



Morphological markers for identification of hybrids and their parental lines in tomato (*Solanum lycopersicum*)

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Received: 01 October 2016; Accepted: 03 March 2017

Key words: Hybrids, Morphological markers, Parental lines, Tomato

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops, whose genetics is thoroughly studied. India ranks second, next to China both in area and production (0.88 mha and 18.23 mt). The productivity level in India (20.72 t/ha) is far less compared to China (51.69 tonnes/ha) and USA (83.80 tonnes/ha) (Anon 2015). Increased availability and adoption of hybrids can be one of the plausible solutions for enhancing the productivity levels. Sudha *et al.* (2006) reported that hybrid seed production technology is adopted to an extent of 40% in tomato making it as predominant among various agricultural systems and are being harnessed for higher incomes under both high input and low input systems. Thus, increased demand for hybrid seeds is pushing the industry for releasing more number of hybrids. The seed production system aims at ensuring supply of good quality seeds to farmers thus making the availability of cultivar descriptors a must for their proper identity and distinctiveness. Being a signatory to WTO agreement on TRIPS, India passed the Protection of Plant Varieties and Farmers Rights Act (2001) for protecting the new varieties. For getting protected, they have to be tested for distinctiveness, uniformity and stability based on accurate and reliable morphological descriptors. The basic objective of varietal characterization is to confirm that the characters to be used in identifying a variety or hybrid should express when grown under varied climatic conditions or generations. The identity of a genotype can be established using morphological, physiological, biochemical and molecular markers. Out of these, morphological markers are primarily used in DUS testing. Morphological markers, both qualitative and quantitative are utilized in characterizing the varieties. Also, they have been universally accepted as undisputable characters for DUS testing when used in sequential fashion thus making them convenient for distinguishing the different varieties. During the process of release and

notification, only a set of certain diagnostic features are released and are being used in seed certification. By and large the complete description of the genotypes is lacking. Thus, present study was taken up to characterize and identify morphological markers for four tomato hybrids and their parental lines developed and released by Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi. The characterization has been performed using conventionally highly heritable morphological descriptors based on seedling, plant, inflorescence, flower, fruit and other agronomic characters.

Genetically pure seed of hybrids, viz PH-1, 2, 4 and 8 and their parental lines (Table 1) was obtained from Division of Vegetable Science, ICAR-IARI, New Delhi. For recording the morphological characters, the material was raised at Experimental Farm, Division of Seed Science and Technology, IARI during both seasons of *rabi* 2014-15 and *rabi* 2015-16. An experiment was laid out in Randomized Complete Block Design following standard recommended agronomic practices including plant protection measures. Data on qualitative (27 characters) and quantitative (12 characters) morphological descriptors listed for tomato by PPV and FRA were recorded at different stages of crop growth. The observations for quantitative characters were recorded using thirty plants, 10 plants from each replicate. For qualitative characters, the observations were made on a group of plants. The quantitative data were subjected to one-way ANOVA followed by mean comparisons at 5% significance level using Least Significant Difference (LSD) in SAS software.

Among the qualitative characters, fourteen characters

Table 1 A list of tomato hybrids along with their parents

Hybrid	Female	Male
PH-1	Female-1	Male-1
PH-2	Female-2	Male-2
PH-4	Female-2	Male-1
PH-8	Female-3	Male-3

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were found to be monomorphic, whereas thirteen were found to be polymorphic. The expression states of all these characters were uniform and consistent during both the years of study (i.e. *rabi* 2014-15 and *rabi* 2015-16). Fourteen characters, viz, seedling anthocyanin pigmentation, growth type, leaf structure, inflorescence type, flower fasciation, flower colour, anther colour, nature of stigma, peduncle abscission layer, intensity of green colour in fruit, fruit shape in cross section, size of blossom scar on fruit, fruit colour at maturity and colour of fruit flesh were observed to be monomorphic (Table 2).

