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ANALYSIS OF INTRA- CULTIVAR HETEROGENEITY IN DASHEHARI MANGO (*MANGIFERA INDICA* L.) BY USING SCANNING ELECTRON MICROSCOPY

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Abstract

The present study aimed at estimating intra-cultivar heterogeneity in Dashehari cultivar of mango (*Mangifera indica* L.) by using Scanning Electron Microscopy (SEM). Forty-five trees of 25-30 years old, from 15 orchards in Malihabad and Mall blocks of district Lucknow, Uttar Pradesh were selected for study which revealed a significant variation in all stomatal characteristics. Phenotypic and Genotypic coefficient of variation (PCV and GCV, respectively) values for these parameters were very close, indicating that they were controlled at genetic level and were independent of environmental effect. Highest phenotypic (41.59) and genotypic (38.27) coefficient of variation, and Genetic advance as percent of mean (GAM) (149.41 %) were recorded for stomatal pore size (width) while highest (97.50) heritability (h^2) and genetic advance (20.86) were observed for stomatal density. Dendrogram clusters of the Dashehari population under study, prepared based on stomatal observations, divided into two main clusters which further divided into sub-clusters. The study on stomatal characters is fully facilitated by SEM and hence, it is suggested that SEM may be a useful tool for identification of intra-varietal variability of Dashehari mango for further crop improvement programme.

Scanning electron microscopy (SEM) is an ideal technique for examining plant anatomical characters at high resolution (Pathan *et al.* 2010) and germplasm identification through morphological and anatomical traits of pollen morphology (Ghanati 2005) and polymorphism (Zhang *et al.* 1999, Li *et al.* 2002) along with other research tools such as molecular marker.

Dashehari is an important export variety of mango from India for its attractive appearance, excellent taste and pleasing flavour. It is cultivated on a commercial level in north India in the Agri-Export zone for mango in Malihabad of Uttar Pradesh state. Orchards have been established through clonal propagation. Yet a large variation has been seen in plants, fruits, stone and kernel morphology, production and quality which show intra-varietal variability even within the same orchards. There are very few reports on the intra-cultivar variability of certain mango cultivars on the basis of morphological traits (Gan *et al.* 1981, Begum 2014) although prominent variation in the landraces Banganapalli, Langra and Dashehri and some variation in the cultivar Mallika were detected on the basis of fruit morphological characters (Singh *et al.* 2009). This intra-cultivar variability may have serious economic ramifications for mango orchardists who may not be procuring plants from standard reliable sources and unknowingly establish mango orchards with less productive morphotypes of a cultivar that may lead to lose its export potential near future.

The prime advantages of studying morphological traits to determine germplasm variability are simple and rapid, inexpensive assays, even from herbarium specimens and other dead tissues, although there are several disadvantages *viz.* limitation in number, lack of decisiveness and morphological expression being influenced by environmental effect besides the limited scope for

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improvement through selection on the basis of these traits (Begum *et al*. 2014).Stomatal characteristics (i.e., size and density) are highly variable depending on the genetic background of the plants as well as on the growth conditions or the leaf ontogeny (Masarovicova 1991) and stomatal density has been shown to vary significantly within individuals, cultivars or ecotypes of a single species, as well as within a community (Jones 1992). Within the *Populus* genus, a wide inter-specific as well as inter-clonal variation in stomatal density, dimension and stomatal index has already been observed (Ferris *et al.* 2002) as well as in wheat (Ahmad 1990, Nayeem and Garskin 1990).

Hence, characterization of intra-varietal heterogeneity in Dashehari studied through morphological traits (Kishor *et al.* 2019) was further established through stomatal characterization in the present study, since, stomatal initiation is regulated at the genetic level (Casson and Hetherington 2010), using scanning electron microscopy as a tool for further elucidation of results.

Three plants each, 25-30 years old from 15 different orchards were selected for study from Agri-Export Zone for mango in the Mall and Malihabad blocks of district Lucknow, Uttar Pradesh, India.

