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MORPHOLOGICAL CHARACTERIZATION OF APPLE ACCESSIONS IN KASHMIR REGION

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Abstract

Variability is the process by which differences occur among individuals of the same plant species. Some characteristics change very little, while others, especially quantitative ones, change rather quickly and readily, even when environmental conditions change slightly. This fact has prompted a study on this diversity. In the present study diversity of apple accessions was studied in Kashmir Valley. Thirty three apple accessions were collected from different locations in Kashmir Valley and their various morphological and pomological characters were studied. The genotypes studied showed variation with respect to tree, leaf and fruit characters. The results showed a significant range in leaf blade length (6.26-12.06 cm), leaf blade width (2.73-7.23 cm), petiole length (1.53-4.30 cm), fruit length(1.14-5.21cm), fruit diameter (1.17-6.42 cm), length of fruit stalk (0.60-5.20 cm), fruit weight (1.06-81.34g), number of seeds (2.12-10.00) per fruit. Regarding chemical analysis of fruit, the TSS varied from6.70-16.30° Brix and acidity varied from 0.06-0.79%. It is thus concluded that wide variation existed in the studied genotypes which can be utilized in future improvement program.

Key words : Apple, variability, size, mass, quality.

Introduction

Apple is the most ubiquitous and well-adapted species of temperate fruit crops. It was known to the Greeks and Romans and mentioned by Theophrastus in the third century B.C. Since then the apple has been distributed into almost all parts of the world. It is grown in high latitude regions of the world where temperatures may reach -40°C at high elevations (Janick, 1974). The apple belongs to the Rosaeceae or rose family and has been classified into the subfamily Pomoideae and genus *Malus*. Malus species are widely distributed throughout North America, Europe, Asia Minor, and Asia and serve as potential genetic resources for the development of new apple cultivars and/or rootstocks adapted to diverse environmental conditions (Hokanson *et al*., 2001). The genus *Malus* consists of about 27 species. Among wild Malus species, *Malus sieversii* Lebed., native to Central Asia, has been recognized as a major progenitor of the domesticated apple *Malus* × *domestica* Borkh (Forsline *et al*., 2003). Himalayas provide suitable ecological niches for the prevalence of large number of temperate fruit germplasm (Sharma and Pramanick, 2012). The indigenous crab apples distributed throughout Himalayan region constitutes different Malus species. The genus *Malus* is characterized by a large diversity resulted from the accumulation of somatic mutations and fostered by the human activities during the long history of the cultivation, artificial crossing and transportation to distant habitats (Muzher *et al*., 2007). Genetic diversity in crop species can be determined using morphological and agronomic characteristics, as well as biochemical and DNA marker analysis (Liu, 1997). Indian Himalayan region is very rich in temperate fruit genetic diversity and stretches from Jammu and Kashmir to the North-eastern hills and harbour vast genetic diversity of temperate fruits like apple, pear, peach, apricot, walnut, almond and other fruits and their wild relatives. Kashmir being a hot spot for apple diversity, the survey was conducted to explore the diversity ofthese apple genotypes. The rich core set developed will be a valuable resource for future genetic studies and crop improvement strategies.

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Materials and Methods

Thirty three apple accessions from Kashmir valley were analyzed in this study using apple descriptor UPOV, (2005). Individual trees of apple genotypes from different locations of Kashmir valley were selected and 30 characters were studied, which comprised of both qualitative and quantitative traits. All the accessions were examined for tree characters *viz*., tree vigour, tree growth habit, type of bearing, one year old shoot pubescence (on distal half of shoot) and number of lenticels present ; leaf characters *viz*., leaf blade length/width ratio, leaf blade: intensity of green colour, pubescence on lower side, incisions of margin (upper half), petiole: extent of anthocyanin colouration from base and fruit characters *viz*., fruit shape, bloom of fruit skin, ground colour, relative area of over colour, colour of flesh, russet amount, firmness, eating quality. Other characters *viz*., leaf blade length and width, petiole length, fruit length, fruit diameter, length of stalk were measured by using Vernier Calliper; flowering characters *viz*., initial bloom and final bloom were studied. The initial bloom was observed when 10% of flowers were open and the date was recorded. The initial bloom was observed when 80% of flowers were open and the date was recorded. Time for harvest (days) was calculated out of time lag between the date of full bloom and the date of actual harvest and the genotypes were classified as early (100-120 days), mid (120-150 days) and late (> 150 days). TSS was calculated by using refractometer and titratable acidity (TA) was determined by neutralization to pH 7.0 with 0.1 N NaOH.