Hybrid tomato PH-1, could be distinguished from its female parent by its features such as less serrated leaves, horizontal attitude of leaf in relation to main stem and weak ribbing of fruit at the peduncle end. Further, it could be distinguished from its male parental line (Male-1), based on fruit shape in longitudinal section and fruit shape at the blossom end. Similarly PH-2 could be distinguished from its female parental line (Female-2), by its dark green leaf, stem anthocyanin pigmentation at upper one-third portion, leaf attitude in relation to main stem, fruit shape at blossom end, whereas it could be distinguished from male parental line (Male-2) based on fruit shape in longitudinal section and depression of fruit at peduncle end. PH-4 differs from its female parental line (Female-2), with respect to leaf colour, leaf serration, stem anthocyanin pigmentation on upper one-third portion, leaf attitude in relation to main stem and fruit ribbing at peduncle end. On the other hand, it could be distinguished from its male parental line (Male-1) based on fruit shape in longitudinal section and at blossom (Fig-3) end. PH-8 was identified from its female parental line (Female-3) with the help of intensity of leaf colour and attitude of leaflet petiole in relation to main axis. Whereas, it differs from its male parental line (Male-3) in characters like stem pubescence, stem anthocyanin pigmentation on upper one-third portion, pubescence of flower style, green shoulder and fruit depression at peduncle end. The stability in expression of these visually assessed characters can be due to high heritability of traits and also low genotype × environment interaction. Most of these characters are controlled by single or a few number of genes having simple dominant or recessive relationship as reported by Kumar *et al.* (2006). Also during the process of developing the parental lines, breeders exert a heavy selection pressure for all these characters to achieve uniformity and stability to a maximum extent thus making these characters most reliable keys for varietal identification. Powell *et al.* (2012) studied presence of green shoulder character in tomato and reported that it is controlled by uniform ripening locus (u). Changes in fruit shape largely controlled by mutation in four genes SUN, OVATE, LOCULE NUMBER, FASCIATED was reported by Rodriguez *et al.* (2011). Langlade *et al.* (2005) reported the high heritability of leaf shape in tomato against its polygenic nature.

The observed data on quantitative characters (Table 3 and 4) were also consistent during both the seasons of study (*rabi* 2014-15 and *rabi* 2015-16) and can be relied

Table 2 Characterization of tomato hybrids and their parents on the basis of morphological descriptors

Character	Female-1	PH-1	Male-1	PH-4	Female-2	PH-2	Male-2	Female-3	PH-8	Male-3
Seedling anthocyanin colour	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Growth type	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate
Stem Pubescence	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
anthocyanin pigmentation of upper 1/3 portion	Weak	Weak	Weak	Weak	Medium	Weak	Weak	Medium	Medium	Weak
Leaf intensity of green colour	Medium	Medium	Medium	Medium	Medium	Light	Light	Medium	Light	Light
Serration	Highly serrated	Less serrated	Less serrated	Less serrated	Highly serrated	Highly serrated	Highly serrated	Highly serrated	Highly serrated	Highly serrated
Structure	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
attitude in relation to main stem	Horizontal	Semi-errect	Semi-errect	Semi-errect	Horizontal	Semi-errect	Semi-errect	Semi-errect	Semi-errect	Semi-errect
leaflet petiole	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Semi-errect	Horizontal	Horizontal
attitude in relation to main axis	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Semi-errect	Horizontal	Horizontal

Contd.

Table 2 (Concluded)

Character	Female-1	PH-1	Male-1	PH-4	Female-2	PH-2	Male-2	Female-3	PH-8	Male-3
Inflorescence type	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous	Uniparous
Flower	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Fasciations	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
pubescence of style	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent
Colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
anther colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
nature of stigma	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted	Non-exserted
number of stigma lobes	4	2	4	6	4-5	2	1	4	3	3
Peduncle: Abscission layer	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
intensity of green colour	Light	Light	Light	Light	Light	Light	Light	Light	Light	Light
green shoulder	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent
shape in longitudinal section	Slightly flattened	Slightly flattened	Cylindrical	Slightly flattened	Slightly flattened	Slightly flattened	Obovoid	Slightly flattened	Slightly flattened	Slightly flattened
ribbing at peduncle end	Absent	Weak	Weak	Weak	Absent	Absent	Absent	Absent	Absent	Absent
shape in cross section	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
depression at peduncle end	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Absent	Absent	Absent	Shallow
size of blossom scar	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
shape at blossom end	Indented to flat	Indented to flat	Flat to Pointed	Indented to flat	Indented to flat	Flat	Flat	Indented to flat	Indented to flat	Indented to flat
colour at maturity	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
colour of flesh at maturity	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red

