Seed source Variations, Genetic Estimates and Cluster Analysis for Physio-chemical Fruit Characters of Himalayan Wild Pomegranate

Divya Mehta* and Tara Gupta
Department of TIGR, College of Forestry,
Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, (Himachal Pradesh), India.

(Corresponding author: Divya Mehta*)

ABSTRACT: Genetic improvement of the Himalayan wild pomegranate through seed source studies is essential to boost the tropical and sub-tropical pomegranate breeding programs. Therefore, the present investigation was carried out in Himachal Pradesh with a target to determine within and between seed source variations, genetic estimates for fruit characters and clustering pattern of the wild pomegranate genotypes. Eight seed sources each with three genotypes were selected and eighteen physio-chemical fruit characters were recorded. The data were then subjected to statistical analysis. For most of the fruit characters, Narag (S_1) as well as the genotypes within the seed source performed best. Maximum environmental (36.7%), genotypic (41.56%) and phenotypic (50.68%) coefficient of variance were observed for aril moisture content, non-reducing sugars and non-reducing sugars, respectively. High heritability was observed for aril weight (0.63), total soluble solids (0.99), ascorbic acid (0.97), total sugars (0.95), non-reducing sugars (0.67) and reducing sugars (0.85). Genetic gain (%) was estimated to be highest for non-reducing sugars (70.21%). Cluster analysis revealed four clusters, among them, Cluster III (S_1G_2, S_8G_2 and S_8G_3) performed best for nine fruit characters *viz.,* fruit size (length = 53.04mm and diameter = 52.34mm), fruit weight (80.83g), rind weight (40.59g), rind weight as a percentage (50.93%), aril weight (40.23g), weight of 100 arils (20.20g), 100 seed weight (5.35g) and rind aril ratio (1.08). Strategies for tree improvement programs can be developed from the present findings.

Keywords: Wild pomegranate, seed sources, fruit characters, genetic estimates, cluster analysis.

INTRODUCTION

Punica granatum L. is an ancient tree mainly known for its valuable fruit. The species is distributed and cultivated throughout central Asia due to its high adaptivity (Morton, 1987). India is the world leader in pomegranate acreage and production with 208.73 thousand ha area and 2442.39 thousand tones of annual production (Anonymous, 2017). In India, *Daru* prefers mid-hill elevations of northern parts (Kumar and Duggal, 2019; Parmar and Kaushal, 1982). Seed source of species comprising of genetically alike individuals, which have become adapted through natural selection (Mohamed *et al*., 2015). The success of tree species in plantations and agroforestry is governed largely by seed sources (Larsen, 1954; Lacaze, 1978). Robust gains in the breeding programs canbe achieved through the selection of appropriate species and seed sources within the species (Zobeland Talbert, 1984). Seed source studies in wild pomegranate were therefore of paramount significance. Assessment of genetic variability is the foremost step and prerequisite for all tree improvement programs (Atta *et al*., 2008). Complex characters like fruit quality are influenced by the environment. It's crucial to divide the observed variation into genotypic, phenotypic and environmental components, which can further be used for genetic estimations.

Traditionally, wild pomegranate is used as a medicinal plant because of the presence of bioactive phytochemicals (Khan *et al*., 2017; Kumar *et al.,* 2018). The phenolic compounds of pomegranate and its derivatives such as ellagitannins, gallic acid, ellagic acid, quercetin, punicalagin and punicalin have been shown the pharmacological property (Khan *et al*., 2015; Sharmaand Thakur, 2018). Different parts of wild pomegranate are used to derive value-added products, which include *anardana* squash, jelly, wine, appetizer, sauce, pesticide, tannins, dye, liquid ink and agricultural apparatuses (Poyrazolu *et al*., 2002; Subhash, 2010). The main economic product of wild pomegranate arils is Anardana, which fetches a market price ranging between ₹ 300 to 400 per Kg (Mushtaq and Gangoo, 2017). Thus, the present study can play an important role in pushing up the livelihood of rural farmers, where the cultivation of the crops is arduous due to poor fruit quality. Wild pomegranate has several other salient features unique to its credit which includes its use in breeding for bacterial blight resistance governed by recessive alleles in *daru* (Kumar *et al.,* 2003; Jalikop *et al*., 2005; Jalikop *et al*., 2006; Singh *et al*., 2008; Chandra *et al*., 2013).

There is high scope to promote in the non-traditional area with sub-tropical or sub-temperate climatic conditions (Chandra *et al.*, 2010). Genetic improvement of the wild pomegranate can boost the tropical and sub-tropical pomegranate breeding programs. Hence, eight seed sources each with three genotypes of *Punica granatum* L. were selected and evaluated for fruit attributes, genetic estimates and clustering patterns to develop strategies for further breeding studies.

MATERIALS AND METHODS

The survey has been conducted in Himachal Pradesh and based on morphological characters of wild pomegranate eight seed sources (Fig. 1) were selected. The experiment was conducted under the department of TIGR, Dr. YSP University of Horticulture and Forestry, Nauni, Solan, in 2019.