Old leaves of uniform age (5-7 months) and physiological maturity were collected as per leaf sampling technique for mango (Poffley 2005) and samples were prepared as per procedure given by Fischer *et al.* (2013) with minor modifications (Fig. 1). Variations in stomatal characters *viz.*, length (µm), width (µm), pore size [length (µm) and width (µm)] and trichome size [length (µm) and width (µm)] of stomata was observed at 5000X magnification and stomata density (μm^{-2}) at 500X magnification by using the scanning electron microscope (SEM) (JSM-6490LV (JEOL, Japan). Analysis of variance using a randomized block design was done for all the characters by ICAR-SPAR (Statistical Package for Agricultural Research). The Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), heritability (h^2), Genetic advance (GA) and Genetic advance as percent of mean (GAM%) were calculated as per standard procedure (Allard, 1960, Singh and Chaudhury 1979 and Falconer 1989). A dendrogram was prepared based on the stomatal characteristics data using SPSS (Statistical package for social science) software for establishing phylogenetic relationship among the sample morphotypes.

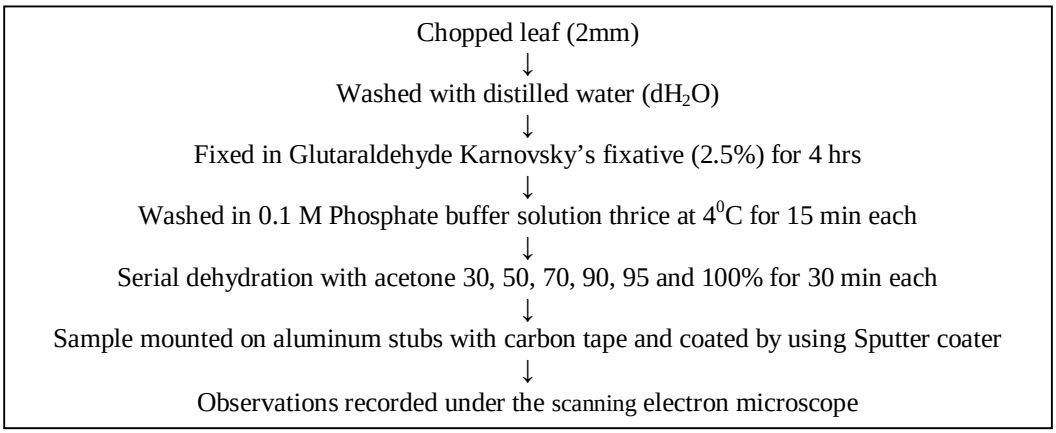

Fig. 1. Procedure for scanning electron microscopy of mango leaf (modified from Fischer *et al.* 2013).

Table 1. Clonal variability in stomatal characteristics of mango (*Mangifera indica* L.) leaf cv. Dashehari.