Results and Discussion

The results of tree characters are given in table 1. Tree vigour as per UPOV apple descriptor was classified as weak, medium and strong with weak observed in 27.27%, medium in 27.27% and strong in 45.45%, tree habit as upright in 36.36% and spreading in 63.63% accessions; the bearing habit as on spurs and long shoots in 75.75%, on spurs only in 18.18% genotypes and on long shoots only in 6.06% genotypes. One year old pubescence on distal half of shoot was observed medium in 48.48%, strong in 36.36% accessions and very strong in 15.15% accessions while few numbers of lenticels on one year old shoot were observed in 42.42%, medium in 30.30% and many in 27.27% accessions. The results of this study were in close confirmation with (Mratinic and Aksic, 2012), who reported that the selections of wild apple showed variability in their tree behaviour and tree size. Wohner *et al*. (2014) also described the morphology of *Malus* × *robusta* as a small to medium-sized tree with a weak to strong vigor and upright to spreading crown and medium to absent pubescence on the upper side of the shoot surface.

Table 1 : Summary of frequency of tree characters of wild apple.

Tree character	Category	Number of selections	Percentage
Tree vigour	Weak	9	27.27
	Medium	9	27.27
	Strong	15	45.45
Tree habit	Upright	12	36.36
	Spreading	21	63.63
	Drooping	0	0
	Weeping	0	0
Type of bearing	On spurs and long shoots	25	75.75
	On spurs only	6	18.18
	On long shoots only	2	6.06
One year old shoot pubescence (on distal half of shoot)	Absent/very weak	0	0
	Weak	0	0
	Medium	16	48.48
	Strong	12	36.36
	Very strong	5	15.15
One year old shoot : Number of lenticels	Few	14	42.42
	Medium	10	30.30
	Many	9	27.27

The results of leaf characters are given in tables 2 & 5. The maximum leaf blade length of 12.06 cm was recorded in Sel-29 while as minimum leaf blade length of 6.26 cm was recorded in Sel-9; leaf blade width was recorded maximum (7.23 cm) in Sel-29 and minimum (2.73cm) in Sel-4. Leaf blade length/width ratio was observed Small in 36.36%, medium in 42.42% and large in 21.21% accessions, Leaf blade intensity of green colour as light in 21.21%, medium in 48.48% and dark in 30.30% accessions, leaf blade pubescence on lower side was observed absent/weak in 87.87%, medium in 9.09% and strong in 1% accessions, Incisions of margins as bicrenate in 18.18%, serrate type 1 in 24.24%, serrate type 2 in 15.15% and biserrate in 42.42% accessions while extent of anthocyanin colouration from base of petiole was observed as small in 15.15%, medium in 30.30% and large in 54.54% accessions and petiole length was maximum (4.30 cm) in Sel-21 and minimum (1.53 cm) in Sel-25. The results in this study were close to results

Table 2 : Summary of frequency of leaf characters of wild apple.

Leaf character	Category	Number of selections	Percentage
Leaf blade length/ width ratio	Small	12	36.36
	Medium	14	42.42
	Large	7	21.21
Leaf blade intensity of green colour	Light	7	21.21
	Medium	16	48.48
	Dark	10	30.30
Leaf blade pubescence on lower side	Absent/ weak	29	87.87
	Medium	3	9.09
	Strong	1	1
Incisions of margins	Crenate	0	0
	Bicrenate	6	18.18
	Serrate type 1	8	24.24
	Serrate type 2	5	15.15
	Biserrate	14	42.42
Petiole:Extent of anthocyanin colouration from base	Small	5	15.15
	Medium	10	30.30
	Large	18	54.54

observed by Reim *et al*. (2012), who reported no pubescence on leaf surface (43%) or only few hairs (37%) in *Malus sylvestris*. The extent of anthocyanin colouration from base of petiole showed variation. Incisions of margins of leaf blade varied between serrate type 1, serrate type 2, biserrate, Bicrenate. Same results regarding leaf morphology were also observed by Wohner *et al*. (2014).