Fruit character: The fully mature fruit samples from every genotype were collected and then carried to the laboratory for physio-chemical examination *viz.,* fruit size (length and diameter; mm), fruit and rind weight (g), rind weight percentage (%), aril weight (g), aril weight percentage (%), 100 aril and seed weight (g), the thickness of the rind (mm), seeds per fruit, rind aril ratio, aril moisture content (%), total soluble solids (°Brix), ascorbic acid (mg/100g), total sugars (%), reducing sugars (%) and non reducing sugars (%). Chemical fruit parameters were examined according to AOAC (2005) method.
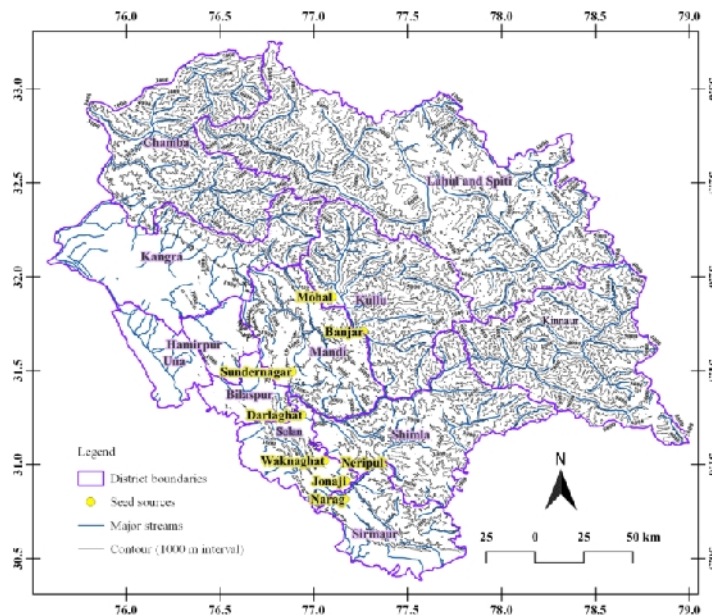

Fig. 1. Map of wild pomegranate seed sources.

B. Statistical analysis

ANOVA: The observed data were analysed using the technique by Panse and Sukhatme (1967).

Genetic estimates: Variability (Burton and De-Vane, 1953; Pillai and Sinha, 1968), heritability (Burton and De-Vane, 1953; Johnson *et al.*, 1955), genetic advance (Lush, 1940; Burton and De-Vane, 1953; Johnson *et al.*, 1955) and genetic advance percentage were computed using formulas given in Table 1.

Table 1: Measures for genetic estimates.

Parameters		Measures
Variability	**Genotypic coefficient of variance (GCV)**	$GCV(\%) = \dfrac{\sqrt{Genotypic\ variance\ (\sigma_g^2)}}{Population\ mean\ of\ character\ (\overline{X})} \times 100$
	Phenotypic coefficient of variance (PCV)	$PCV(\%) = \dfrac{\sqrt{Phenotypic\ variance\ (\sigma_p^2)}}{Population\ mean\ of\ character\ (\overline{X})} \times 100$
	Environmental coefficient of variance (ECV)	$ECV(\%) = \dfrac{\sqrt{Environmental\ variance\ (\sigma_e^2)}}{Population\ mean\ of\ character\ (\overline{X})} \times 100$
Broad Sense Heritability (H_{BS}^2)		$H_{BS}^2 = \dfrac{Genotypic\ variance\ (\sigma_g^2)}{Phenotypic\ variance\ (\sigma_p^2)}$
Genetic advance (GA)		$GA = H_{BS}^2 \times \sigma_p \times K$ *where,* $H_{BS}^2 = $ *Broad Sense Heritability (%)* σ_p = *Phenotypic standard deviation* K = 2.06(*selection differential at 5% selection intensity*)
Genetic advance expressed as percent mean (GAM)		$GAM(\%) = \dfrac{Genetic\ advance\ (GA)}{Population\ mean\ of\ character\ (\overline{X})} \times 100$

Cluster analysis: Genetic difference of all twenty-four genotypes was calculated using Mahalanobis D^2 statistics, further the genotypes were grouped into clusters using Tocher's technique (Rao, 1952; Mahalanobis, 1928).

RESULTS AND DISCUSSION

Fruit analysis: Fruit characters act as classical markers for the assortment of parents in a breeding program (Karimi *et al*., 2009). The analysis revealed significant variation among different seed sources as well as genotypes for eighteen fruit characters, which are described under the following subsections.

Fruit length (mm): The fruit length varied considerably in seed sources and genotypes. Among seed sources, Narag (S_1) performed best in fruit length (56.1mm). However minimum fruit length (39.7mm) was observed in Neripul (S_2). Among different genotypes, S_1G_2 expressed ultimate fruit length (56.7mm) and the lowest fruit length (35.1mm) was obtained in S_2G_1. Larger fruit length is associated with higher fruit quality. The fruit lengths were in line with the study carried out by Singh (2019), who reported wild pomegranate fruit lengths ranging between 38.57-56.36mm. However, Khadivi *et al.* (2020), Negi (2019), Thakur *et al*. (2011) and Pant (1995) reported different results for fruit length, which were ranging between 27.20-63.27mm, 29.71-38.83mm, 46.90-62.80mm and 53.10-75.30mm, respectively.

Fruit diameter (mm): Among eight seed sources, Narag (S_1) exhibited the largest fruit diameter (55.0mm) and the smallest (39.0mm) by Neripul (S_2). Genotypes S_1G_1 revealed maximum fruit diameter (56.7mm) and the lowermost fruit diameter was observed for S_2G_2 (36.2mm). Fruit diameter is another important factor in the determination of good fruit size. Several researchers *viz.*, Khadivi *et al.* (2020) in Iran (30.98-71.40mm), Negi (2019) in Himachal Pradesh (29.09-39.53mm), Singh (2019) in Himachal Pradesh (38.11-53.34mm), Thakur *et al*. (2011) in Himachal Pradesh (44.60-54.20mm) and Pant (1995) in Himachal Pradesh (43.50-65.00mm), had found different ranges for fruit diameter, however, results of the present study were in agreement with the findings of Singh (2019).

Fruit weight (g): Among all seed sources studied, Narag (S_1) was recorded with the heaviest fruit (86.54g) and the lightest fruit (36.82g) by Neripul (S_2). Genotypes S_1G_2 showed the highest fruit weight (91.76g) and S_5G_3 recorded the lowest (31.57g). Our study showed a wider range for fruit weights as compared to other studies *viz.*, Negi, 2019 (22.72-41.69g), Singh, 2019 (40.19-48.56g), Thakur *et al*., 2011 (55.10-83.50g) and Pant, 1995 (59.77-101.00g), in Himachal Pradesh.

