RESEARCH ARTICLE

The Journal of Agriculture and Environment, Vol: 24, June, 2023 | *(179-186)*

MORPHOLOGICAL CHARACTERIZATION OF POMEGRANATE *(Punica granatum L.)* GENOTYPES AT DAILEKH

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ARTICLE INFO

Keywords:

Aril weight,

flowers,

marketable fruit,

pomological characters,

Punica granatum L.

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ABSTRACT

Pomegranate is an emerging potential fruit crop of Nepal and it can be cultivated successfully from tropical to warm temperate climatic condition of terai to mid-hills. This research was conducted to study the morphological traits and identify the superior pomegranate genotypes at Horticulture Research Station (HRS), Dailekh in 2019. Three pomegranate genotypes (HRDPOM001, HRDPOM004 and HRDPOM004M) were studied for their growth, flowers and pomological characters. Variation in plants, flower and pomological characters was observed in pomegranate genotypes. The highest marketable fruit weight (1.4 kg/plant) produced in HRDPOM001, followed by HRDPOM004 (1.2 kg/plant). The highest aril weight (60.5%) was measured in HRDPOM004. Highest TSS (12.8°Brix) and good taste preferences were recorded in the fruits of HRDPOM004. Pomological attributes for HRDPOM004 was found better than other studied genotypes. Individual fruit weight, fruit length, and fruit diameter showed the significant positive association with total fruit weight, and selection of these traits could improve the fruit yield. Based on the major pomological attributes, HRDPOM004 was found promising and selected to cultivate at the similar agro-climatic regions of Nepal.

1. INTRODUCTION

Pomegranate is one of the important ancient domesticated fruits. Asia Minor, Transcaucasia, Iran and Turkmenistan are reported to be the center of origin of pomegranate (Melgarejo and Salazar, 2003). Pomegranate fruit has a high nutritional and medicinal importance, and its cultivation has increased particularly in Iran, India, China, Turkey and the United States. Fruits contain enormous fibers, protein, vitamin C, vitamin K, folate and potassium. It prevents cardiovascular disease, lower the blood pressure and strengthens the digestive system. In Nepal, it is one of the important emerging potential commercial fruit (Atreya *et al*., 2020).

Pomegranate is well-adapted in sub-tropical, tropical and warm-temperate climate, and it can be grown successfully from terai to an elevation of 1,600 m above sea level (asl). It can be thrived well in drought condition (Atreya, 2014). Pomegranate is considered as deciduous in warm-temperate and temperate climate, but it is an evergreen or partially deciduous fruit in tropical and sub-tropical climate. But high temperature during fruit ripening (August to October) is necessary to get high quality and quantity of fruits (Karimi and Mirdehghan, 2013). Globally, more than 760 varieties of pomegranate are cultivated (Muhammad *et al*., 2019). In Nepal, Safeda, Bedana, Spanish Ruby, Local (Darim), Kandhari, Paper Shell, Muscat Red, Ganesh, Mridula, Red Dyana and Bagwa are important varieties and cultivated in different regions. Based on the flowering behavior, it can be categorized into Ambe bahar (February-March), Mrig-bahar (June-July) and Hasta bahar (September-October). In general, one bahar fruiting is regulated to maintain productivity of the plants, but the fruit regulation also depends on market factors and availability of water (Poudel *et al*., 2017).

In Nepal, pomegranate is cultivated 762 ha with productivity of 6.6 mt/ha, but in Karnali Province, the productive area is estimated to be around 22 ha with the productivity of 7.5 mt/ha (MoALD, 2021). Different varieties are cultivated across the country, but information on fruit traits such as fruit size, peel color,

taste, aril color and seed hardness in different varieties are not available yet. Pomological attributes differ in genotypes. The edible part of pomegranate fruit is arils, which constitute about 50% of the whole fruit. Different pomegranate genotypes have variability for aril-related traits and taste, color, size and seed hardiness (Barone *et al*., 2001). Variability in pomegranate is characterized in terms of domestic, wild, and ornamental types which show the differences in characters such as peel, and aril color, succulence, sugar/acid ratio, fatty acid content, anthocyanin, phenols, and antioxidant activity (Onias *et al*., 2021). Fruit weight and skin color are the dominant traits for genotypes discrimination of pomegranate (Orhan *et al*., 2014). Adequate flowering, fruiting, high and regular yield, large fruit size, red peel genotype, red and soft aril, and excellent taste are important selection criteria (Khadivi and Arab, 2021). Besides, no fruit cracking, high and attractive juice, and sour, sweet and sour-sweet taste are considered to choose the desired fruit in pomegranates (Muradoglu *et al*., 2006).