Male -1 common for PH-1 and PH-4; Female-2 common for PH-2 and PH-4

Table 3 Morphological (quantitative) characterization of tomato hybrids and their parental lines during rabi 2014 and 2015

Genotype	Internode length		Leaf length (cm)		Leaf width (cm)		Leaflet length (cm)	
	I year	II year	I year	II year	I year	II year	I year	II year
Female-1	25.5 ± 0.2a	22.7 ± 0.4a	21.0 ± 0.9bc	20.8 ± 0.9b	15.9 ± 0.5ab	17.3 ± 0.5ab	7.9 ± 0.2bc	8.2 ± 0.2bc
PH-1	16.6 ± 0.3f	18.0 ± 0.5bcd	22.6 ± 0.4a	23.7 ± 0.3a	16.6 ± 0.4a	17.8 ± 0.3a	9.1 ± 0.2a	9.5 ± 0.1a
Male-1	18.6 ± 0.3de	18.7 ± 0.3bc	21.7 ± 0.7ab	21.9 ± 0.5b	15.1 ± 0.7bcd	16.0 ± 0.6cde	8.4 ± 0.3ab	8.6 ± 0.3b
PH-4	15.3 ± 0.2g	16.0 ± 0.2d	20.1 ± 0.4c	20.8 ± 0.3b	15.3 ± 0.3abcd	16.6 ± 0.3abc	8.3 ± 0.2b	8.6 ± 0.1b
Female-2	19.1 ± 0.4d	18.3 ± 0.3bc	20.3 ± 0.4bc	21.0 ± 0.4b	15.9 ± 0.3ab	16.9 ± 0.3abc	7.5 ± 0.2cd	7.7 ± 0.1c
PH-2	15.2 ± 0.3g	16.9 ± 0.4cd	20.2 ± 0.4c	21.0 ± 0.3b	15.8 ± 0.3ab	17.0 ± 0.2abc	7.8 ± 0.1bc	8.1 ± 0.1bc
Male-2	23.7 ± 0.3b	22.5 ± 0.4a	18.0 ± 0.3d	18.9 ± 0.3cd	14.0 ± 0.3d	14.8 ± 0.2e	7.2 ± 0.1de	8.2 ± 0.3bc
Female-3	17.5 ± 0.5ef	17.4 ± 0.5cd	14.8 ± 0.5e	14.9 ± 0.4e	11.6 ± 0.3e	12.0 ± 0.2f	5.3 ± 0.1f	5.6 ± 0.1c
PH-8	17.4 ± 0.3ef	18.5 ± 0.4bc	17.6 ± 0.4d	18.2 ± 0.4d	14.4 ± 0.3cd	15.2 ± 0.3ed	6.6 ± 0.1e	7.0 ± 0.1d
Male-3	20.4 ± 0.4c	19.8 ± 0.3b	19.6 ± 0.4bc	20.3 ± 0.4bc	15.7 ± 0.4abc	16.2 ± 0.3bcd	7.5 ± 0.1cd	7.8 ± 0.1c
	Leaflet width (cm)		Calyx size (cm)		Peduncle length (cm)		Fruit Weight (g)	
	I year	II year	I year	II year	I year	II year	I year	II year
Female-1	4.6 ± 0.1abc	4.8 ± 0.1bcd	1.29 ± 0.05de	1.33 ± 0.03cd	0.48 ± 0.03ed	0.45 ± 0.02f	491 ± 18d	504 ± 16f
PH-1	5.0 ± 0.1a	5.3 ± 0.1a	1.12 ± 0.03f	1.16 ± 0.03e	0.53 ± 0.02cd	0.54 ± 0.02de	536 ± 17cd	567 ± 16de
Male-1	4.8 ± 0.1ab	4.9 ± 0.1abc	1.23 ± 0.03ef	1.18 ± 0.03e	0.44 ± 0.02e	0.45 ± 0.02f	468 ± 23d	510 ± 21ef
PH-4	4.7 ± 0.1abc	5.0 ± 0.1ab	1.11 ± 0.03gf	1.12 ± 0.03e	0.61 ± 0.02b	0.62 ± 0.02c	613 ± 20b	640 ± 19bc
Female-2	4.4 ± 0.1bcd	4.5 ± 0.1cde	1.73 ± 0.05a	1.75 ± 0.03a	0.75 ± 0.02a	0.81 ± 0.02a	846 ± 25a	858 ± 18a
PH-2	4.1 ± 0.1cd	4.7 ± 0.1bcde	1.19 ± 0.03ef	1.21 ± 0.03dc	0.63 ± 0.01b	0.63 ± 0.02c	570 ± 13bc	598 ± 13cd
Male-2	4.2 ± 0.1cd	4.3 ± 0.1e	0.97 ± 0.03g	0.97 ± 0.03f	0.50 ± 0.02ed	0.49 ± 0.02ef	336 ± 26e	383 ± 15g
Female-3	2.9 ± 0.1f	3.0 ± 0.1f	1.39 ± 0.03cd	1.39 ± 0.03c	0.71 ± 0.02a	0.73 ± 0.02b	339 ± 26e	357 ± 23g
PH-8	3.7 ± 0.3e	4.3 ± 0.1de	1.46 ± 0.03bc	1.45 ± 0.03bc	0.58 ± 0.02bc	0.58 ± 0.02cd	621 ± 36b	665 ± 34b
Male-3	4.9 ± 0.1a	4.9 ± 0.1abc	1.54 ± 0.05b	1.57 ± 0.03b	0.62 ± 0.02b	0.61 ± 0.02c	606 ± 12bc	635 ± 12bc