The initial bloom ranged from 31st March in Sel-12 to 26th April in Sel-29 (table 3). On the basis of beginning of bloom the accessions were divided as early bloomers (30th March- 14th April), mid (15th April- 23rd April) and late bloomers (24th April- 7th May). The final bloom in apple genotypes studied ranged from 23rd April to 11th May. In Sel-12 bloom retained till 23rd April and in Sel-29 till 11th May. The flowering date and period may vary depending upon the cultivar aptitude as well as ecological and cultural conditions (Facteau *et al*., 1986). Our results were in close confirmation with that of Kumar *et al*. (1997), who reported that flowering time in apple started from last week of March to first week of April and Mratinic and Aksic (2011) reported that the earliest initial bloom was recorded in some apple cultivars on 22nd April and lasted till 6th May and also reported an approximate 16 day of difference in full bloom between the earliest and latest cultivars. However, most of the accessions in present study were early or mid bloomers but late bloomers should be favoured because of its possibility to avoid freezing injury. Our results were also in close confirmation with Pirlak and Bozbuga (2012). However, the flower colour showed very less variation and majority of genotypes studied were white except in genotypes Sel-5, Sel-13, Sel-14, Sel-15, Sel-16, Sel-19 and Sel-27 in which the colour of flowers was not white.

Table 3 : Flower characteristics of wild apple genotypes as per UPOV descriptor.

S. no.	Selection	Initial bloom (10% bloom)	Final bloom (80% bloom)	Flower colour
1.	Sel-1	12th April	2nd May	White
2.	Sel-2	24th April	08 May	White
3.	Sel-3	11th April	03 May	White
4.	Sel-4	10th April	29th April	White
5.	Sel-5	9th April	29th April	Pink
6.	Sel-6	5th April	26th April	White
7.	Sel-7	5th April	29th April	White
8.	Sel-8	1st April	25th April	White
9.	Sel-9	6th April	29th April	White
10.	Sel-10	2nd April	25th April	White
11.	Sel-11	3rd April	26th April	White
12.	Sel-12	31st March	23rd April	White
13.	Sel-13	7th April	28th April	Pink
14.	Sel-14	8th April	28th April	Pink
15.	Sel-15	15th April	3rd May	Pink
16.	Sel-16	15th April	4th May	Pink
17.	Sel-17	18th April	4th May	White
18.	Sel-18	19th April	5th May	White
19.	Sel-19	13th April	29th April	Pink
20.	Sel-20	14th April	30th April	White
21.	Sel-21	7th April	27th April	White
22.	Sel-22	8th April	27th April	White
23.	Sel-23	22nd April	7th May	White
24.	Sel-24	22nd April	8th May	White
25.	Sel-25	20th April	5th May	White
26.	Sel-26	23rd April	3rd May	White
27.	Sel-27	12th April	28th April	Pink
28.	Sel-28	23rd April	10th May	White
29.	Sel-29	26th April	11th May	White
30.	Sel-30	22nd April	9th May	White
31.	Sel-31	20th April	6th May	White
32.	Sel-32	6th April	27th April	White
33.	Sel-33	8th April	27th April	White

Wide variation in fruit characters were also recorded during the study (tables 4 & 5). The maximum fruit length