HRS, Dailekh is assigned to undertake pomegranate research and variety selection as a collaborating research station of Karnali Province (Gotame *et al*., 2020). Selection of potential genetic resources of pomegranate in the agro-climatic region of Dailekh will help to increase its productivity and income of farmers. The morphological characterization of different pomegranate genotypes is important to select new cultivar of promising characters (Onias *et al*., 2021; Khadivi and Arab, 2021). Previous studies on morphological and physico-chemical characteristics have been done in several countries of the world (Gazde, 2012; Zaouay *et al*., 2012; Ferrara *et al*., 2014; Poudel *et al*., 2017; Souza *et al*., 2020). But study on phenotypic characterization of pomegranate genotypes at Dailekh, Karnali Province of Nepal is limited. Therefore, this research was done to characterize plant and pomological attributes, and to select the best pomegranate genotypes for commercial production at the similar agro-climatic regions of Nepal.

2. MATERIALS AND METHODS

2.1 Experimental site and plant materials

Morphological characters of pomegranate genotypes were evaluated at HRS, Dailekh which is located at 28˚ 50’ 49.8” N longitude and 81˚ 43’ 19.4” E latitude with an elevation of 1, 255m asl in Karnali Province, Nepal. The annual rainfall amount ranged between 140 and 160 mm and its distribution is high around June-July. The average monthly temperature in fruit bearing period (April-September) at Dailekh ranged from 14.6 to 24.1°C (HRS, 2019). Three genotypes (HRDPOM001, HRDPOM004 and HRDPOM004M) were introduced from National Horticulture Research Centre (NHRC), Khumaltar at HRS, Dailekh for the multi-location evaluation. Six plants of each genotype were planted at the spacing of 5m *x* 5m in 2015. The trial was designed in randomized complete block and replicated three times, where each replication consisted of two plants. Nutrition, irrigation and pests, and diseases control were done regularly and uniformly for all the genotypes.

2.2 Morphological characterization

Plant growth habit, tree growth vigor and canopy density were taken using the descriptor of International Plant Genetic Resources Institute (IPGRI, 2001). Plant growth habit was visually assessed and classified into three types; erect, semi-erect and spreading. Likewise, tree growth habit was also classified into three types; weak, moderate and high. Canopy density was visually assessed and categorized into three types; weak, moderate and high. Flowering and fruit setting were visually observed. Fruits were harvested from each genotype and weighted. Cracked, insect and diseases damaged fruits were considered as non-marketable, and weighted. Uniform and un-cracked fruits were considered as marketable and weighted. Five individual fruits were weighted using digital electronic balance (H-Honda™, India) and averaged it. Fruit length (mm), fruit diameter (mm) and fruit pericarp thickness (mm) were measured using digital caliper (150mm, Model: DC-515). Pericarp weight and aril were calculated on fruit weight basis and expressed as percentage. Number of segments were counted after horizontal cutting of fruits. The total soluble solids (TSS) were determined using a digital refractometer (Model ATAGO, Tokyo, Japan) and expressed in °Brix. For each reading, the refractometer was washed with distilled water each time after use and dried with blotting paper to avoid contamination. Aril color was visually examined and noted using IPGRI (2001) descriptor. Fruit taste was assessed by judge panel of 12 personnel using 1-5 scales (1 = very poor, 2 = poor, 3 = fair, 4 = Good, and 5 = Excellent) and overall preferences for fruit taste were ranked. Seed hardness was assessed using 1-3 scales (1 = Very hard, 2 = Semi-Soft and 3 = Soft) (Khadivi and Arab, 2021). The qualitative traits including fruit shape, fruit peel color, fruit symmetry, fruit feel cracking, aril shape, aril color and fruit quality (based on qualitative

traits) were visually examined and coded using the method described by Mars and Marrakchi (1999).

2.3 Data analysis

Plant and fruits data were edited and processed using MS Excel (version 16.0, Microsoft, Redmond, WA, USA) and then, one-way analysis of variance (ANOVA) followed by Tukey's Test, Honestly Significant Difference (HSD) was done using SPSS (SPSS Inc., Chicago, Ill., USA). Phenotypic correlation coefficients among quantitative traits were computed using R version 4.2.2 [R Core Team (2022). R: A language and environment for Statistical Computing, Vienna, Austria. URL, https:///www.R-project.org/].