Rind weight (g): Narag (S_1) demonstrated the highest rind weight (35.45g) among all seed sources and Neripul (S_2) exhibited the lowest rind weight (17.68g). Out of twenty-four genotypes, S_1G_2 showed maximum rind weight (43.46g), however, S_2G_2 recorded minimum rind weight (14.23g). The mean rind weight (20.73g) observed by Khadivi *et al.,* (2020) was comparable with the present observations.

Rind weight percentage (%): Sundernagar (S_8) was witnessed with maximum rind weight percentage (55.18%) and minimum (39.23%) by Banjar (S_6). Genotype S_8G_1 was recorded for the highest rind weight percentage (60.08%) and genotype S_6G_3 (35.2%) was the lowest. Similarly, rind weights were one-fourth to half of the total fruit weight in the recent studies on wild pomegranate in Himachal Pradesh (Negi, 2019 and Singh, 2019).

Aril weight (g): Narag (S_1) accounted for the heaviest arils (51.09g) and Neripul (S_2) for the lightest arils (19.14g), statistical at par with Jonaji (S_4; 24.9g). Among genotypes, S_1G_1 (53.00g), showed the highest aril weight. Genotype S_5G_3 (14.66g) showed the lowest aril weight observed for a genotype. Sharma and Thakur, 2016 (32.73g), Thakur *et al*., 2011 (33.00-48.70g) and Thakur *et al.,* 2010 (33.2g) reported similar results.

Aril weight percentage (%): Seed source Banjar (S_6) showed a maximum aril weight percentage (60.77%). Contrariwise, Sundernagar (S_8) demonstrated a minimum aril weight percentage (44.82%). Among all genotypes studied S_6G_3 recorded for highest aril weight percentage (64.8%) and S_8G_1 demonstrated the lowest (39.92%). Similarly, Khadivi *et al*. (2020) reported 40.85% to 78.82% rind in the wild pomegranate of Northern Iran.

100 aril weight (g): Sundernagar (S_8) showed a maximum 100 aril weight (20.82g), however Jonaji (S_4) was recorded with a minimum (13.36g). Genotype S_8G_3 (22.11g) showed the highest 100 aril weight, on the opposite side, the lowest was recorded for S_4G_3 (12.11g). Our findings were in agreement with the results of Negi (2019), who observed 9.16 to 21.36g 100 aril weight in different seed sources of Himachal Pradesh.

100 seed weight (g): Sundernagar (S_8) and Neripul (S_2) demonstrated the highest (5.35g) and lowest (3.07g) 100 seed weight, respectively. Among genotypes, S_8G_3 and S_2G_3 demonstrated the highest (6.44g) and lowest (2.88g) 100 seed weight, respectively. Similarly, Negi (2019) reported 3.00 to 3.53g 100 seed weight.

Rind thickness (mm): Among seed sources, Banjar (S_6) showed the thickest fruit rind (3.57mm), on the other side, Sundernagar (S_8) showed the thinnest rind (2.64mm). Among all genotypes studied, the fruit rind of genotype S_2G_3 was the thickest (4.63mm), however, genotype S_1G_3 was having the thinnest fruit rind (1.93mm). Singh (2019) reported a higher range (4.33 to 6.50mm) and Parashuram *et al*. (2018) observed a lower range (1.83-3.69mm) for rind thickness as compared to the present study.

Seeds per fruit: Seed source Narag (S_1) and Neripul (S_2), accounted for the largest (294.82) and smallest (137.06) number of seeds per fruit, respectively. Among genotypes, S_1G_1 showed the maximum number of seeds per fruit (308.18), however, S_8G_1 showed the minimum number of seeds per fruit (95.4). Comparably, Thakur *et al.* (2011) observed a higher (339.00) number of seeds per fruit for the wild pomegranate collected from Narag.

Table 2: Physio-chemical fruit characters of wild pomegranate.

