought and salinity and grows well in well drained soils (Hussain *et al*., 2012). The fruit is commercially available from October to February in the Northern Hemisphere while from March to May in Southern Hemisphere (Morton, 1987). Pomegranates are adapted to arid to semi-arid zones globally and are considered tolerant to water scarcity (Rodríguez *et al*., 2012; Galindo *et al*., 2017). Pakistan is blessed with wide range of best agro ecological pockets which are cultivation of diverse horticultural crop species including fruits, vegetable and different flowers and ornamental plants. The wide climatic conditions of Pakistan are considered optimum for lucrative and successful cultivation of pomegranate. Meanwhile, utilization of pomegranate is obsessed by both fresh marketplace as well as processing industry, it is vital to recognize whole fruit quality traits to not only categorize all the varieties from a botanical view point, but also to meet present market requirement for quality fruits. A number of research studies have been conducted and many published reports are available on the morpho-biochemical features of the pomegranate varieties that are different from other genotypes under

study. It is not only nutritionally significant but also highly valued for the farmers to fetch high price in the market. Shortly, pomegranate fruit production is a long-standing approach to eradicate hunger, poverty and access to healthy food Thus, its significance and requirement in the present situation, the current experiment was started to explore the superlative strain of pomegranate which can be grown with successful and high quality production in the region.

Materials and method

The experiment was carried out at Fruit plant nursery Horticultural Research Institute, Ayub Agricultural Research Institute Faisalabad during 2017-188 year old mature and healthy plants of five varieties (Tarnabgulabi, NARC-1, NARC-2, Kandhari and Sultan) were selected for assessment of morho-biochemical traits. All the plants were subjected to uniform cultural practices. The layout plan of experimentation was Randomized Complete Block Design (RCBD) with five treatments each replicated four times. Data regarding plant height, leaf size was collected in the field. Fruit was harvested at physiological maturity and brought to laboratory at Horticultural Research Institute for further qualitative and quantitative analysis. Plant height was measured with the help of a wooden measuring pole by placing it parallel to the tree trunk. Leaf size was calculated by measuring the length and width of twelve leaves of each experimental tree. Then average was calculated and expressed as mm. Five number of fruits were selected for fruit physical and biochemical analysis. Size of fruit was estimated by measuring the fruit length and fruit diameter of each fruit sample**.** Samples of fruits were weighed through digital weighing balance and mean fruit weight was calculated by dividing total weights of fruits by number of all fruits in the sample (5). Five fruits were weighed on the digital weighing balance and the mean weight per fruit was recorded. Subsequently, yield per plant was estimated by weighing the total harvest.

The average peel weight percentage was calculated by using following formula:

$$\text{Percent Peel weight} = \frac{\text{Mean peel weight}}{\text{Mean fruit weight}} \times 100$$

Juice of each separate fruit sample was extracted and sieved to remove fruit pulp and then weight of each juice sample was measured. The juice %age was calculated by formula given as under:

$$\text{Juice weight}(\%) = \frac{\text{Mean juice weight}}{\text{Mean fruit weight}} \times 100$$

For determination of pH of pomegranate juice, approximately 20 mL of juice sample was pour in a glass beaker and pH (potential hydrogen) was determined with the help of digital pH meter. TSS was recorded with the help of refractometer (BX-1,Japan). Titratable acidity and ascorbic acid contents were measured by following the methodology devised by AOAC (2005). The sugars were determined according to the procedure described by Ranganna (2001). The estimations of the antioxidant activities were carried out as radical foraging activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by means of procedure devised by Brand-Williams *et al*. (1995) and Sánchez-Moreno *et al*. (1998). During the biochemical study, total phenolic contents (TPC) were examined by using a Folin-Ciocaltau colometric method (Singleton and Rossi, 1999) and expresses the findings as Gallic acid equivalents (GAE).

Table. 1: Soil analysis report of experimental area (fruit plants nursery, Horticultural Research Institute, Ayub Agricultural Research Institute, Faisalabad)

Components	Quantity
Sand	50.3%
Silt	27.7%
Nitrogen (N)	0.030%
Clay	22.0%
Texture type	Sandy to clay loam
Saturation percentage	39.0%
pH	7.9
EC	1.4 dSm^{-1}
Organic matter	0.76%
Available phosphorus (P)	7.2 mg/ kg
Extractable potassium (K)	120 mg /kg

Variance analysis was used for statistical analysis while difference in the means were equated using Least Significance Difference Test (Jan *et al*. 2009). The data were analyzed using statistical software MSTATC (Michigan State University).