Morphotypes	Stomatal length (µm)	Stomatal width (µm)	Stomatal pore size		Trichome Size		Stomatal density (μm^{-2})
			Length (µm)	Width (µm)	Length (µm)	Width (µm)	
DM_1	11.13	11.12	1.96	4.03	38.85	45.20	29.0
DM_2	12.07	10.83	3.97	5.17	40.07	40.80	43.0
DM_3	9.75	8.48	2.40	4.24	30.22	39.20	37.0
DM_4	10.44	8.26	2.56	2.97	32.21	31.57	37.0
DM_5	16.30	15.00	2.40	4.01	33.66	34.80	44.0
DM_6	8.73	7.36	3.89	2.40	31.64	32.80	39.0
DM_7	10.39	10.45	3.20	5.15	32.69	39.30	40.0
DM_8	8.93	7.76	3.32	4.56	36.01	39.60	36.0
DM_9	8.48	7.12	2.92	4.64	32.02	32.00	40.0
DM_{10}	8.00	7.68	2.00	3.21	28.98	30.41	25.0
DM_{11}	9.08	9.08	2.60	4.72	32.40	34.80	35.0
DM_{12}	9.52	8.88	3.72	5.12	32.80	33.21	33.0
DM_{13}	8.32	7.44	3.32	4.64	29.67	30.82	36.0
DM_{14}	7.92	7.48	2.52	3.24	33.20	32.41	31.0
DM_{15}	7.24	7.80	2.44	2.92	32.00	31.20	27.0
DM_{16}	6.00	5.32	1.80	2.32	35.20	37.20	46.0
DM_{17}	9.32	6.52	2.68	5.45	34.86	35.20	37.0
DM_{18}	7.00	6.80	2.80	4.08	34.40	32.00	31.0
DM_{19}	6.04	5.12	2.72	4.00	29.62	31.20	40.0
DM_{20}	6.53	5.76	2.52	3.52	36.88	34.00	35.0
DM_{21}	6.84	5.68	3.56	3.80	36.88	34.00	28.0
DM_{22}	6.36	6.88	2.88	3.92	30.00	32.00	35.0
DM_{23}	6.88	5.80	2.40	4.04	35.60	35.20	38.0
DM_{24}	6.84	6.12	2.68	3.24	27.60	28.81	30.0
DM_{25}	7.00	5.92	2.72	4.08	33.66	38.40	28.0
DM_{26}	7.36	7.00	2.68	4.12	36.80	41.60	31.0
DM_{27}	8.93	6.40	2.80	3.76	34.86	40.43	42.0
DM_{28}	17.56	12.72	6.56	11.70	31.20	32.81	24.0
DM_{29}	6.59	7.48	2.16	3.52	38.82	40.01	32.0
DM_{30}	7.36	6.08	2.60	3.48	42.82	40.40	35.0
DM_{31}	7.01	6.40	3.32	3.16	32.60	32.81	33.0
DM_{32}	15.62	11.20	6.16	10.59	41.83	40.80	26.0
DM_{33}	8.03	6.91	2.41	4.22	32.40	30.80	35.0
DM_{34}	7.72	8.00	3.24	3.57	34.88	34.04	34.3
DM_{35}	7.69	8.16	3.52	4.28	35.60	33.60	33.0
DM_{36}	7.48	8.51	4.52	4.06	32.82	32.81	35.0
DM_{37}	6.68	6.84	3.20	2.60	33.20	33.20	39.0
DM_{38}	7.12	5.92	2.44	5.92	32.02	30.41	39.0
DM_{39}	7.84	7.28	3.24	4.68	33.20	35.61	31.0
DM_{40}	7.08	7.40	3.30	3.60	29.62	34.00	36.0
DM_{41}	7.62	8.03	3.62	3.79	34.41	33.21	41.0
DM_{42}	7.43	7.08	3.80	3.77	40.40	38.40	35.0
DM_{43}	6.48	6.76	2.88	4.00	34.06	35.20	36.0
DM_{44}	6.28	5.64	2.40	2.96	28.40	29.60	36.0
DM_{45}	8.00	6.08	1.68	4.00	28.81	28.00	37.3
SE(m)±	0.516	0.385	0.444	0.537	0.470	0.496	0.827
CD (P=0.05)	1.021	0.762	0.879	1.063	0.930	0.982	1.637

SE(m) ±: Standard Error of the Mean, CD: Critical deference at 5% level of significance.

The analysis of variability in stomatal characters studied during the experiment was found significant among the 45 Dashehari morphotypes (Table 1 and Fig. 2A and 2B). The maximum stomatal length (17.56 µm), stomatal pore size (length 6.56 and width 11.70 µm) were recorded for morphotype DM_{28} while stomatal width (15 µm) for morphotype DM_5. The morphotypes DM_{16} showed highest stomatal density (46 μm^{-2}). However, the morphotype $DM_{30}$and DM_1 showed maximum (length 42.82 µm and width 45.20 µm) trichome size. This trend was also seen in earlier studies in stomatal length and density in poplar (Ferris *et al*. 2002, Tognetti *et al.* 2004) which has been used as a parameter for existence of the large clonal variability. Marron (2006) postulated that stomatal traits could be used as early indicators of growth potential in poplar as well as a criterion for clonal discrimination in the genus and stomatal density is reported to differ significantly even among clones belonging to different parentages, between different canopy positions and on leaf surfaces besides varying within leaves, plants and individuals of a single species (Afas *et al.* 2006). Stomatal length has also been reported to correlate with genome size (Aasamaa *et al.* 2001, Beaulieu *et al*. 2008, Xu and Zhou 2008). Therefore, the genetic and developmental basis for high stomatal density and stomatal conductance and its application in germplasm studies is exploited as a research priority in plant physiology, agriculture and paleo-biology (Rocha 2015, Wang *et al.* 2015).

Table 2. Analysis of variances for different stomatal characters in mango (*Mangifera indica* L.) leaf cv. Dashehari.