Seed sources	Genotypes	FL (mm)	FD (mm)	FW (g)	RW (g)	RWP (%)	AW (g)	AWP (%)	100AW (g)	100SW (g)	RT (mm)	SPF	RAR	AMC (%)	TSS (°B)	AA (mg/100g)	TS (%)	RS (%)	NRS (%)
Narag (S₁)	S₁G₁	55.3	56.7	84.61	31.61	37.35	53.00	62.65	17.31	3.61	2.55	308.18	0.60	79.01 (2.89)	15.72	18.57	9.15	7.15	2.00
	S₁G₂	56.7	54.1	91.76	43.46	47.32	48.30	52.68	17.18	4.50	4.33	290.82	0.90	73.04 (2.2)	13.50	20.79	8.36	6.89	1.46
	S₁G₃	56.4	54.3	83.25	31.29	37.88	51.96	62.12	18.57	3.61	1.93	285.47	0.61	80.1 (3.08)	15.22	20.29	8.04	5.85	2.19
	Mean	**56.1**	**55.0**	**86.54**	**35.45**	**40.85**	**51.09**	**59.15**	**17.69**	**3.91**	**2.94**	**294.82**	**0.70**	77.38 (2.73)	**14.82**	**19.88**	**8.51**	**6.63**	**1.88**
Neripul (S₂)	S₂G₁	35.1	37.6	31.63	14.97	46.87	16.66	53.13	16.26	3.22	2.97	104.25	0.90	79.3 (2.96)	10.47	16.32	7.04	5.88	1.16
	S₂G₂	39.8	36.2	33.81	14.23	42.33	19.58	57.67	14.79	3.12	2.83	135.24	0.75	78.67 (2.87)	12.37	20.68	8.37	7.38	0.99
	S₂G₃	44.2	43.2	45.02	23.84	53.08	21.19	46.92	12.30	2.88	4.63	171.7	1.14	76.64 (2.6)	11.54	19.37	7.90	6.70	1.20
	Mean	**39.7**	**39.0**	**36.82**	**17.68**	**47.43**	**19.14**	**52.57**	**14.45**	**3.07**	**3.48**	**137.06**	**0.93**	78.2 (2.81)	**11.46**	**18.79**	**7.77**	**6.65**	**1.12**
Wakna-ghat (S₃)	S₃G₁	42.6	46.4	55.11	22.03	40.51	33.07	59.49	15.50	4.10	2.99	214.82	0.69	72.82 (2.17)	12.44	20.57	7.58	6.55	1.03
	S₃G₂	45.1	46.9	60.39	31.93	51.93	28.46	48.07	15.00	3.31	3.65	196.62	1.12	76.28 (2.67)	10.53	19.71	7.55	5.83	1.72
	S₃G₃	42.3	45.5	55.16	23.38	42.32	31.78	57.68	14.95	4.13	2.44	216.88	0.74	72.04 (2.06)	15.59	19.50	9.59	8.20	1.39
	Mean	**43.3**	**46.3**	**56.89**	**25.78**	**44.92**	**31.10**	**55.08**	**15.15**	**3.85**	**3.02**	**209.44**	**0.85**	73.71 (2.3)	**12.85**	**19.93**	**8.24**	**6.86**	**1.38**
Jonaji (S₄)	S₄G₁	42.3	44.7	53.33	26.14	48.65	27.19	51.35	15.26	3.51	2.76	183.42	1.00	75.55 (2.58)	12.32	17.38	6.18	5.15	1.03
	S₄G₂	40.5	39.6	38.58	19.52	50.98	19.06	49.02	12.70	4.26	3.03	153.02	1.08	65.99 (1.46)	17.55	19.52	6.88	6.01	0.87
	S₄G₃	43.3	44.5	53.35	24.91	46.40	28.44	53.6	12.11	3.96	3.37	250.37	0.87	65.6 (1.45)	17.40	18.26	6.39	5.88	0.51
	Mean	**42.0**	**42.9**	**48.42**	**23.52**	**48.68**	**24.90**	**51.32**	**13.36**	**3.91**	**3.05**	**195.61**	**0.98**	69.05 (1.83)	**15.76**	**18.39**	**6.49**	**5.68**	**0.80**
Darla-ghat (S₅)	S₅G₁	46.4	49.5	74.88	34.46	45.42	40.43	54.58	20.55	4.30	3.52	196.98	0.84	78.75 (2.85)	18.7	18.51	10.31	7.49	2.82
	S₅G₂	40.7	44.0	50.33	24.21	47.68	26.13	52.32	14.39	4.32	3.18	181.68	0.92	69.62 (1.82)	18.41	18.4	10.20	8.00	2.20
	S₅G₃	36.4	37.8	31.57	16.91	53.99	14.66	46.01	12.59	3.47	2.76	116.69	1.19	72.03 (2.07)	16.47	19.36	8.09	6.94	1.15
	Mean	**41.2**	**43.7**	**52.26**	**25.19**	**49.03**	**27.07**	**50.97**	**15.84**	**4.03**	**3.15**	**165.12**	**0.98**	73.47 (2.25)	**17.86**	**18.76**	**9.53**	**7.48**	**2.06**
Banjar (S₆)	S₆G₁	43.9	46.3	61.05	23.1	37.54	37.95	62.46	16.14	4.74	3.02	232.17	0.61	71.07 (2.02)	14.47	20.55	7.73	6.66	1.07
	S₆G₂	40.4	41.8	53.38	24.32	44.96	29.06	55.04	18.24	4.07	3.71	161.8	0.84	78.24 (3.01)	14.41	22.4	7.77	6.87	0.90
	S₆G₃	37.8	41.0	41.33	14.58	35.2	26.75	64.8	15.61	3.23	3.98	171.93	0.55	79.09 (2.91)	15.49	20.3	7.36	6.53	0.83
	Mean	**40.7**	**43.0**	**51.92**	**20.67**	**39.23**	**31.25**	**60.77**	**16.67**	**4.02**	**3.57**	**188.63**	**0.66**	76.14 (2.65)	**14.79**	**21.08**	**7.62**	**6.69**	**0.93**
Mohal (S₇)	S₇G₁	41.7	43.7	45.04	22.88	51.3	22.16	48.7	12.38	3.68	2.68	182.85	1.06	70.26 (1.87)	10.43	17.68	6.65	6.36	0.30
	S₇G₂	49.5	49.6	66.88	24.9	37.07	41.97	62.93	17.88	4.79	3.45	235.65	0.6	73.11 (2.18)	12.52	17.26	8.41	6.94	1.47
	S₇G₃	48.8	45.9	54.11	24.84	46.19	29.27	53.81	15.49	5.13	2.88	188.07	0.89	66.37 (1.6)	11.48	16.34	6.5	5.59	0.91
	Mean	**46.7**	**46.4**	**55.34**	**24.21**	**44.85**	**31.13**	**55.15**	**15.25**	**4.53**	**3.00**	**202.19**	**0.85**	69.91 (1.89)	**11.48**	**17.09**	**7.19**	**6.29**	**0.89**
Sunder-nagar (S₈)	S₈G₁	41.0	41.9	44.9	26.94	60.08	17.97	39.92	19.06	4.52	2.62	95.40	1.52	75.24 (2.52)	15.59	22.24	8.72	7.79	0.93
	S₈G₂	53.1	53.7	88.02	43.35	49.82	44.67	50.18	21.30	5.11	3.03	219.07	1.04	75.75 (2.5)	13.57	16.41	7.72	6.63	1.09
	S₈G₃	49.3	49.2	62.7	34.96	55.65	27.74	44.35	22.11	6.44	2.26	138.72	1.31	65.71 (1.53)	11.4	18.41	7.28	5.95	1.33
	Mean	**47.8**	**48.3**	**65.21**	**35.08**	**55.18**	**30.12**	**44.82**	**20.82**	**5.35**	**2.64**	**151.07**	**1.29**	72.24 (2.18)	**13.52**	**19.02**	**7.91**	**6.79**	**1.12**
Treatments										CD₀.₀₅									
Seed sources		3.62	3.15	9.96	5.05	3.83	5.88	3.83	2.40	0.65	0.47	40.68	0.16	0.12	0.21	0.27	0.25	0.30	0.35
Genotypes		6.27	5.46	17.26	8.75	6.63	10.18	6.63	4.15	1.13	0.81	70.46	0.27	0.21	0.36	0.46	0.43	0.53	0.61