3. RESULTS AND DISCUSSION

3.1 Morphological description

Morphological characters, flowering and fruiting traits of pomegranate genotypes are given in Table 1. Growth habit varied from erect, semi-erect to spreading type (Figure 1). HRDPOM004M, HRDPOM001 and HRDPOM004 had semi-erect, spreading and erect growth habit, respectively. HRDPOM004 had high tree growth vigor whereas remaining two genotypes had moderate types. Canopy density in HRDPOM001 had high and rests of two genotypes had moderate types. Variability for growth habit, tree growth vigor and canopy density in pomegranate genotypes has also described by previous researchers (Dandachi *et al*., 2017; Khadivi and Arab, 2021). Flowering was started earlier i.e. second week of March in two genotypes (HRDPOM001 and HRDPOM004) and delayed flowering (second week, April) was observed in HRDPOM004M. Two genotypes (HRDPOM001 and HRDPOM004) had 50% flowered in second week of April. All genotypes started to flower in spring season, but flowering was continued till rainy season (June-July) in HRDPOM004. With respect to first fruiting setting, HRDPOM004M and HRDPOM004 found earlier i.e. third week of April.

Table 1. Growth habit, flowering and fruit setting traits of pomegranate genotypes

Genotypes	Growth habit	Tree growth vigor	Canopy density	First flower opening	50% flowering	Flowering time	First fruit setting
HRDPOM001	Spreading	Moderate	High	Second week, March	Second week, April	Spring	Fourth week, April
HRDPOM004	Erect	High	Moderate	Second week, March	Second week, April	Spring-Rainy	Third week, April
HRDPOM004M	Semi-erect	Moderate	Moderate	Second week, April	Third week, April	Spring	Third week, April

3.2 Pomological description

The results of the fruit yield and quality traits are presented in Table 2. Genotypes showed significant ($P<0.05$) differences in total fruit weight, non-marketable and marketable fruit weight, fruit weight, fruit length and diameter, and fruit pericarp thickness. HRDPOM001 produced the highest fruit weight (2.8 kg/plant) and marketable fruit weight (1.2 kg/plant). The highest individual fruit weight was measured in HRDPOM001 (240.4 g), followed by HRDPOM004 (229.4 g). In contrast, in a study of Poudel *et al*. (2017), they reported the highest (355.4 g) fruit weight in Safeda cultivar. Fruit weight is an economic trait which is important for genotype selection (Orhan *et al*., 2014). Dandachi *et al*. (2017) have explained the variation in fruit weight in pomegranate genotypes. Variation in fruit weight is mainly governed by genetics, but it also depends on soil and climatic condition (Martinez *et al*., 2006). Besides, variation in fruit weight ranged from 150.0 to 568.0 g in pomegranate cultivars has been reported (Kazankaya *et al*., 2003; Ozkan, 2005). In this study, the fruit length and diameter between HRDPOM001 and HRDPOM004 were not significantly different. Fruit size is an essential trait to attract consumers' attention in the fresh food market. Many researchers (Dandachi *et al*., 2017; Poudel *et al*., 2017) have reported the variation in fruit length and diameter in pomegranate genotypes. Pericarp thickness was the highest (4.5 mm) in HRDPOM001, but it was not significantly different from HRDPOM004M (4.0 mm). The variation in fruit pericarp thickness in pomegranate genotypes has also reported in previous studies (Dandachi *et al*., 2017; Khadivi and Arab, 2021).

Table 2. Fruit yield and quality traits of pomegranate genotypes

Genotypes	Total fruit wt. (kg/ plant)	Non-market. fruit wt. (kg/plant)	Marketable fruit wt. (kg/plant)	Fruit wt. (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit pericarp thickness (mm)
HRDPOM001	2.8±0.7 a	1.6±0.4 a	1.4±0.4 a	240.4±27.0 a	72.6±1.9 a	77.0±2.6 a	4.5±0.1 a
HRDPOM004	2.0±0.6 a	1.1±0.5 a	1.2±0.1 a	229.4±26.5 b	71.1±0.9 a	75.4±2.7 a	3.4±0.7 b
HRDPOM004M	1.2±0.4 b	0.7±0.3 b	0.6±0.1 b	179.7±33.0 c	67.2±4.7 b	69.1±4.9 b	4.0±0.6 a

Mean ± SE (n=5). Means in a column followed by same letter (s) are not significant different at P<0.05 according to Tukey's Test, HSD.