(5.21 cm) was recorded in Sel-31 followed by Sel-24 (5.19 cm) and minimum (1.14 cm) was recorded in Sel-3, the maximum fruit diameter of 6.42cm was recorded in Sel-20 followed by Sel-31(6.02cm) while the minimum fruit diameter (1.17cm) was recorded in Sel-3. Fruit size is also important parameter for selection of superior genotypes through breeding programmes (Westwood and Blaney, 1963). The results were close to results found by Reim *et al*. (2012), who recorded the fruit size between 1.8-5.1cm and Reim *et al*. (2013) also reported that the majority of the trees had fruit size under 3.5cm thus indicating a true type *Malus sylvestris*. Variation in fruit size and weight might be under control of genetic factors involving their phylogenic behaviour (Cowman *et al*., 2001 and Harada *et al*., 2005). Fruit weight was maximum (81.34 g) in Sel-20 and minimum in (1.06 g). It is a well known fact that the genetics, environment and cultural practices all interact to determine fruit weight. Producing bigger fruits might be the inherent ability of genotype to utilize resources efficiently to achieve a certain fruit size (Stanley *et al*., 2000). The results of this study were close to results of Mratinic and Aksic (2011), who reported fruit weight of 70-00g to 193.33g in some Turkish Malus species and Gordana (2013), who reported that fruit weight in some wild apples was 3.828 and 3.668g.

Fruit shape showed significant variation with maximum number of genotypes having globose shape (63.63%), obloid (18.18%), ellipsoid (12.12%), ovoid (3.03%) and conic (3.03%), bloom of fruit skin was recorded as absent/weak in 66.66%, moderate in 24.24% and strong in 9.09% accessions. Fruit shape though is influenced by the type of pollen parent (Abdel Aziz *et al*., 1999). Hofer *et al*. (2012) studied fruit shape and reported flat globose in 65%, flat globose in 14% and the globose shape in 11% accessions.

Fruit ground colour was observed as not visible in 3.03%, yellow in 27.27%, yellow green in 42.42% and green in 27.27% accessions. The relative area of over colour was also recorded as small in 33.33%, medium in 6.06%, large in 15.15% and very large in 45.45% accessions. Colour of flesh was also recorded as white in 15.15%, cream in 15.15%, yellowish in 48.48% and pinkish in 21.21% genotypes. Fruit colour is an important parameter to evaluate fruit characters, which directly correlate with environmental conditions in prevailing localities. Fruit colour is significantly influenced by temperature, location of plant, light penetration and growth habit of tree. Sunlight is main factor responsible for synthesis of anthocyanin synthesis in fruit skin (Erez and Flore, 1986) and thus fruit colour (Marini *et al*., 1991). The fruit color of *M. sylvestris* may vary from green to

Table 4 : Summary of frequency of fruit characters of wild apple.

Fruit character	Category	Number of selections	Percentage
Fruit shape	Cylindrical waisted	0	0
	Conic	1	3.03
	Ovoid	1	3.03
	Cylindrical	0	0
	Ellipsoid	4	12.12
	Globose	21	63.63
	Obloid	6	18.18
Fruit : bloom of skin	Absent/weak	22	66.66
	Moderate	8	24.24
	Strong	3	9.09
Fruit ground colour	Not visible	1	3.03
	Whitish yellow	0	0
	Yellow	9	27.27
	Whitish green	0	0
	Yellow green	14	42.42
	Green	9	27.27
Relative area of over colour	Small	11	33.33
	Medium	2	6.06
	Large	5	15.15
	Very large	15	45.45
Fruit: colour of flesh	White	5	15.15
	Cream	5	15.15
	Yellowish	16	48.48
	Greenish	0	0
	Pinkish	7	21.21
	Reddish	0	0
Fruit russet amount	Absent or small	28	84.84
	Medium	4	12.12
	Large	1	3.03
Fruit: firmness of flesh	Very soft	0	0
	Soft	8	24.24
	Medium	8	24.24
	Firm	17	51.51
	Very firm	0	0
Eating quality	Bitter	9	27.27
	Slightly acidic	3	9.09
	Acidic	16	48.48
	Sweet	5	15.15
Harvesting date	Early (100-120 days)	0	0
	Mid (120-150 days)	24	72.72
	Late (> 150 days)	9	27.27

Table 5 : Mean values of different traits of wild apple genotypes.