(I year : rabi 2014-15 and II year : rabi 2015-16) where, values are mean values ± standard error, Values with same letters statistically on par; and with different letters are significantly different.

Table 4 Morphological (quantitative) characterization of tomato hybrids and their parental lines during rabi 2014 and 2015

Genotype	Fruit length (cm)		Fruit width (cm)		Scar diameter (cm)		Pericarp thickness (cm)	
	I year	II year	I year	II year	I year	II year	I year	II year
Female-1	3.1 ± 0.03e	3.5 ± 0.1d	4.4 ± 0.03cd	5.4 ± 0.05c	0.8 ± 0.03c	0.8 ± 0.02d	0.49 ± 0.02d	0.51 ± 0.02e
PH-1	3.7 ± 0.05c	4.3 ± 0.1bc	4.7 ± 0.05bcd	5.6 ± 0.08bc	0.7 ± 0.02d	0.7 ± 0.02de	0.61 ± 0.02b	0.62 ± 0.02c
Male-1	4.2 ± 0.08a	5.1 ± 0.1a	4.2 ± 0.08d	4.4 ± 0.08d	0.6 ± 0.03d	0.7 ± 0.03e	0.76 ± 0.02a	0.81 ± 0.02a
PH-4	3.8 ± 0.05bc	4.5 ± 0.1b	4.8 ± 0.08bc	5.8 ± 0.08b	0.9 ± 0.03c	1.0 ± 0.03c	0.70 ± 0.02a	0.71 ± 0.03b
Female-2	3.7 ± 0.05cd	4.4 ± 0.1b	5.9 ± 0.10a	7.0 ± 0.13a	1.4 ± 0.05a	1.4 ± 0.05a	0.48 ± 0.02d	0.54 ± 0.03de
PH-2	4.1 ± 0.05a	5.1 ± 0.1a	4.8 ± 0.08bc	5.8 ± 0.08b	0.8 ± 0.03c	0.9 ± 0.03c	0.56 ± 0.02bc	0.58 ± 0.02cd
Male-2	4.0 ± 0.05ab	4.9 ± 0.1a	3.6 ± 0.03e	4.1 ± 0.05e	0.3 ± 0.01e	0.4 ± 0.01f	0.51 ± 0.02cd	0.51 ± 0.02de
Female-3	3.2 ± 0.10e	3.9 ± 0.1c	4.5 ± 0.10bcd	5.4 ± 0.08c	1.1 ± 0.05b	1.2 ± 0.05b	0.35 ± 0.02e	0.36 ± 0.02f
PH-8	3.5 ± 0.05d	4.6 ± 0.1b	4.9 ± 0.13b	5.9 ± 0.13b	1.0 ± 0.05b	1.0 ± 0.03c	0.53 ± 0.02cd	0.58 ± 0.02cd
Male-3	3.7 ± 0.08cd	4.3 ± 0.1bc	4.7 ± 0.10cd	5.3 ± 0.08c	0.9 ± 0.05c	0.9 ± 0.03c	0.61 ± 0.02b	0.61 ± 0.02c