Table 2: Weather of Faisalabad during experimental period 1st year

		Temperature	Humidity	Pressure
March -2018	Maximum value	36 °C (30 Mar, 17:00)	85% (21 Mar, 08:00)	1016 mbar (21 Mar, 08:00)
	Minimum value	14 °C (4 Mar, 08:00)	14% (30 Mar, 11:00)	1001 mbar (3 Mar, 20:00)
	Mean value	24 °C	55%	1011 mbar
April- 2018	Maximum value	40 °C (30 Apr, 14:00)	77% (9 Apr, 05:00)	1013 mbar (9 Apr, 05:00)
	Minimum value	18 °C (21 Apr, 05:00)	16% (23 Apr, 14:00)	997 mbar (30 Apr, 17:00)
	Mean value	30 °C	42%	1006 mbar
May- 2018	Maximum value	44 °C (29 May, 14:00)	76% (3 May, 05:00)	1011 mbar (3 May, 05:00)
	Minimum value	22 °C (7 May, 05:00)	13% (25 May, 11:00)	991 mbar (29 May, 17:00)
	Mean value	33 °C	36%	1002 mbar
June- 2018	Maximum value	44 °C (13 Jun, 14:00)	96% (29 Jun, 05:00)	1006 mbar (29 Jun, 05:00)
	Minimum value	23 °C (26 Jun, 23:00)	20% (13 Jun, 14:00)	988 mbar (12 Jun, 17:00)
	Mean value	33 °C	55%	996 mbar
July-2018	Maximum value	39 °C (9 Jul, 17:00)	100% (3 Jul, 02:00)	1000 mbar (3 Jul, 02:00)
	Minimum value	22 °C (3 Jul, 14:00)	41% (9 Jul, 17:00)	973 mbar (2 Jul, 05:00)
	Mean value	31 °C	75%	996 mbar
August -2018	Maximum value	38 °C (30 Aug, 14:00)	100% (25 Aug, 20:00)	1003 mbar (25 Aug, 20:00)
	Minimum value	26 °C (14 Aug, 08:00)	44% (30 Aug, 17:00)	992 mbar (12 Aug, 05:00)
	Mean value	32 °C	69%	998 mbar
September- 2018	Maximum value	36 °C (6 Sep, 14:00)	100% (9 Sep, 14:00)	1012 mbar (9 Sep, 14:00)
	Minimum value	21 °C (29 Sep, 05:00)	43% (19 Sep, 14:00)	998 mbar (6 Sep, 17:00)
	Mean value	29 °C	69%	1005 mbar
		Temperature	**Humidity**	**Pressure**
March- 2017	Maximum value	31 °C (13 Mar, 17:00)	88% (4 Mar, 05:00)	1020 mbar (4 Mar, 05:00)
	Minimum value	12 °C (28 Feb, 05:00)	32% (11 Mar, 14:00)	1008 mbar (13 Mar, 17:00)
	Mean value	21 °C	58%	1014 mbar
April- 2017	Maximum value	42 °C (20 Apr, 14:00)	86% (6 Apr, 05:00)	1015 mbar (6 Apr, 05:00)
	Minimum value	20 °C (8 Apr, 02:00)	14% (12 Apr, 17:00)	994 mbar (20 Apr, 17:00)
	Mean value	29 °C	40%	1005 mbar
May- 2017	Maximum value	43 °C (26 May, 17:00)	69% (23 May, 05:00)	1010 mbar (23 May, 05:00)
	Minimum value	23 °C (1 May, 05:00)	18% (1 May, 17:00)	993 mbar (30 May, 20:00)
	Mean value	33 °C	38%	1002 mbar
June- 2017	Maximum value	44 °C (4 Jun, 17:00)	100% (8 Jun, 23:00)	1008 mbar (8 Jun, 23:00)
	Minimum value	20 °C (9 Jun, 02:00)	20% (3 Jun, 17:00)	993 mbar (25 Jun, 17:00)
	Mean value	32 °C	56%	999 mbar
July- 2017	Maximum value	38 °C (6 Jul, 14:00)	96% (14 Jul, 17:00)	1004 mbar (14 Jul, 17:00)