S. no.	Selection	Fruit length (cm)	Fruit diameter (cm)	Length of stalk (cm)	Fruit weight (gm)	Number of seeds	Leaf blade length (cm)	Leaf blade width (cm)	Petiole length (cm)	TSS (°Brix)	Acidity (%)
1	Sel-1	1.14	1.37	3.60	1.37	2.32	8.24	4.20	4.06	10.20	0.58
2	Sel-2	2.76	2.66	1.63	6.77	2.51	9.67	7.16	2.90	15.60	0.54
3	Sel-3	1.43	1.17	3.60	1.06	2.12	8.44	4.23	1.90	15.50	0.43
4	Sel-4	1.30	1.34	3.96	2.22	8.44	10.60	5.66	2.73	16.30	0.79
5	Sel-5	2.54	2.49	3.53	7.70	3.25	9.53	4.30	3.40	12.00	0.46
6	Sel-6	1.21	1.36	3.50	1.19	3.56	9.90	5.00	1.86	15.80	0.48
7	Sel-7	3.38	3.38	2.43	20.48	10.00	9.50	5.60	2.60	12.60	0.26
8	Sel-8	2.23	2.29	0.90	6.20	6.89	10.70	5.46	3.73	9.70	0.39
9	Sel-9	2.11	2.60	1.80	18.76	4.91	6.26	3.20	3.86	14.70	0.20
10	Sel-10	1.18	1.32	3.40	1.34	5.34	8.56	5.33	3.23	6.70	0.72
11	Sel-11	2.26	2.73	2.73	9.17	6.72	7.00	3.50	3.46	13.00	0.25
12	Sel-12	3.16	3.79	1.06	46.85	7.33	8.900	4.90	2.30	11.00	0.37
13	Sel-13	1.78	1.95	2.60	3.68	3.21	9.73	5.93	2.50	11.00	0.40
14	Sel-14	1.82	2.00	2.70	3.75	6.53	7.70	3.26	3.20	11.10	0.44
15	Sel-15	2.16	2.66	5.20	8.66	4.23	9.06	4.30	3.13	11.60	0.38
16	Sel-16	2.18	2.56	4.33	10.34	5.29	9.06	4.46	2.66	10.50	0.42
17	Sel-17	2.92	4.05	0.60	27.08	3.26	8.83	4.26	2.73	7.40	0.10
18	Sel-18	1.72	2.09	1.46	5.16	2.31	7.36	4.76	1.90	14.90	0.40
19	Sel-19	2.49	2.87	4.33	11.08	7.56	7.20	4.50	2.53	11.10	0.50
20	Sel-20	4.79	6.42	0.93	81.34	7.00	7.30	5.13	2.00	11.90	0.06
21	Sel-21	1.94	2.33	3.73	6.06	5.26	7.50	4.30	4.30	14.30	0.48
22	Sel-22	2.48	2.94	2.03	14.60	3.33	8.06	4.26	2.56	16.00	0.18
23	Sel-23	4.29	4.67	1.10	47.43	2.51	7.93	4.33	1.66	13.27	0.10
24	Sel-24	5.19	5.48	1.76	75.55	6.00	7.90	4.26	2.23	13.70	0.11
25	Sel-25	2.54	3.40	0.80	16.41	2.51	6.56	2.73	1.53	12.60	0.08
26	Sel-26	3.52	3.92	2.23	26.92	3.22	7.21	4.00	4.00	13.00	0.21
27	Sel-27	1.97	2.14	2.86	3.91	3.53	9.16	3.70	3.86	11.30	0.53
28	Sel-28	4.98	5.83	0.90	80.68	4.21	7.30	4.86	2.10	14.00	0.17
29	Sel-29	3.23	3.85	3.30	26.20	3.71	12.06	7.23	4.13	11.60	0.24
30	Sel-30	4.23	4.37	1.50	32.69	5.00	7.00	5.00	2.50	10.10	0.36
31	Sel-31	5.21	6.02	1.90	43.75	6.33	7.60	4.40	1.76	12.50	0.67
32	Sel-32	1.23	1.45	3.14	1.45	2.27	7.43	3.82	2.90	10.10	0.51
33	Sel-33	2.86	2.78	2.33	6.34	2.52	9.23	5.21	2.63	11.40	0.57
	Mean	**2.67**	**3.04**	**2.48**	**19.88**	**4.64**	**8.44**	**4.64**	**2.81**	**12.31**	**0.37**
	CV	**17.23**	**16.03**	**15.41**	**3.66**	**23.46**	**6.63**	**8.13**	**15.52**	**7.34**	**5.29**
	CD@5%	**0.75**	**0.79**	**0.62**	**1.18**	**1.63**	**0.91**	**0.61**	**0.71**	**1.47**	**0.03**