*Figures in the parentheses are logit transformed values

Abbreviations	Full forms	Abbreviations	Full forms	Abbreviations	Full forms	Abbreviations	Full forms
FL (mm)=	Fruit length	**RWP (%)=**	Rind weight percentage	**100 SW (g)=**	100 seed weight	**TSS (°B)=**	Total soluble solids
FD (mm)=	Fruit diameter	**AW (g)=**	Aril weight	**RT (mm)=**	Rind thickness	**AA (mg/100gm)=**	Ascorbic acid
FW (g)=	Fruit weight	**AWP (%)=**	Aril weight percentage	**SPF=**	Seeds per fruit	**TS (%)=**	Total sugars
RW (g)=	Rind weight	**100 AW (g)=**	100 aril weight	**RAR=**	Rind aril ratio	**RS (%)=**	Reducing sugars
				AMC (%)=	Aril moisture content	**NRS (%)=**	Non-reducing sugars

Table 3: Genetic estimates for physio-chemical fruit characters of wild pomegranate.

Fruit characters	ECV (%)	GCV (%)	PCV (%)	H^2_{BS}	GA	GAM (%)
Fruit length (mm)	11.17	12.69	16.91	0.56	8.77	19.63
Fruit diameter (mm)	9.54	11.31	14.79	0.58	8.11	17.80
Fruit weight (g)	24.25	28.91	37.73	0.59	25.85	45.62
Rind weight (g)	26.85	27.84	38.68	0.52	10.71	41.28
Rind weight percentage (%)	11.42	13.20	17.45	0.57	9.51	20.56
Aril weight (g)	26.40	34.23	43.23	0.63	17.16	55.83
Aril weight percentage (%)	9.83	11.37	15.03	0.57	9.51	17.71
100 aril weight (g)	20.47	14.98	25.37	0.35	2.95	18.23
100 seed weight (g)	22.04	17.06	27.88	0.37	0.88	21.51
Rind thickness (mm)	20.74	18.27	27.64	0.44	0.77	24.88
Seeds per fruit	29.08	26.16	39.11	0.45	69.55	36.04
Rind aril ratio	24.13	25.04	34.77	0.52	0.34	37.13
Aril moisture content (%)	10.19	4.47	11.13	0.16	2.73	3.71
Total soluble solids (°B)	2.03	18.18	18.29	0.99	5.24	37.22
Ascorbic acid (mg/100g)	1.47	8.91	9.03	0.97	3.46	18.10
Total sugars (%)	3.34	13.92	14.31	0.95	2.20	27.88
Reducing sugars (%)	4.85	11.49	12.47	0.85	1.45	21.80
Non-reducing sugars (%)	29.01	41.56	50.68	0.67	0.89	70.21

Rind aril ratio: Among seed sources, it ranged from 1.29 for Sundernagar (S_8) to 0.66 for Banjar (S_6). For genotypes, it was maximum for S_8G_1 (1.52), statistically similar to S_8G_3 (1.31) and minimum for S_6G_3 (0.55). Rind aril ratio lesser than one indicated more aril weight as compared to ring weight and vice versa.

Aril moisture content (%): Seed source Neripul (S_2) was observed with the highest (78.2%) aril moisture content, on the contrary, Jonaji (S_4) showed the lowest aril moisture content (69.05%). Genotype S_1G_3 demonstrated the highest aril moisture content (80.1%). Contrarily, S_4G_3 showed the lowest aril moisture content (65.6%). Studies on wild pomegranate in Himachal Pradesh had reported similar aril moisture content (%) viz., Thakur et al., 2011 (73.2%), Thakur et al., 2010 (72.3%) and Sharma and Thakur, 2016 (71.1%).

Total soluble solids (°B): Darlaghat (S_5) performed best in terms of TSS (17.86°B), however, Neripul (S_2) was recorded with the lowest TSS (11.46°B). Among genotypes, the performance of S_5G_1 was great (18.7°B). Genotype S_7G_1 (10.43°B) exhibited the lowest TSS (10.43°B). Total soluble solids (°B) estimated by several researchers for fruits collected from different locations in Himachal Pradesh were comparable viz., Negi, 2019 (9.61-15.01°B), Singh, 2019 (13.40-17.7°B) and Pant, 1995 (10.60-14.00°B).

Ascorbic acid (mg/100g): Among seed sources, its value varied from 21.08mg/100g for Banjar (S_6) to 17.09mg/100g for Mohal (S_7). Among genotypes, S_6G_2 showed the highest ascorbic acid (22.4mg/100g). The lowest was observed for S_2G_1 (16.32mg/100g). In Himachal Pradesh, ascorbic acid (mg/100g) of wild pomegranate fruits had been reported to range between 16.83 to 21.14 mg/100g (Singh, 2019; Thakur et al., 2011), which were similar to present results.