	Minimum value	24 °C (7 Jul, 05:00)	43% (8 Jul, 14:00)	992 mbar (1 Jul, 17:00)
	Mean value	31 °C	72%	998 mbar
August- 2017	Maximum value	38 °C (18 Aug, 14:00)	92% (30 Aug, 17:00)	1006 mbar (30 Aug, 17:00)
	Minimum value	24 °C (25 Aug, 11:00)	40% (18 Aug, 14:00)	993 mbar (3 Aug, 17:00)
	Mean value	31 °C	70%	1000 mbar
September- 2017	Maximum value	37 °C (29 Sep, 14:00)	100% (5 Sep, 08:00)	1009 mbar (5 Sep, 08:00)
	Minimum value	21 °C (5 Sep, 08:00)	34% (17 Sep, 17:00)	999 mbar (1 Sep, 17:00)
	Mean value	30 °C	65%	1005 mbar

Table 3: Weather of Faisalabad during experimental period 2nd year

Varieties	PLANT HEIGHT (feet)	LEAF AREA (mm)	fruit weight (g)	Fruit size (cm)	Plant yield (Kg)
Tarnab gulabi	11.22a	1026.6 a	143.97 e	7.61a	24.80a
NARC-I	10.51a	1025.4 a	224.07 a	6.84 c	22.30a
NARC-II	10.18a	988.4 b	199.40 b	7.22 b	23.26a
kandhari	8.86b	970.6 c	160.10 d	6.71 c	21.00a
sultan	11.23a	1031.4 a	188.73 c	5.95 d	23.06a
CV	3.92	0.38	0.81	1.63	7.31

Results

Data regarding various vegetative and biochemical parameters is presented in table 4 & 5. Plant height ranges from 8.86 to 11.23 feet. Tarnab gulabi and sultan found to be at par (11.22 & 11.23 feet respectively) while; Kandhari had lowest plant height (8.86feet). Leaf area of five varieties of pomegranate ranges from 1031.4 mm to 970.6mm with Sultan had highest leaf area and Kandhari showed lowest. NARC-I had largest fruit weight (224.07g) while minimum fruit weight was recorded in Kandhari (160.10g). Fruit size ranges from 7.61cm in Tarnab gulabi to 6.71 in Kandhari.

Table 4: Plant Height, Leaf Area, Fruit Weight, Fruit Size and Plant yield of five varieties of pomegranate grown under Faisalabad conditions

Varieties	Plant Height (feet)	Leaf Area (mm)	Fruit Weight (g)	Fruit Size (cm)	Plant Yield (kg)
Tarnab gulabi	11.22a	1026.6 a	143.97 e	7.61a	24.80a
NARC-I	10.51a	1025.4 a	224.07 a	6.84 c	22.30a
NARC-II	10.18a	988.4 b	199.40 b	7.22 b	23.26a
kandhari	8.86b	970.6 c	160.10 d	6.71 c	21.00a
sultan	11.23a	1031.4 a	188.73 c	5.95 d	23.06a
CV	3.92	0.38	0.81	1.63	7.31

Mean values having same letter are significantly alike at p<5%

Highest fruit yield was obtained from Tarnab Gulabi while lowest from Kandhari. Five varieties found to be non-significant difference with respect to fruit yield. Juice percentage ranges from 34.33% in NARC-I to 18.10% in Kandhari. Highest seed percent (42.67%) and peel percent (39.23%) was recorded in Kandhari. NARC-I had lowest seed percent (27.77%) while minimum peel percent (33.23 %) was obtained from fruits of Tarnab Gulabi. Value for pH ranges from 3.43 to 3.23 and found non-significant in all varieties. Fruits harvested from sultan exhibited highest TSS (19.20 ^{o}brix) followed by Tarnab Gulabi (17.03^{o}brix). NARC-I and NARC-II found to be at par

.**Table 5: Juice%, seed% and peel% of five varieties of pomegranate grown under Faisalabad conditions**