Fruit pericarp weight was significantly (P<0.05) different among the genotypes (Table 3). The highest fruit pericarp weight (47.8%) and aril weight (60.5%) was recorded in HRDPOM004. Aril is the fundamental part of fruit which constitute about 50% of the whole fruit and our study showed more than 60% aril weight of the fruit in HRDPOM004 and this might be due to genetic trait. Fruit segments were not significantly different among the genotypes. TSS was measured the highest (12.8°Brix) in HRDPOM004, but it was statistically similar to HRDPOM004M (11.0°Brix), and the lowest TSS (9.4°Brx) was measured in HRDPOM001. TSS values more than 12% and the juice extracted from the fruits in all genotypes are suitable for commercial uses (Vazquez-Araujo *et al*., 2014). The superior selected genotype of pomegranate is shown in Figure 1 (D, E and F). Compared to HRDPOM001 and HRDPOM004M, fruits taste of HRDPOM004 was good (4.0) and seed hardiness was also soft in HRDPOM004.

Table 3. Fruit quality traits of pomegranate genotypes evaluated at HRS, Dailekh, 2019

Genotypes	Fruit pericarp wt. (%)	Aril wt. (%)	Fruit segments (no.)	TSS (°Brix)	Fruit taste (1-5)	Seed hardness (1-3)
HRDPOM001	44.7±1.6 b	55.3±1.6 b	5.6±0.4 a	9.4± 0.4 b	2.0	2.0
HRDPOM004	47.8±1.4 a	60.5±1.9 a	5.3±0.3 a	12.8±0.3 a	4.0	3.0
HRDPOM004M	44.8±2.5 b	55.2±2.4 b	5.4±0.6 a	11.0±0.3 a	3.0	2.0

Mean ± SE (n = 5). Means in a column followed by same letter (s) are not significant different at P<0.05 according to Tukey's Test, HSD. Fruit taste (1-5 scales); 1; Very poor, 2; Poor, 3; Fair, 4; Good, and 5; Excellent. Seed hardness (1-3 scales); 1; Very hard, 2; Semi-soft and 3; Soft.

Fruit pomological traits of studied genotypes are given in Table 4. Fruit morphology (fruit shape and fruit peel color) and aril color of pomegranate genotypes are also presented in Figure 1. Fruit shape of HRDPOM004M and HRDPOM001 had round, while the fruits of HRDPOM004 had oval- shaped. Two genotypes (HRDPOM004M and HRDPOM001) contained yellow-red fruit peel color, while HRDPOM004 had red-brown fruit peel color. Fruit skin color is dominant trait for the genotypes discrimination in pomegranate (Orhan *et al*., 2014). Peel color is one of the important criteria for famers to decide the appropriate harvesting time (Melgarejo-Sanchez *et al*., 2015). Fruit symmetry was present in HRDPOM004 and remaining two genotypes did not show any fruit symmetry. Fruit peel cracking was present in HRDPOM004M and HRDPOM001 and it was absent in HRDPOM004. Triangular aril shape was recorded in HRDPOM004M, whereas aril of HRDPOM001 and HRDPOM004 contained oval and prismatic aril shape, respectively. Aril color was red in HRDPOM004M and it was white-red in HRDPOM001 and dark red in HRDPOM004. Dark red aril color is one the important traits to be considered for the variety selection in pomegranate (Ozgen *et al*., 2008). Aril color is also the predominant trait for cultivar selection (Stover and Mercure, 2007). Diverse aril color from light milky, pink, white red, red and red-black has reported in a study of Khadivi and Arab (2021), but in our study observed white-red, red and dark-red aril color. Fruit qualities (good fruit shape, absence of fruit cracking, and dark-red aril color etc.) was higher in HRDPOM004 than HRDPOM004M and HRDPOM001. Fruit shape, fruit peel color, absence of cracking and aril color are the important traits for commercial importance. No fruit cracking, peel and aril colors are important traits of fruit quality in pomegranate marketing (Mena *et al*., 2011).

Table 4. Fruit pomological traits of pomegranate genotypes

Genotypes	Fruit shape	Fruit peel color	Fruit symmetry	Fruit peel cracking	Aril shape	Aril color	Fruit quality
HRDPOM001	Round	Yellow-red	Absent	Presence	Oval	White-red	Moderate
HRDPOM004	Oval	Red-brown	Present	Absence	Prismatic	Dark red	High
HRDPOM004M	Round	Yellow-red	Absent	Presence	Triangular	Red	Moderate

Figure 1. Trees, fruits and aril of pomegranate genotypes. A, B, C represent tree, fruit and aril of HRDPOM001, respectively. D, E, F represent tree, fruit and aril of HRDPOM004, respectively and G, H, I represent tree, fruit and aril of HRDPOM004M, respectively.

