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Morphological characterization of clonal rootstocks of apple

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Abstract
Identification of clonal rootstocks of apple was carried out to characterize clonal rootstocks based on morphological characters using International Union for the Protection of New Varieties of Plants (UPOV) descriptor during two consecutive years 2016 and 2017. A wide variation was observed among different selections for various morphological traits. A total of 32 UPOV attributes were observed among which 15 traits were found more informative during the course of investigation. Maximum range was observed for number of spines (5.45-9.10) followed by leaf blade width (3.76 - 4.56) while as minimum range was observed for ratio of petiole length to blade length (0.27-.0.28). The phenotypic coefficient of variation and genotypic coefficient of variation was highest for number of spines (27.10% & 22.42%) followed by leaf blade length of tip (24.34% & 12.24%) and lowest for leaf blade length (6.75% & 4.82%). Phenotypic coefficient of variation was slightly higher than genotypic coefficient of variation, which indicates the minor role of environment in the expression of traits under observation.

Keywords: Apple rootstocks, morphological traits, clonal rootstocks, UPOV

Introduction
Apple is the most ubiquitous and well-adapted species of temperate fruit crops. 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 [3]. In Indian subcontinent, Jammu and Kashmir state is bestowed with climatic conditions which are best suited for temperate fruit production particularly apple. Kashmir has for long been called the home of apples The fruit industry is the backbone of economy in Jammu and Kashmir State and has a great potential to ameliorate the living conditions of rural people. Despite the fact that Jammu and Kashmir is the leader in apple productivity in the country, it is far below the productivity of developed countries. One of the main reasons of this low productivity is the uneven and unpredictable yields registered by the use of incompatible and seedling rootstocks.

Clonal rootstocks which offer a viable solution to this problem have been used by the fruit growers in scientifically advanced countries for better management and quality fruit production. Clonal rootstocks are genetically identical offshoots or clones of a mother rootstock type with certain desirable characteristics such as disease resistance, tolerance of winter cold, seasonal flooding and summer droughts, or reducing tree size e.g. M9 reduces the tree size up to 70% as compared to seedling rootstocks while as MM106 reduces tree size up to 40%. Hence the clonal rootstocks in combination with specific scion variety offer great potential to improve both quality and productivity of apple. The M and MM series were used as parent materials of rootstock breeding. The apple dwarf rootstocks '7734' and '7848', which had cold and drought resistance and early flowers and fruit, were selected from M series Li *et al*., 2000 [7]. The apple dwarf rootstocks 'NM.11', 'GM.256', and 'MD.001' had strong cold resistance and their lineages were thought to have M or MM heritage Han *et al*., 2005 [4].

The existence of a very large number of rootstocks reinforces the need for a reliable verification system to identify them properly by the nurserymen and also the growers. This represents a very important aspect in the fruit industry, as initial planting and establishing orchards incur huge investments of time and money Oliveira *et al*., 1999. Accurate identification of rootstocks is also essential for patent protection of these materials.

In this study we use UPOV apple rootstock descriptor for morphological characterization of M9, MM106 and MM111 clonal rootstocks.

Materials and Methods

The experiments were carried out at Ambri Apple Research Centre, Pahnoo, Shopian, SKUAST-Kashmir during two consecutive years 2016 and 2017. 85 clonal rootstocks of apple were analysed in this study using apple rootstock descriptor UPOV, 2015. The rootstocks were collected from 5 different locations viz. SKUAST-Kashmir, ACHD Zainapora, AARC Pahnoo, CITH and private nursery from Pulwama. A total of 32 UPOV traits were studied. All the clonal rootstocks were examined for plant characters viz., plant vigour, number of branches, plant habit and number of spines. The various observations recorded on one-year-old shoot included growth pattern, pubescence, glossiness, thickness, length of internode, number of lenticels, size of lenticels, colour on sunny side, size of vegetative bud and shape of apex of vegetative bud. The various observations recorded on leaf included attitude in relation to shoot, leaf length, leaf width, ratio length/ width, profile of cross section, length of tip, incisions of margin, depth of incisions of margin, undulation of margins, pubescence on lower side, glossiness of upper side, intensity of Green colour, petiole length, petiole length relative to length of blade, petiole extend of anthocyanin from base and stipule size.