Varieties	Juice %	Seed (%)	Peel (%)	pH	TSS (^{O}Brix)
Tarnab gulabi	32.03 a	34.73 b	33.23 b	3.3 ab	17.03 b

NARC-I	34.33 a	27.77 d	37.90 a	3.23 b	15.20 d
NARC-II	28.03 b	35.83 b	36.13 ab	3.47 a	15.67 cd
kandhari	18.10 c	42.67 a	39.23 a	3.23 b	16.13 c
sultan	31.30 ab	30.87 c	37.83 a	3.43 a	19.20 a
cv	4.86	2.64	3.92	2.05	1.61

Mean values having same letter are significantly alike at p<5%

Data of various biochemical parameters was statistically analyzed and presented in table 6. Sultan had lowest value for non-reducing sugars (3.45) and highest recorded value for reducing sugars (11.70). Highest value of Total sugars (16.30) and ascorbic acid (12.30) was obtained from Tarnab Gulabi. NARC-I exhibited maximum titratable acidity (1.67) while minimum recorded from Sultan (0.93).

Table 6: Non-reducing sugars, reducing sugars, total sugars, titratable acidity and ascorbic acid contents of five varieties of pomegranate grown under Faisalabad conditions

Varieties	non-reducing sugars (%)	reducing sugars (%)	total sugars (%)	titarteable acidity (%)	ASCORBIC ACID (mg/100mL)
Tarnab gulabi	4.15 ab	11.33 ab	16.30 a	1.57 a	12.30 a
NARC-I	4.53 a	10.73 b	16.07 a	1.67 a	12.13 a
NARC-II	4.16 ab	9.93 c	14.83 b	1.53 a	11.07 b
kandhari	3.69 bc	11.13 ab	15.60 ab	1.20 b	12.27 a
sultan	3.45 c	11.70 a	15.93 a	0.93 c	11.90 a
cv	7.05	3.20	3.10	5.29	3.44

Mean values having same letter are significantly alike at p<5%

Results of total antioxidant and total phenols of five varieties of pomegranate is presented in table7 which clearly shows that total antioxidants ranges from 4.53 (DPPH inhibition %age) in NARC-I to 3.45 (DPPH inhibition %age) in Sultan. Sultan exhibited highest value (11.70 µg GAE L^{-1}) for total phenols while lowest was recorded from NARC-II (9.93 µg GAE L^{-1})

Table 7: Total antioxidants and total phenols of five varieties of pomegranate grown under Faisalabad conditions

Varieties	Total Antioxidant (DPPH Inhibition %age)	Total Phenols (µg GAE L^{-1})
Tarnab Gulabi	4.15 ab	11.33 ab
NARC-I	4.53 a	10.73 b
NARC-II	4.16 ab	9.93 c
Kandhari	3.69 bc	11.13 ab
Sultan	3.45 c	11.70 a
cv	3.38	0.30

Mean values having same letter are significantly alike at p<5%

Discussion

Our studies majorly focused on genetic diversity of *punica granatum* germplasm for physical and biochemical characteristics. Values of some physical characteristics showed significant variation in current study (Table. 1). The outcomes are in line with the results of (Abbas *et al*., 2018) who studied performance of eight pomegranate varieties. A higher degree Brix directs a greater soluble sugars and more sweeter the fruit juice will be. Total soluble solids or degree of Brix is a crucial criterion for quality fruit juice, and it has been stated to range (12% to 18%). Therefore, the Sultan among the five pomegranate genotypes assessed in this research were found to have outstanding degree of brix (19.20) and to be appropriate for sipping as a juice or to be eaten as fresh fruit. Our maximum value for Brix is higher than the values of Alcaraz-Marmol *et al*. (2017) who described TSS values ranging from 15 to 17 percent in twenty pomegranate cultivars cultivated in Spain, one of the foremost global producers of pomegranate. These Brix standards are within the array of values published by present investigation, indicating that saleable sweeter pomegranates can be grown in sub-tropical environments also. These results paralleled to those described by (Hasnaoui *et al*., 2011.; Martínez *et al*.