Total sugars (%): Darlaghat (S_5) seed source achieved maximum value total sugars (9.53%) and the minimum by (6.49%) Jonaji (S_4). Among genotypes, S_5G_1 was examined with the highest total sugars (10.31%).

Moreover, genotype S_4G_1 showed the lowest total sugars (6.18%). Similarly, the mean total sugars (%) of wild pomegranate for different sites in Himachal Pradesh was found to be varying between 6.82% to 13.23%, as per several studies (Negi, 2019; Singh, 2019; Thakur et al., 2011; Pant, 1995).

Reducing sugars (%): In seed sources, it demonstrated the highest (7.48%) and lowest (5.68%) values for Darlaghat (S_5) and Jonaji (S_4), respectively. Genotype S_3G_3 out performed in terms of reducing sugars (8.20%), on the other side, genotype S_4G_1 (5.15%) revealed the lowest reducing sugars. The results were in line with earlier studies, which reported 4.47% to 9.21% reducing sugars in wild pomegranate from Himachal Pradesh (Singh, 2019; Sharma and Thakur, 2016; Thakur et al., 2010; Pant, 1995).

Non-reducing sugars (%): Among seed sources, Darlaghat (S_5) excelled (2.06%) and on the contrary, Jonaji (S_4) demonstrated the lowest non-reducing sugars (0.80%). In genotypes, the value fluctuated from 2.82% (S_5G_1) to S_7G_1 (0.30%). Likewise, Negi (2019) and Singh (2019) reported non-reducing sugars (%) ranging between 1.51% to 5.68% for different locations in Himachal Pradesh.

Genetic estimates: Maximum environmental (36.7%), genotypic (41.56%) and phenotypic (50.68%) coefficient of variance were observed for aril moisture content, non-reducing sugar and non-reducing sugar, respectively. High heritability was observed for aril weight (0.63), total soluble solids (0.99), ascorbic acid (0.97), total sugar (0.95), reducing sugar (0.85) and non-reducing sugar (0.67). Moderate heritability was recorded for fruit length (0.56), fruit diameter (0.58), fruit weight (0.59), rind weight (0.52), rind weight percentage (0.57), aril weight percentage (0.57), 100 aril weight (0.35), 100 seed weight (0.37), rind thickness (0.44), seeds per fruit (0.45) and rind aril ratio (0.52). Low heritability was demonstrated only by aril moisture content (0.16). Genetic gain as a percentage of mean was estimated to be highest for non-reducing sugar (70.21%) followed by aril weight (55.83%), fruit

weight (45.62%), rind weight (41.28%) and total soluble solids (37.22%). Effects of selection were best predicted when heritability with genetic advance is used (Patil *et al.*, 1996; Ramanjinappa *et al.*, 2011). Genetic advance alone was also of huge significance since it predicted the probable genetic gain from one cycle of selection (Hamdi *et al.*, 2003).

Cluster analysis: Perusal of dendrogram (Fig. 2.) revealed four clusters of the genotypes. Custer I, cluster II, cluster III and cluster IV was comprised of six genotypes (S_2G_3, S_3G_2, S_4G_1, S_4G_3, S_7G_1 and S_7G_3), seven genotypes (S_2G_1, S_2G_2, S_4G_2, S_5G_3, S_6G_2, S_6G_3 and S_8G_1), three genotypes (S_1G_2, S_8G_2 and S_8G_3) and eight genotypes (S_1G_1, S_1G_3, S_3G_1, S_3G_3, S_5G_1, S_5G_2, S_6G_1 and S_7G_2), respectively. However, genotypes in cluster I, cluster II, cluster III and cluster IV belonged to four (S_2, S_3, S_4, and S_7), five (S_2, S_4, S_5, S_6 and S_8), two (S_1 and S_8) and five (S_1, S_3, S_5, S_6 and S_7) seed sources, respectively. The observed pattern suggests that the genetic divergence of genotypes was independent of geographical distribution. Cluster III performed best for nine fruit characters (Table 4) *viz.,* fruit length (53.04mm), fruit diameter (52.34mm), fruit weight (80.83g), rind weight (40.59g), rind weight percentage (50.93%), aril weight (40.23g), 100 aril weight (20.20g), 100 seed weight (5.35g) and rind aril ratio (1.08). However, cluster IV demonstrated the highest cluster means for six fruit characters (Table 4) *viz.,* aril weight percentage (49.07%), seeds per fruit (216.20), total soluble solids (12.83°B), total sugar (7.79%), reducing sugar (6.49%) and non-reducing sugar (1.29%). Inter-cluster hybridization between genotypes can result in heterosis for the desirable fruit character.
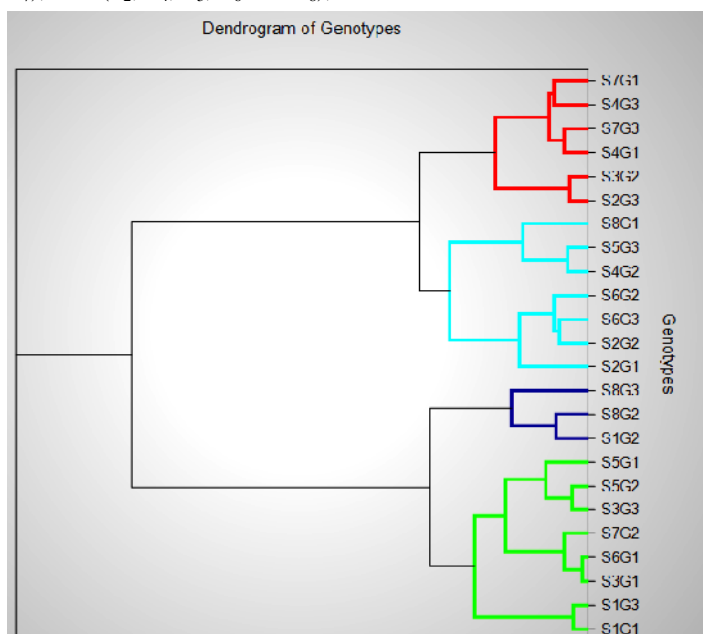

Fig. 2. Dendrogram of wild pomegranate genotypes.