The recording of observations on plant was carried as under:

- Plant: Observations on the plant was made in the dormant season.
- One-year-old shoot: Observations on the shoot was made on the middle third of the one-year-old shoot in the dormant season.
- Young shoot: Observation of the young shoot was made on the upper third of the one-year-old shoot during rapid growth.
- Leaf: Observations on the leave was made on fully developed leaves from the middle third of vigorous current season shoots.

Results and Discussion

Plant Characters

The apple clonal rootstocks selected for study were grown in same environmental conditions. In the present study, 14 out of 16 traits (table-1) showed variability in plant characters. Based on the tree vigour the rootstocks varied between weak, medium and strong. Number of branches in plants varied between few and medium among the clonal rootstocks under study. However, plant habit of all rootstocks was found same i.e., upright to spreading. Based on number of spines in plants, rootstocks varied between few and many. The observations recorded on one-year-old shoot also showed variability in plant characters. The various observations recorded on one-year-old shoot included growth pattern, pubescence, glossiness, thickness, length of internode, number of lenticels, size of lenticels, colour on sunny side, size of vegetative bud and shape of apex of vegetative bud. Growth pattern was found straight in all selected clonal rootstocks of apple under study. Pubescence ranged between strong and very strong, while as glossiness was found medium and strong in apple clonal rootstocks under observation. Based on length of internode the clonal rootstocks of apple varied between short medium and long. Number of lenticels ranged between few, medium and many while as size of lenticels varied between medium and large in clonal rootstocks of apple under observation. Based on colour on sunny side rootstocks ranged between reddish brown, medium brown and dark brown. Size of vegetative bud ranged between large and small while as shape of apex of vegetative bud varied between acute and rounded. Based on position of vegetative bud in relation to young shoot rootstocks ranged between adpressed and slightly held out. The results of this study were in close confirmation with Mratinic and Aksic, 2011 [14], who reported that the selections of wild apple showed variability in their tree behaviour and tree size. Wohner *et al*. 2014 [15] also described the morphology of *Malus* × *robusta* as a small to medium-sized tree with a weak to strong vigour and upright to spreading crown and medium to absent pubescence on the upper side of the shoot surface. Hassan *et al*. 2017 [5] reported similar variations in tree vigour, tree habit, pubescence on one-year-old shoot and number of lenticels on one-year-old shoot in wild apple. Koc *et al*. 2009 [6] also reported similar variations in various UPOV criterions in apple rootstocks. Shyamali 2006 [11] reported that variation in tree characters of different pear genotypes and these variations might be due to genetic makeup of the pear cultivar/rootstock.

Leaf Characteristics

Leaves play an important role in plant growth and development as leaves are the part of plant which serves as a source of food to the plants. In present study a wide variation has been observed in various leaf characters of clonal rootstocks of apple (Table-1). Leaf length, length of tip of leaf blade, petiole length and length of petiole relative to length of blade varied between short, medium and long. Attitude in relation to shoot of leaf blade was found upwards and outwards. Based on width of leaf blade rootstocks ranged between narrow, medium and broad. The ratio of leaf blade length to blade width varied between slightly elongated, moderately elongated and strongly elongated. On the bases of profile of cross section of leaf blade clonal rootstocks of apple varied between flat and concave. Incisions of margin of leaf blade varied between serrate type 1 and biserriate while as depth of incision of margin of leaf blade ranged between shallow, medium and deep. However, undulation of margins of leaf blade varied between absent or very weak, weak and strong. Based on pubescence on lower side of leaf blade clonal rootstocks of apple varied between weak, medium and strong while as glossiness of upper side of leaf blade ranged between weak and medium. However, intensity of green colour of leaf blade varied between light, medium and dark. On the bases of extend of anthocyanin from base of petiole of clonal rootstocks of apple ranged between small, medium and large. Finally, size of stipule of clonal rootstocks of apple varied between small, medium and large. The results in this study were close to results observed by Reim *et al*. 2012, who reported variation on pubescence on leaf surface in *Malus sylvestris*. Koc *et al*. 2009 [6] also reported similar variations in various UPOV criterions in apple rootstocks. Wohner *et al*. 2014 [15] reported variation in extent of anthocyanin colouration from base of petiole along with variation in incisions of margins in wild apple. Hassan *et al*. 2017 [5] also reported similar variations leaf blade intensity of green colour, leaf blade pubescence on lower side, incisions of margins, extend of anthocyanin colouration from base of petiole and ratio of leaf blade length to width in wild apple.

