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P Sridevi
Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

M Sankaran
Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

KV Ravishankar
Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

KS Shivashankara
Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru, Karnataka, India

P Vinay Kumar Reddy
Department of Horticulture, College of Horticulture, Venkataramannagudem, West Godavari, Andhra Pradesh, India

Corresponding Author:
P Sridevi
Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

Studies on physiological and biochemical characterization of certain selected half sib populations in mango (*Mangifera indica* L.)

P Sridevi, M Sankaran, KV Ravishankar, KS Shivashankara and P Vinay Kumar Reddy

Abstract
The present study was conducted at IIHR, Bengaluru during 2019 & 2020 with 8 mango half sib populations consists of 200 progenies (Lazzat Baksh, Kesar, Goa Mankurad, Malanji, Dashehari, Sensation, Himsagar and Mandoor Khatta) were assessed and compared using physiological and biochemical characters such as stomata number, stomata size, total chlorophyll content and total phenol content and descriptive statistics analysis was done. Among the eight half sib groups studied, the highest mean stomata number (1244 / mm^2) and total phenol content (197.81 mg / 100g F.W) was recorded by Goa mankurad half sib population. Highest mean stomata size was noticed with Malanji half sib population (252.16 µm). Mandoor khatta half sib group recorded highest mean total chlorophyll content (6.69 mg/100 g F.W).

Keywords: Mango, half sibs, characterization, physiological and biochemical characters

Introduction
Mango *(Mangifera indica* L.) is one of the ancient fruits of India, and its cultivation dates back to 4000 years (Mukherjee, 1953; Kostermans and Bompard, 1993) [13, 12]. The genus *Mangifera* originated from the Indo-Burma region. It is one of the choicest and admired fruit crops of the tropical and subtropical areas of the world. It is considered the "King of fruits" due to consumers' preference, production scale, exquisite flavour, savoury taste, appealing colour, and high nutritive value. The cross-pollination nature and a wide range of prevailing agro climatic conditions have contributed to its wide genetic diversity in India in mango. Mango has been reported to have extensive diversity due to alloploidy, outbreeding and phenotypic differences arising from varied agro-climatic conditions in different mango growing regions. Leaf stomata are considered as a gate between plant and atmosphere which play an important role in response to different environmental conditions. Understanding stomatal functioning and its role is important since stomata control both photosynthesis, i.e. the production potential, and transpiration, which in turn influences irrigation management. Moreover, it is well known that stressing conditions which induce stomatal closure are needed for flowering in mango. The first striking feature is that mango seems to be well protected against excessive losses of water through transpiration. Stomatal frequency is high but stomata are small, which allows for quick responses to relevant stimuli. At the same time stomatal numbers are not directly related to photosynthesis but stomata have a direct relationship with water use efficiency and transpiration in plants.
Photosynthesis is the basis for growth, development and yield in plants, but perennial trees like mango have very low orchard efficiency (Chacko and Randhawa, 1971) [4]. Earlier studies on different mango genotypes showed significant variation in gas exchange parameters, production and translocation of photosynthates which are important in meeting the urgent requirement of sink (Singh and Rajan, 2009) [17]. Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. It is also reported that, photosynthesis, has a strong association with chlorophyll content, photosynthate production, total sugar concentration and specific leaf weight (SLW) in mango and other crops (Saini and Joshi, 1989; Nii *et al.*, 1995) [18]. Phenolic compounds are a group of antioxidants that have the function of suppressing cell damage, caused by free radicals, by giving electrons speedily and transforming the free radicals into stable forms, in order to prevent oxidative damage that cause ailments.

Many mango cultivars are being popularly grown in different agro-ecological regions of India for several decades. However, limited studies have been carried out on leaf physiological characteristics of these cultivars. Such studies are not only important for the identification of species, but also to determine their genetic divergence. Therefore, the present investigation was conducted to determine the variation in leaf physiological and biochemical characteristics across the selected mango half sib groups viz., Lazzat baksh, Kesar, Goa Mankurad, Malanji, Dashehari, Sensation, Himsagar and Mandoor Khatta).