I year : rabi 2014-15 and II year : rabi 2015-16, where, values are mean values ± standard error, Values with same letters statistically on par; and with different letters are significantly different

upon for varietal identification which is coherent with the findings of Kumar *et al.* (2004) in pearl millet. Significant differences were found for all the characters studied among the genotypes indicating good amount of variability and distinguishability among hybrids and their parental lines.

The phenotypes were found to be consistently expressing under both the crop growth seasons, indicated that these traits could be used for characterizing and distinguishing tomato genotypes. Of the twelve characters studied, seven characters, viz. internode length between 1st and 4th

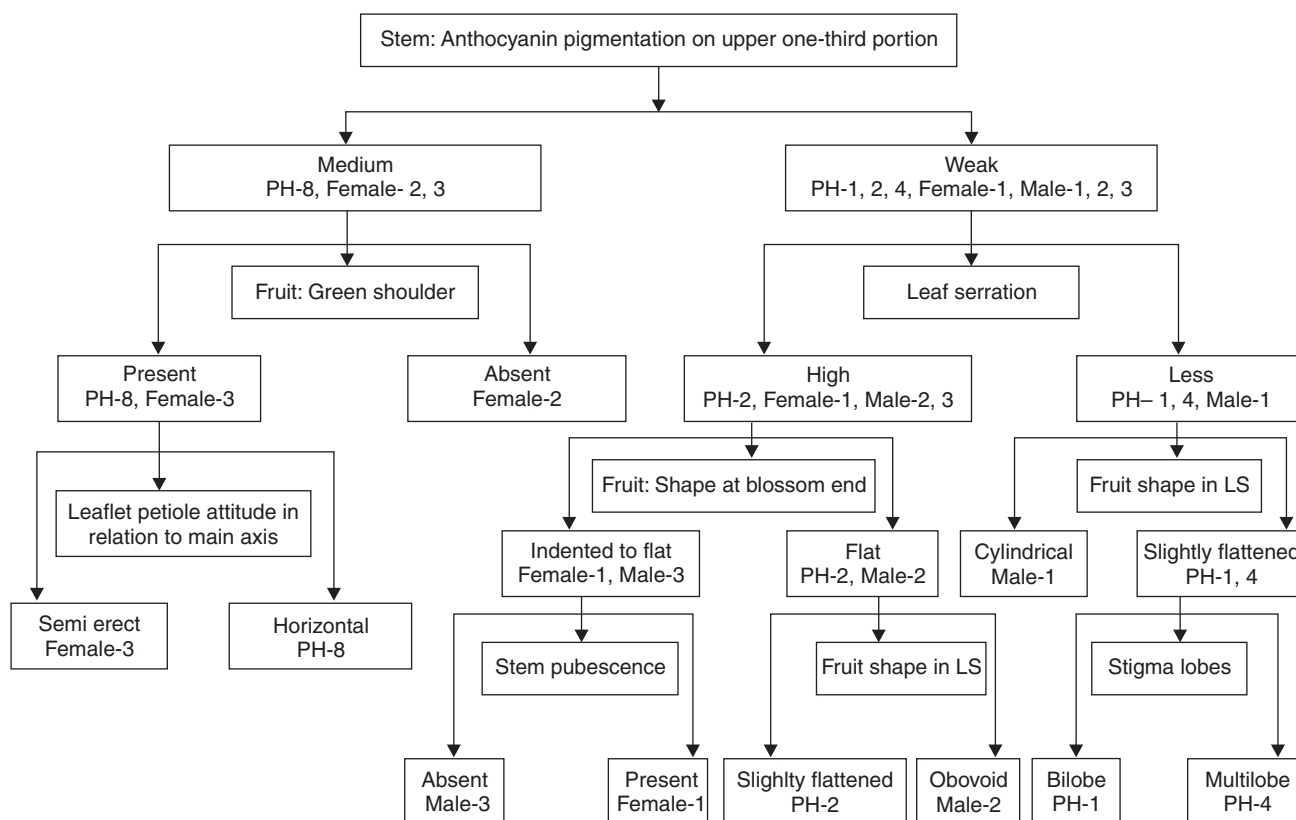


Fig 1 Morphological markers for identification of tomato hybrids and their parental lines.

inflorescence, leaf width, leaflet width, calyx size, fruit width, fruit scar diameter and pericarp thickness were polymorphic. Remaining five characters, viz. leaf length, leaflet length, peduncle length, fruit weight and fruit length were found to be monomorphic. Hence, these characters could be employed for clear distinction between hybrids with their parental lines. The inconsistencies in data for rest of the characters did not qualify for establishing distinctness of the hybrids with their parental lines.

The basic objective of varietal characterization is to establish the distinctiveness among the genotypes, which could be handy for the official regulatory bodies, to have a basis for granting rights and thereby to protect the interests of the breeders and farmers (Joshi *et al.