Characters	Grand mean	Mean range		CV	PCV	GCV	h^2	GA	GAM%
		minimum	maximum						
Stomata length (µm)	8.45	6.0	17.56	7.69	30.79	29.81	93.80	10.36	122.60
Stomata width (µm)	7.60	5.12	15.0	6.65	27.13	26.35	94.30	8.26	108.68
Stomatapore length (µm)	3.04	1.68	6.56	18.46	34.28	28.88	71.0	3.13	102.96
Stomatapore width (µm)	4.27	2.32	11.70	16.29	41.59	38.27	84.70	6.38	149.41
Trichome length (µm)	33.76	27.60	42.82	1.72	10.48	10.34	97.30	14.60	43.24
Trichome width (µm)	34.78	30.41	45.20	1.78	11.33	11.19	95.90	16.31	46.89
Stomata density (μm^{-2})	35.03	24.0	46.0	2.97	14.64	14.34	97.50	20.86	59.54

CV: Coefficient of variation.

The extent of variability among the morphotypes was determined in terms of PCV, GCV, heritability, genetic advance and GAM% (Table 2). The PCV for all the characters was slightly higher than the GCV. The highest PCV (41.59), GCV (38.27) and GAM% (149.41) were observed for stomatal pore size (width) indicating higher degree of genetic variability among the stomatal characteristics and the germplasm. In general, stomatal initiation is controlled by both environmental and genetic factors (Casson and Hetherington 2010) and is indicative of clonal variability. As a quantitative trait, stomatal density is genetically determined (Gailing *et al.* 2008) and stomatal length has been reported to correlate with genome size (Xu and Zhou 2008). In the present study all stomatal characteristics showed narrow differences between PCV and GCV (Table 2) which indicates the negligible environmental effect on these characters and a greater regulation at the genetic level creating scope for further crop improvement through selection at an early stage on the basis of these characters (Majumder *et al.* 2012). These results are in agreement with the findings of Riaz and Chaudhary (2003) who observed genotypic and phenotypic coefficient of variation (7.43 and 7.29%) for stomatal size in wheat indicating that all of the variation for the trait was due to genetic causes and highly heritable.