The variability parameters viz., mean, range and coefficients of variation are presented in table-2. Phenotypic coefficient of variation was highest for number of spines (27.10%) followed leaf blade length of tip (24.34%) and was lowest (6.75%) leaf blade length. The estimates of PCV suggesting that the scope for identification of clonal rootstocks of apple by these traits during selection could be based on phenotypic variability. The

small difference between GCV and PCV indicates that the observed variations for the trait were mostly due to genetic factors. However, the environment played a little role on the expression of this trait. On the other hand, large difference between GCV and PCV indicated the role of environmental influence over the characters under study. The higher values of both PCV and GCV for characters like number of spines, length of internode and shoot diameter indicate that these traits are expected to help in identification of clonal rootstocks of apple through morphological means. Miletic *et al*. (2003) [8] studied variability of pomological characteristics of the Sumatovoka apples and found the 12.95% coefficient of variance for fruit length, 11.4% for fruit width and 16.4% for pedicel length. For diversity studies coefficient of variation was also studied by Bisati (2012) [2]. Barua and Sharma (2002) [1] reported significant differences between genotypes of apple (*M. domestica*) for various traits and phenotypic and genotypic variations were highest for yield/tree. Similar results were reported by Silva *et al*. (1998) [12] and Sharma and Sharma *et al*. (2007) [10].

Table 1: The most distinct morphological characters of rootstocks

S. No	Morphological Traits	Apple rootstocks				
		M9-Pajam	M9-T337	M9-T339	MM106	MM111
1	Plant: Vigour	weak			medium	strong
2	One-year-old shoot: Thickness	thin			medium	thick
3	One-year-old shoot: Length of internode	short			medium	long
4	One-year-old shoot: No. of lenticles	few			medium	many
5	One-year-old shoot: Colour on sunny side	reddish brown			medium brown	dark brown
6	Leaf blade: Length	long			medium	Short
7	Leaf blade: Width	medium			broad	narrow
8	Leaf blade: Length of tip	long			medium	short
9	Leaf blade: Undulation of margins	strong			weak	absent or very week
10	Leaf blade: Pubescence on lower side	weak			strong	medium
11	Leaf blade: Intensity of Green colour	light			dark	medium
12	Petiole: Length	long			medium	short
13	Petiole: Length relative to length of blade	long			medium	short
14	Petiole: Extend of anthocyanin from base	large			medium	small
15	Stipule: Size	small			large	medium

Table 2: Analysis of variance for various traits in clonal rootstocks of apple (Pooled)

Characters	Range Min. Max.	Mean ± S.E	Coefficient of variation (%)	
			GCV	PCV
Leaf blade length of tip (cm)	0.46 - 0.68	0.58 ± 0.04	12.48	24.34
Leaf blade length (cm)	6.00 - 6.71	6.43 ± 0.10	4.82	6.75
Leaf blade width (cm)	3.76 - 4.56	4.26 ± 0.12	6.96	11.27
Petiole length (cm)	1.62 - 1.84	1.76 ± 0.03	4.73	7.60
Number of spines	5.45 - 9.10	6.79 ± 0.33	22.42	27.10
Length of internode (cm)	1.69 - 2.26	1.92 ± 0.03	12.40	13.58
Shoot diameter cm	0.48 - 0.59	0.52 ± 0.01	8.46	10.15
Blade length/ Blade width	1.37 - 1.65	1.54 ± 0.04	6.54	11.20
Petiole length/ blade length	0.27 - 0.28	0.27 ± 0.01	2.18	7.46