Table 4: Cluster means of physio-chemical fruit characters of wild pomegranate.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Number of genotypes	6	7	3	8
Fruit length (mm)	44.24	38.71	53.04	47.13
Fruit diameter (mm)	44.81	39.41	52.34	49.03
Fruit weight (g)	51.88	39.32	80.83	66.41
Rind weight (g)	25.76	18.78	40.59	26.87
Rind weight percentage (%)	49.59	47.77	50.93	40.72
Aril weight (g)	26.12	20.53	40.23	39.54
Aril weight percentage (%)	50.41	52.23	49.07	59.28
100 aril weight (g)	13.76	15.61	20.20	16.91
100 seed weight (g)	3.75	3.70	5.35	4.20
Rind thickness (mm)	3.33	3.13	3.21	2.88
Seeds per fruit	195.51	134.05	216.20	233.98
Rind aril ratio	1.01	0.97	1.08	0.70
Aril moisture content (%)	71.78	75.51	71.50	74.57
Total soluble solids (°B)	12.28	14.62	12.83	15.38
Ascorbic acid (mg/100g)	18.12	20.12	18.54	19.21
Total sugars (%)	6.86	7.75	7.79	8.87
Reducing sugars (%)	5.92	6.77	6.49	7.11
Non-reducing sugars (%)	0.95	0.98	1.29	1.77

CONCLUSIONS

The present investigation was carried out in Himachal Pradesh with a target to determine within and between seed source variations, genetic estimates for fruit characters and clustering pattern of the wild pomegranate genotypes. Although there were significant variations among the different seed sources, great variability was also found within the seed sources. For most of the physical fruit characters Narag as well as the genotypes within the seed source performed best, however, Darlaghat seed source performed better in chemical parameters. High heritability was observed for aril weight, total soluble solids, ascorbic acid, total sugar, reducing sugar and non-reducing sugar. Genetic gain as a percentage of mean was estimated to be highest for non-reducing sugar followed by aril weight, fruit weight and rind weight. Clustering analysis revealed four clusters of genotypes *viz.*, Custer I, cluster II, cluster III and cluster IV, which were comprised of six, seven, three and eight genotypes, respectively. However, genotypes in each cluster belonged to different seed sources. The observed pattern suggests that the genetic divergence of genotypes was independent of geographical distribution. Cluster III performed best for nine physical fruit characters, however, cluster IV demonstrated the highest cluster means for two physical and four chemical fruit characters. Inter-cluster hybridization between genotypes can result in heterosis for the desirable fruit character.

Conflict of Interest. There was no conflict of interest between the authors.

REFERENCES

A.O.A.C. (2005). *Official methods of analysis*. 18th edn. Association of Official Analytical Chemists, Washington, USA. 1018p.

Anonymous. (2017). *Horticultural statistics at a glance*. Horticulture Statistics Division, Department of Agriculture, Cooperation & Farmers Welfare Ministry of Agriculture & Farmers Welfare, India. 514p.

Atta, B. M., Haq, M. A., and Shah, T. M. (2008). Variation and inter relationships of quantitative traits in chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany*, *40*: 637-647.

Burton, G.W. and De-vane, E. W. (1953). Estimating heritability in tall Fescue (*Festucaar uandinacea*) from replicated clonal material. *Agronomy Journal, 1*: 78-81.

Chandra, R., Jadhav, V. T., and Sharma, J. (2010). Global scenario of pomegranate (*Punica granatum* L.) culture with special reference to India. *Fruit, Vegetable and Cereal Science and Biotechnology*, *4*: 7-18.

Chandra, R., Lohakare, A. S., Babu, K. D., Maity, A., Singh, N. V. and Jadhav V. T. (2013). Variability studies of physico-chemical properties of pomegranate (*Punica granatum* L.) using a scoring technique. *Fruits, 68*: 135-146.

Hamdi, A., El-Ghareib, A. A., Shafey, S. A. and Ibrahim, M. A. M. (2003). Genetic variability, heritability and expected genetic advance for earliness and seed yield from selection in lentil. *The Egyptian Journal of Agricultural Research*, *81*: 125-137.

Jalikop, S. H., Kumar, P. S., Rawal, R. D., and Kumar, R. (2006). Breeding pomegranate for fruit attributes and resistance to bacterial blight. *Indian Journal of Horticulture*, *63*: 352-358.

Jalikop, S. H., Rawal, R. D. and Kumar, R. (2005). Exploitation of sub-temperate pomegranate Daru in breeding tropical varieties. *Acta Horticulture, 696*: 107-112.

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. *Agronomy journal*, *47*(7), 314-318.

Karimi, H. R., Zamani, Z., Ebadi, A. and Fatahi, M.R. (2009). Morphological diversity of *Pistacia* species in Iran. *Genetic Resources and Crop Evolution*, *56*: 561–571.

Khadivi, A., Mirheidari, F., Moradi, Y., Paryan, S. (2020). Morphological variability of wild pomegranate (*Punica granatum* L.) accessions from natural habitats in the Northern parts of Iran. *Scientia Horticulturae, 264*: 109-165.

Khan, M. P. Z., Ahmad, M., Zafar, M., Sultana, S., Ali, M. I., and Sun, H. (2015). Ethnomedicinal uses of edible wild fruits (EWFs) in Swat Valley, Northern Pakistan. *Journal of Ethnopharmacology, 173*: 191-203.