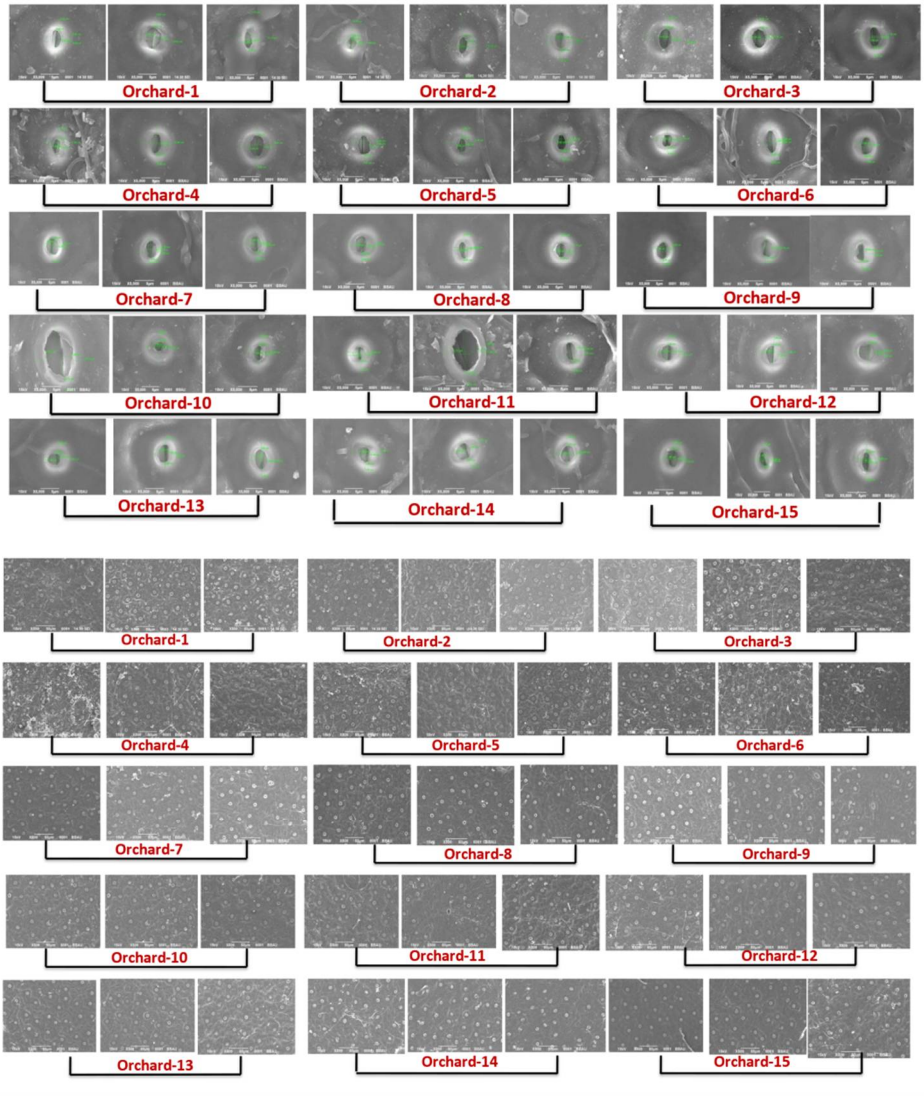

Fig. 2. Intra-varietal variability in stomatal pore size (A) and stomatal density (B) of 45 Dashehari mango morphotypes. (Group marking indicates 3 samples from each selected orchard; green line marked the pore space).

Although the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are the measures of genetic variability however, the amount of genetic gain can be estimated from genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) along with heritability (Majumder *et al.* 2012).Similarly in the present study, higher heritability (97.50) and genetic advance (20.86) were observed for stomatal density (Table 2) which is reported to be genetically determined and controlled by additive genes(Gailing *et al.* 2008). These results are consonance with those of Riaz and Chaudhary (2003) that high estimates of heritability for stomatal characters indicate that these traits are transmitted to the offspring and

were governed by additive gene. Trichome is an epidermal cell and hair like appendages on the plant surface of many angiosperms primarily involved in plant defence mechanism. Irrespective of genetic control of trichomes distribution, their density also depends on tissue (Kang *et al.*2010) and environmental conditions (Wilkens *et al.* 1996). In the present investigation the trichome length showed PCV (10.48%), GCV (10.34%), h^2 (97.30%), GA (14.60%) and GAM (43.24%). However, the trichome width showed PCV (11.33%), GCV (11.19%), h^2 (95.90%), GA (16.31%) and GAM (46.89%). Trichomes, in general, have been proven to be an excellent phenotypic trait for finding evolutionary and taxonomic relationships among species (Khosroshahi and Salmaki 2019). The present results are also consistent with the results of Van Dam *et al* (1999), who found significant variation in the phenotype of trichomes among the populations of *Datura wrightii*, another species in Solanaceae, commonly used in ecological studies (Nunez-Farfan and Dirzo 1994, Valverde *et al*. 2001). Usefulness of any character is related to its onward transmission to the progeny and characters with high heritable are easy to select for breeding purpose. Higher values of heritability of stomatal characters in the present study indicates that either these were simply inherited characters governed by a few major genes or additive gene effects even if, they were under polygenic control and therefore, selection of these characters would be more effective for improvement and can be exploited at an early stage of development of the plants. Thus, heritability and genetic gain (GA) aid in referring valuable conclusion for effective selection in a germplasm. Since SEM is an advanced technique and is precise in the measurements of the stomatal characters which are clearly indicative of variations in a germplasm, hence, this SEM technique could be utilised effectively for the analysis of intra-varietal heterogeneity in mango.

Although, Casson and Hetherington (2010) opined that some stomatal characteristics are generally controlled by both environmental and genetic factors, however, as a quantitative trait, stomatal density is genetically determined (Gailing *et al.* 2008) and stomatal length has been reported to correlate with genome size (Xu and Zhou 2008). Considering the stability of stomatal characteristics at the genetic level as above, a dendrogram was prepared on the basis of stomatal characteristics (Table 3 and Fig. 3) of 45 Dashehari morphotypes in order to establish their relatedness to each other. The 45 Dashehari morphotypes under study were found to be very closely related and grouped into only two major clusters (cluster I and II) with additional sub-clusters, differentiating the morphotypes collected from different areas. Cluster-I consisted of 43 morphotypes which further divided into five sub-groups (cluster IA, IB, IC, ID and IE) while cluster-II comprised two morphotypes which was divided into two sub-groups (cluster IIA and IIB) (Table 3 and Fig. 3).

Table 3. Non-hierarchicaleuclidean cluster analysis in 45 Dashehari mango morphotypes.