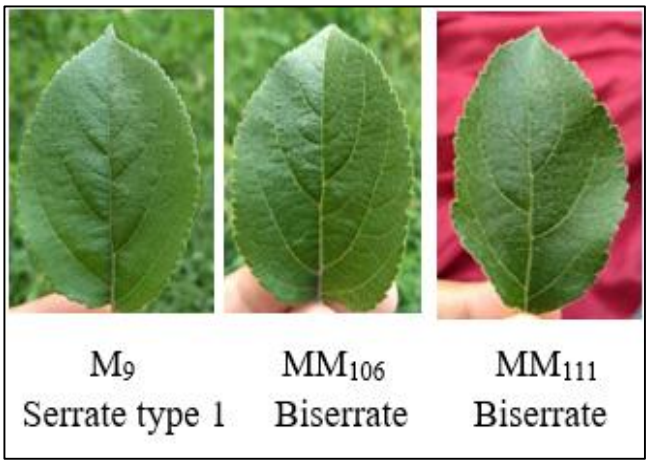

Plate 1: Incisions and depth of margin of leaf

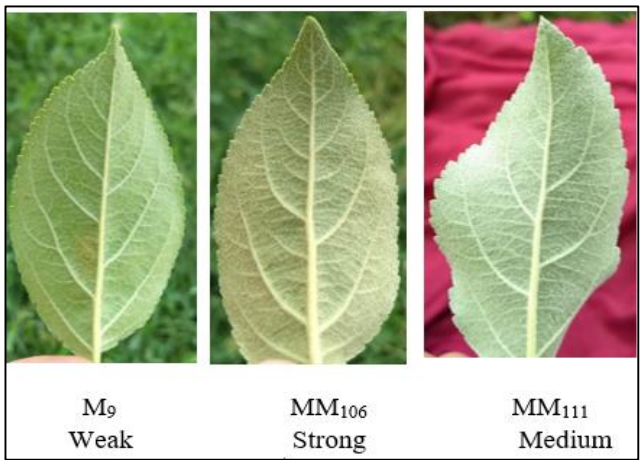

Plate 2: Pubescence on lower side of leaf

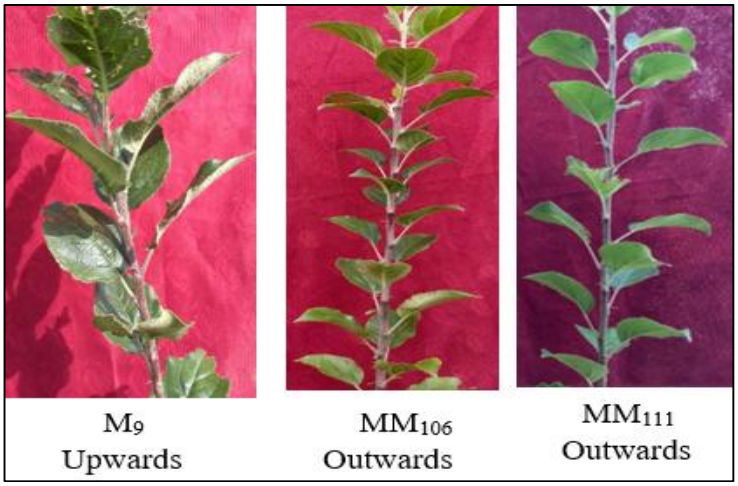

Plate 3: Leaf blade attitude in relation to shoot

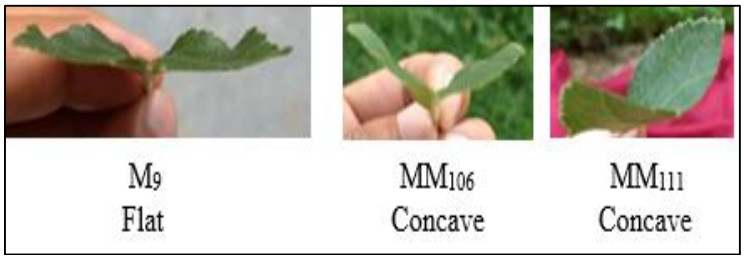

Plate 4: Profile of cross section of leaf blade

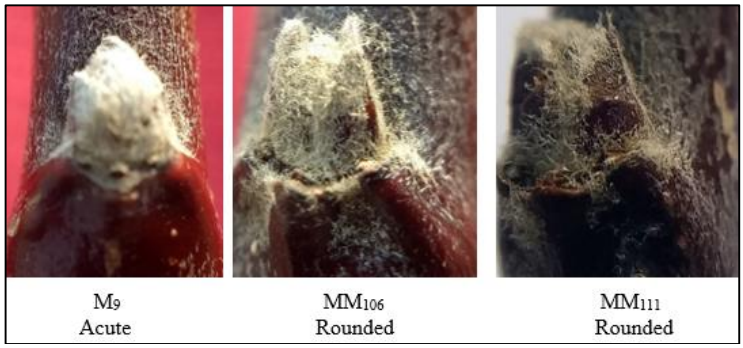

Plate 5: Shape of apex of vegetative bud