Khan, S., Khan, N., Khan, W., Rahman, I. U., Ullah, F. and Nisar, M. (2017). Ethnobotanical and ecological study of *Punica granatum* in Dir district, Khyber Pakhtunkhwa, Pakistan. *Regulatory Mechanisms in Biosystems*, *4*: 656-661.

Kumar, G., & Duggal, S. (2019). Ethnomedicinal Diversity of Aromatic Plants in Foot Hill Regions of Himachal Pradesh, India. *International Journal of Theoretical & Applied Sciences*, *11*(1), 18-39.

Kumar, N., Kumar, N. and Kumar, S. (2018). Functional Properties of Pomegranate (*Punica granatum* L.). *Journal of Pharmaceutical Innovation*, *7*: 71-81.

Kumar, R., Jalikop, S. H. and Rawal R. D. (2003). Exploitation of Sub-temperate Pomegranate Daru in Breeding Tropical Varieties. In: *VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics,* pp. 107-112.

Lacaze, J. F. (1978). Advances in species and provenance selection. *Unasylva, 30:* 17-20.

Larsen, C. S. (1954). *Provenance testing and forest tree breeding proceedings*. 11th Congress, IUFRO, Rome. pp. 467-473.

Lush, J. C. (1940). Intersire correlation and regression of offspring on damsana method of estimating heritability character. Proc. American Society on Animal Production*, USA. 33*: 293-301.

Mahalanobis, P. C. (1928). *On the generali1zed distance in statistics.* National Institute of Science, India, pp. 49-55.

Mohamed, M. N., Parthiban, K. T., Ravi, R., and Kumar, P. (2015). Provenance variation in growth and genetic potential of *Aquilaria malaccensis* under nursery condition. *African Journal of Biotechnology*, *14*: 2005-2013.

Morton, J. (1987). Pomegranate. In: *Fruits of warm climates*. Florida Fair Books, Miami, pp. 352-355.

Mushtaq, S. M. and Gangoo, S. A. (2017). Anardana (dehydrated wild pomegranate arils) as livelihood option for rural communities in Chenab valley of

Jammu and Kashmir. *Indian Journal of Hortic*ulture, *74*: 306-309.

Negi, A. (2019). Seed source studies in wild pomegranate (*Punica granatum* L.) in Himachal Pradesh. M.Sc. thesis, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India. 68p.

Panse, V. G. and Sukhatme, P. V. (1967). *Statistical Methods for Agricultural Workers*, ICAR, New Delhi. 695p.

Pant, K. S. (1995). Variation studies and provenance evaluation of wild pomegranate (*Punica granatum L.*). Ph.D. thesis, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India. 148p.

Parashuram, S., Singh, N. V., Roopasowjanya, P., Babu, K. D., Sangnure, V., Singh, S., Sharma, J. and Pal R. K. (2018). Germplasm conservation and phenotypic characterization of pomegranate (*Punica granatum* L.) germplasm accessions for various morphological and physico-chemical characters. *Journal of Pharmacognosy and Phytochemistry, 2*: 114-118.

Parmar, C. and Kaushal, M. K. (1982). *Punica granatum*. In: *Wild Fruits*. Kalyani Publishers, New Delhi, India, pp. 74-77.

Patil, Y. B., Madalageri, B. B., Biradar, B. D. and Hoshmani R. M. (1996). Variability studies in okra (*Abelmoschus esculentus* L. Moench.). *Karnataka Journal of Agricultural Science, 9:* 289-293.

Pillai, S.K. and Sinha, H.C. (1968). *Statistical Methods for Biological Workers*, ICAR, New Delhi. 502p.

Poyrazolu, E., Gokmen, V., and Artik, N. (2002). Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. *Journal of Food Composition and Analysis,15*: 567-575.

Ramanjinappa, V., Arunkumar, K. H., Hugar, A. and Shashibhaskar, M. S. (2011). Genetic variability in okra (*Abelmoschus esculentus* L. Moench.). *Plant Archives*, *11*: 435-437.

Rao, C. R. (1952). *Advanced statistical methods in biometrical research,* John Wiley and Sons, New York. pp. 357-363.

Sharma, A. and Thakur N. S. (2016). Influence of active packaging on quality attributes of dried wild pomegranate (*Punica granatum* L.) arils during storage. *Journal of Natural and Applied Sciences*, *8*: 398-404.

Sharma, A. and Thakur N. S. (2018). Wild pomegranate (*Punica granatum* L.): A review on physical and chemical attributes of Himalayan wild pomegranate fruit. *Journal of Pharmacognosy and Phytochemistry, 7*: 1518-1524.

Singh, D. B. and Kingsly, A. R. P. (2008). Effect of drying on quality of anardana. *Indian Journal of Hortic*ulture, *65*: 413-416.1

Singh, T. J. (2019). Progeny analysis in wild pomegranate (*Punica granatum* L.). Ph.D. thesis, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India. 136p.

Subhash, D. G. (2016). Value Added Products from Wild Pomegranate (*Punica granatum* L.). Ph.D. thesis, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP) India. 167p.

Thakur, N. S., Bhat, M. M., Rana, N. and Joshi, V. K. (2010). Standardization of pre-treatments for the preparation of dried arils from wild pomegranate. *Journal of Food Science and Technology 47*: 620-625.

Thakur, N. S., Dhaygude, G. S. and Gupta, A. (2011). Physiochemical characters of wild pomegranate fruits in different Locations of HP. *International Journal of Farm Sciences, 1*: 37-44.

Zobel, B. and Talbert, J. (1984). *Applied Forest Tree Improvement*, John Wiley and Sons, New York. 505p.