2006; Tehranifar *et al*. 2010; Abbas *et al*., 2018). The range of present soluble sugar contents was higher than those of the reported °Brix by (Tehranifar *et al*. 2010), but very closer to 13.7–19.1 Brix in other reports (Dafny-Yalin *et al*. 2010; Ferrara *et al*., 2011, Ferrara *et al*., 2014; Martínez *et al*. 2012; Alcaraz-Mármol *et al*. 2017; Khadivi *et al*. 2018). Substantial genetic variation was observed with respect to fruit weight in this study. The difference in fruit weight of the varieties might be due to genetic behavior as explained previously by Zaouay *et al*. (2012) who determined that difference in fruit weight depended on the variety and environmental conditions. The Abbas *et al*., 2018 reported diversity in 8 different pomegranate varieties collected from various localities of Punjab, Pakistan. The varieties investigated during the study, Sultan and NARC-1 having maximum juice percentages (32.8 and 32.5 correspondingly), could be more auspicious than other genotypes because juice contents are highly demanded trait in beverage and food processing industry (Rajasekar *et al*., 2012). The significant variations among various varieties were noticed in values of pH and total acidity (TA) might be owing to the pattern of phenolic contents of juice extracted from different varieties of pomegranate (Gil *et al*., 2000). Natural sugars found abundantly in pomegranate fruits like almost all other fruit crops. The values of total sugars in various varieties of pomegranate are in agreement with those experiential by Poyrazoglu *et al*. (2002) and Aviram *et al*. (2000). Although, the most imperative antioxidant, vitamin C is commonly observed as a nutritive attribute to assess the quality of fruit. Ascorbic acid (Vit. C) contents in Chinese commercial cultivars and Iranian pomegranate genotypes were ranged 9.91–20.92 mg 100 g^{-1} (Aarabi *et al*. 2008) (Tehranifar *et al*. 2010). Findings of Youssef *et al*. (2007) concerning Ascorbic acid, supported the results of the current research. The findings of the research represented that the juice configuration of pomegranate fruit and its biochemically active composites depends on maturity index and variety as examined by Miguel *et al*. (2004). Total phenolic contents (TPC) and antioxidants of the juice of five evaluated cultivars differs significantly and parallel to the findings gained by Faria and Calhau (2010). Nevertheless, wholly the variabilities contained antioxidants which make them promising for healthy for drinking.

The length and diameter of fruits defines the pomegranate size. There were substantial variations in mean fruit weight among numerous genotypes evaluated in this experiment. The difference in fruit weight was probably due to variance in genetic make-up of those varieties as published by (Kumar and Khosla, 2012: Abbas *et al*., 2018) who tested performance of pomegranate 14 varieties at Hill station of India and 8 pomegranate varieties in Punjab, Pakistan, correspondingly. Among the fruit nectars, pomegranate juice is measured to be one of the great antioxidant source. (Elfalleh *et al*., 2011). These compounds (antioxidants) may help to decrease the threat of many heart issues and evade some cancer types (Afaq *et al*., 2005; Khan *et al*., 2007; Malik and Mukhtar, 2006). Pomegranate nectar is pretty good source of mineral elements (Opara *et al*., 2009; Melgarejo *et al*., 2011). Pomegranate is also well known for being a good phenolics source that are full of different antioxidants, that are associated to possible health aids (Mphahlele *et al*., 2014). Presently, there is great interest in phenolics and their free radical scavenging activities among customers and the researchers in previous years due to the epidemiological studies concerning the intake of antioxidant rich diets with reduced threats of cancer and heart problems (Pourreza, 2013). The total phenolic (TP) content in the pomegranate juice ranged from 9.93 to 11.70 (µg GAE L^{-1}) (Table. 7), which was lower than that reported in a previous reported (40.91 to 132.47 µg/mL) of thirty seven Chinese cultivars (Peng *et al*., 2020). These differences than those described in this study, possibly due to genetic variations, and show significant diversity in phytonutritional compounds among different pomegranate varieties.

Conclusion

Our results indicate that there is substantial phenological diversity among these pomegranate varieties. Based on results of present study Sultan genotype can be recommend for subtropical climates as it recorded maximum values in most of the parameters studied (plant height, leaf area, juice percentage, pH, total soluble solids and Total phenols).

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