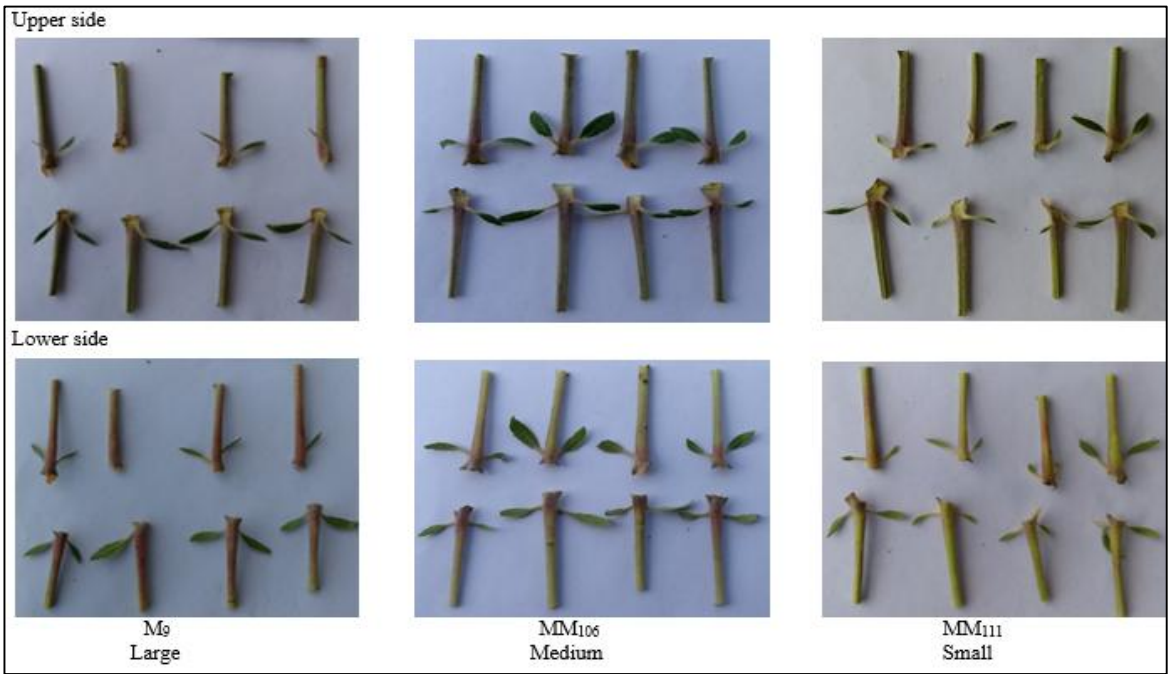

Plate 6: Extend of anthocyanin Colouration for base of petiole

Concussion

From the study it can be concluded that clonal rootstocks of apple can be identified on the bases of UPOV traits. However, marker assisted identification will always be preferred as it completely removes confusion for identification of clonal rootstocks.

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