* 2007). All the ten genotypes were characterized individually by employing characters, viz. stem anthocyanin pigmentation on upper one-third portion, fruit green shoulder, leaflet petiole attitude with respect to main axis, leaf serration, stem pubescence, fruit shape at blossom end, fruit shape in longitudinal section and stigma lobes, which resulted in distinctive profiles for all the hybrids and their parental lines studied (Fig 1). Distinctive profiles to identify different genotypes were made in oats (Sumathi *et al.* 2014), jute (Begum and Kumar 2014), pearl millet (Singh *et al.* 2016) and coriander (Singh and Verma 2015).

Thus, the morphological characters in their stable expression forms could be successfully employed for identification of hybrids and their parental lines of tomato in the present study. Further, the distinctive profiles could be

registered for plant breeders or farmers rights for registration under PPV and FRA. A narrow genetic base as shown by the monomorphism in bulk of the traits studied necessitates for complementation of morphological markers in establishing the distinctiveness of closely related genotypes.

SUMMARY

Four hybrids, viz. PH-1, 2, 4 and 8 and their parental lines were studied for developing identification keys using morphological characters. Out of 39 characters studied, fourteen qualitative and six quantitative characters were polymorphic for identifying the parents from the hybrids and also among themselves. Characters like stem anthocyanin pigmentation on upper one-third portion, fruit green shoulder, leaflet petiole attitude with respect to main axis, leaf serration, stem pubescence, fruit shape at blossom end, fruit shape in longitudinal section and stigma lobes were important for development of identification keys. These characters were stable during two growing seasons (*rabi* 2014-15 and *rabi* 2015-16) and no variation was observed for any character in any genotype. A combination of these morphological markers could effectively utilized for distinguishing all the hybrids and their parental lines under study

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