Clusters	Morphotypes
Cluster I	DM_1, DM_2, DM_3, DM_4, DM_5, DM_6, DM_7, DM_8, DM_9, DM_{10}, DM_{11}, DM_{12}, DM_{13}, DM_{14}, DM_{15}, DM_{16}, DM_{17}, DM_{18}, DM_{19}, DM_{20}, DM_{21}, DM_{22}, DM_{23}, DM_{24}, DM_{25}, DM_{26}, DM_{27}, DM_{29}, DM_{30}, DM_{31}, DM_{33}, DM_{34}, DM_{35}, DM_{36}, DM_{37}, DM_{38}, DM_{39}, DM_{40}, DM_{42}, DM_{43}, DM_{44}, DM_{45}
Cluster II	DM_{28}, DM_{32}

The information obtained from intra-varietal diversity analysis can be utilized in making crosses and selection of divergent parents to maximize heterosis in future intra-varietal breeding programmes on the basis of fruit, stone and kernel morphology (Anatov 2020, Kishor *et al.* 2020) and stomatal characteristics.

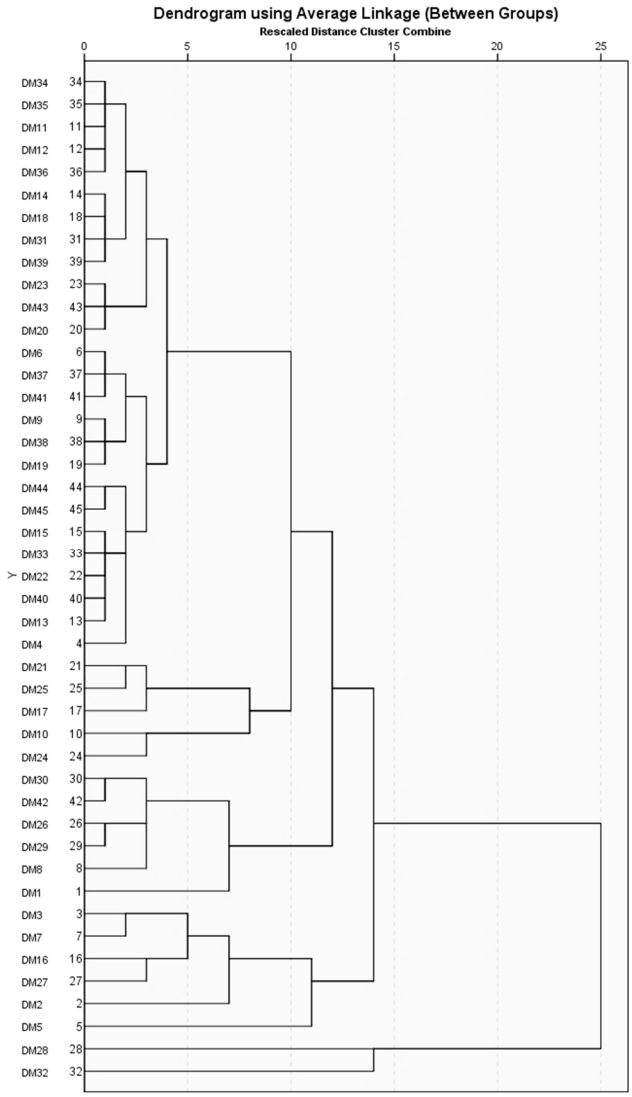

Fig. 3. Dendrogram of 45 Dashehari mango morphotypes based on stomatal characteristics.

Variability in stomatal characters studied was found significant among the 45 Dashehari morphotypes of mango cv. Dashehari, an important export variety from the state of Uttar Pradesh in North India. The highest PCV (41.59), GCV (38.27) and GAM% (149.41) were observed for stomatal pore size (width) while higher heritability (97.50%) and genetic advance (20.86) were observed for stomatal density indicating higher degree of genetic variability among the stomatal characteristics of the studied morphotypes. This statistically significant intra-varietal variation was recorded in stomatal characteristics possibly because of lack of availability of true-to-type planting material at the time of establishment of these orchards about 30 years ago. It could also be an expression of the adaptations of trees to variable microenvironment and edaphic factors of the orchards under study or of the stionic effect in the plants since they are all propagated on seedling

rootstocks through approach grafting. Accordingly, the samples grouped into two clusters which subdivide into further groups.

Stomatal characters indicate that these traits are transmitted to offspring and are governed by additive genes. Usefulness of any character is related to its onward transmission to progeny and characters with high heritability are easy to select for breeding purpose (Riaz and Chowdhry 2003). Since SEM is an advanced technique and is precise in the measurements of the stomatal characters which are clearly indicative of variations in a germplasm, hence, this SEM technique could be utilised effectively for the analysis of intra-varietal heterogeneity in mango.

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