green-yellow and without or only a touch of red over color (Wagner, 1996). Mratinic and Aksic (2012) reported the background colour from cream white (38.89%), over yellow (16.67%) to green yellow(44.44%), while over colour ranged from red(50.0%) to dark red or purple (5.56%) in some Malus species in South Serbia. The russet amount was absent/small in 84.84%, medium in 12.12% and large in 3.03% accessions. This russet usually develops in early stage of fruit development shortly after bloom, corresponding to the period of tangential growth and can also be genetic in nature such as in Cox's orange Pippin (Faust and Shear, 1972). Russet formed in response to the damage of epidermal cells may result from application of crop protection agents, particularly those having a high degree of oil solubility as these can penetrate the cuticle directly (Goffinet and Pearson, 1991)

and russeting has been shown to be controlled by a major dominant gene (*Ru*) with minor modifying and polygenic effects (Brown, 1992). Firmness ranged from 4 Kg/m^2 (soft), 5 Kg/m^2 (medium) and > 7 Kg/m^2 (firm). It was recorded soft in 24.24%, medium in 24.24% and firm in 51.51% accessions. Damyar *et al*. (2007) reported that fruit firmness varied greatly and was classified in 6 groups ranging from very soft (4%) to very firm (7%). Kaya *et al*. (2015) on their studies on fruit quality characters and genetic variability of apple germplasm in Turkey reported that the fruit flesh firmness had a range of 3.99–14.05 kgcm^{-2}.

The TSS of the wild genotypes studied ranged from 6.70-16.30ºBrix. The maximum TSS (16.30ºBrix) was recorded in Sel-4 followed by Sel-2 (15.60ºBrix). Minimum TSS of 6.70ºBrix was recorded in Sel-10.The acidity of 33 wild genotypes studied ranged from 0.06-0.79%. The maximum (0.79%) was recorded in Sel-4 where as minimum (0.06%) in Sel-20.Our results were similar to Mratinic and Aksic (2012) who in their studies on phenotypic diversity of apple germplasm in South Serbia reported that the soluble solid in ºBrix content varied from 12.55 to 19.24 and titratable acidity varied between 0.10 and 0.82%. Kaya *et al*. (2015) in their studies on fruit quality characters and genetic variability of apple germplasm in Turkey reported that the range for soluble solids content was 9.0%–14.4% and 0.15%–1.75% for titratable acidity. Eating quality varied from bitter (27.27% accessions), slightly acidic (9.09% accessions), acidic (48.48% accessions), sweet (15.15% accessions). Same results were found by Dzhangaliev *et al*. (2003), who reported that the fruits of both *Malus kirghisorum* and *Malus sieversii* are diverse in flavor, ranging from sour-sweet to acid to bitter and astringent and also Kushmarenko *et al*. (2009) in their studies on *Malus sieversii* concluded that the flavours were acidic, bitter, sweet, sweet-sour. Harvesting date was recorded as mid (120-150 days) in 72.72% accessions to late (> 150 days) in 27.27% accessions however early varieties were not recorded. Maximum (10) number of seeds were recorded in Sel-7 where as minimum (2.12) number of seeds was recorded in Sel-3. The number of days from full bloom to maturity was more reliable index for determining maturity of Red Delicious than other factors studied and reported that 124-129 days from full bloom to maturity was found most reliable index for determining maturity of Red Delicious apple cultivar (Tripathi, 1984).

Conclusion

The present study confirmed that there is huge diversity of apple in Kashmir, thus it becomes necessary for preserving these unique genetic resources and continuing its study to ensure its conservation, exchange and utilization in future breeding programmes for future development of innovative, market-driven cultivars.

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