3.3 Correlation among the measured traits

Phenotypic correlation coefficients of 11 quantitative traits in pomegranate genotypes are presented in Table 4. Total fruit weight/plant was positively and significantly correlated with non-marketable fruit weight/plant (r = 0.94), marketable fruit weight (r = 0.87), fruit weight (0.86), fruit length (r = 0.71) and fruit diameter (r = 0.80). Non-marketable fruit weight showed positive and significant correlation with fruit weight (r = 0.90), fruit length (r = 0.76), and fruit diameter (r = 0.86). Fruit weight exhibited positive and significant strong correlation with fruit length (r = 0.90), fruit diameter (r = 0.98), and aril weight (r = 0.50). Positive and significant correlation of fruit weight with fruit length and fruit diameter in pomegranate have been reported by previous researchers (Karimi and Mirdehghan, 2013; Khadivi-Khub *et al*., 2015; Khadivi and Arab, 2021). Positive and significant correlation between fruit weight and aril weight has also mentioned by Waliullah *et al*. (2021). Fruit length had a significant positive association with fruit diameter (r = 0.96) and aril weight (r = 0.48) while fruit diameter was positively and significantly correlated with aril weight (r = 0.40) and was in line with the previous findings in pomegranate (Khadivi and Arab, 2021). There was a negative and significant correlation between pericarp weight and aril weight (r = -0.98). Pericarp weight also showed positive and significant correlation with TSS (r = 0.68). In contrast, a negative and significant correlation between aril weight and TSS was observed. Phenotypic correlation of morphological characters gives important information to breeders that they can use in designing a high-performance design

to explore genotypes (Tancred *et al*., 1995). Besides, this coefficient can help to compare the direct and indirect selections and develop a plan for the traits that are difficult to select and study (Falconer and Mackay, 1996).

Table 5. Phenotypic correlation coefficient of 11 quantitative traits in pomegranate genotypes

Traits	TFW	NMF	MWT	FW	FL	FD	FPT	PWT	AWT	FS	TSS
TFW	1.0	0.94**	0.87**	0.86**	0.71*	0.80**	0.43	-0.26	0.27	-0.62	-0.01
NMF		1.0	0.66	0.90**	0.76*	0.86**	0.33	-0.23	0.24	-0.47	0.01
MWT			1.0	0.63	0.48	0.54	0.48	-0.24	0.25	-0.74*	-0.03
FW				1.0	0.90**	0.98**	0.44	0.04	0.50*	-0.39	0.11
FL					1.0	0.96**	0.31	-0.06	0.48*	-0.27	-0.06
FD						1.0	0.41	0.04	0.40*	-0.34	0.06
FPT							1.0	0.35	-0.35	0.04	0.52
PWT								1.0	-0.98**	0.21	0.68*
AWT									1.0	-0.21	-0.68*
FS										1.0	0.33
TSS											1.0

**Significant at P<0.05, **Highly significant at P<0.01. TFW; Total fruit weight/plant (kg), NMF; Non-marketable fruit weight (kg/plant), MWT; Marketable fruit weight (kg/plant), FW; Fruit weight (g), FL; Fruit length (mm), FD; Fruit diameter (mm), FPT; Fruit pericarp thickness (mm), PWT; Pericarp weight (%), AWT; Aril weight (%), FS; Fruit segment (no./fruit), and TSS; Total soluble solids (°Brix).*

4. CONCLUSION

This study morphologically characterized the three pomegranate genotypes and found significant variability for plants, flowers and pomological characters. Marketable fruit weight/plant, fruit and aril weight, TSS and fruit taste showed significant variation among the genotypes. Based on the pomological and important traits of commercial importance including high marketable weight, high fruit weight, high aril weight, high TSS, good taste with soft seed, red-brown fruit peel color and dark red aril and high fruit quality, genotype HRDPOM004 was found to be promising and can be recommended to cultivate directly in the farmer's field of similar agro-climatic regions of Nepal.

ACKNOWLEDGEMENTS:

The authors would like to thank Nepal Agricultural Research Council for the financial support under 'Multi-location Evaluation of Pomegranate Genotypes' project of HRS, Dailekh.

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