Experimental material and methods

The experimental material for present investigation was comprised of 200 progenies (8 half sib mango populations) collected from different mother varieties maintained at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru. These eight half sib groups include Lazzat baksh, Kesar, Goa mankurad, Malanji, Dashehari, Sensation, Himsagar and Mandoor khatta. All the half sib groups were provided with standard agronomic practices such as irrigation, nutrient and pest management. Data were recorded on four important parameters includes stomata number / mm^2, stomata size (µm), total chlorophyll content and total phenol content. At the beginning of experiment, the age of half sib groups was 2 years old. The statistical mean was calculated using the method suggested by Goulden (1952) [8]. Range was calculated based on the difference between the lowest and the highest values present in the half sib population. The coefficient of variation was computed according to Burton and Devane (1953) [2].

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Results and Discussion

Significant differences were observed in stomata number among the half sib populations. On analyzing the data from table 1, the stomata number varied from 750 to 1600 stomata / mm^2 among the 8 half sib populations. In Lazzat baksh half sib group maximum and minimum stomata number recorded were 750 / mm^2, 1550 / mm^2 respectively. In Kesar half sib group maximum stomata number recorded was 1600/ mm^2 and minimum stomata number recorded was 850 / mm^2. In Goa mankurad half sib group maximum and minimum stomata number observed were 1700/ mm^2, 900 / mm^2 respectively. In case of Malanji half sib group, maximum stomata number recorded was 1400/ mm^2 and minimum stomata number recorded was 800/ mm^2 cm. Dashehari half sib group had maximum stomata number of 1400 / mm^2 and minimum stomata number of 650 / mm^2. In Sensation half sib population, maximum and minimum stomata numbers recorded were 1300 / mm^2 & 950 / mm^2 respectively. In Himsagar half sib population, maximum stomata number recorded was 1400 / mm^2 cm and minimum stomata number recorded was 950 / mm^2. In case of Mandoor khatta half sib population, maximum and minimum stomata number recorded were 1350 / mm^2, 950 / mm^2 respectively. Mean stomata number among the half sib populations varied from 1080 / mm^2 to 1244 / mm^2 as illustrated in Fig 1. Highest mean stomata number was recorded by Goa mankurad half sib group (1244 / mm^2) followed by Kesar (1232 / mm^2), Lazzat Baksh and Malanji (1184/ mm^2), Dashehari (1180/ mm^2). Lowest mean stomata number was recorded by Sensation (1184/ mm^2) followed by Himsagar (1118/ mm^2) and Mandoor khatta (1126/ mm^2) as depicted in figure 1. Highest coefficient of variation for stomata number was recorded by Dashehari (18.46%) half sib population followed by Lazzat Baksh half sib group (17.48%). Leaf stomata are considered as a gate between plant and atmosphere which play an important role in response to different environmental conditions. At the same time stomatal numbers are not directly related to photosynthesis but stomata have a direct relationship with water use efficiency and transpiration in plants. It is evident from the data that significant variations were detected for stomata number among the half sib families. The stomatal density and patterning is variable among the species, but it is regulated by a mechanism that sustains a minimum of one cell spacing between stomata. These results were in conformity with the findings reported by Ganopichyagrai *et al*. (2016) [9] investigated macroscopic, microscopic leaf characteristics, and genetic relationship among 17 cultivars selected from six groups of mango in Thailand. Selected 17 Thai mango cultivars had individually macroscopic characteristics based on fruits and leaves. For microscopic characteristics, the stomatal number, veinlet termination number, and palisade ratio were slightly differentiable. Cahyanto *et al.* (2017) [3] reported that stomatal density, size and index are less powerful to be applied as characters to identify cultivars of mangoes but they are important means of gas exchange in plants.

Data in table 2 exhibited the stomata size varied from 36.73 cm to 517.99 µm among the half sib populations. In Lazzat Baksh half sib group maximum and minimum stomata number recorded were 348.90 µm, 146.63 µm respectively. In Kesar half sib group maximum stomata size recorded was 347.70 µm and minimum stomata size recorded was 97.33 µm. In Goa mankurad half sib group maximum and minimum stomata sizes observed were 517.99 µm, 117.30 µm respectively. In case of Malanji half sib group, maximum stomata size recorded was 389.64 µm and minimum stomata size recorded was 110.12 µm. Dashehari half sib group had maximum stomata size of 350.53 µm and minimum stomata size of 145.97 µm. In Sensation half sib population, maximum and minimum stomata size recorded were 185.22 µm & 106.23 µm respectively. In Himsagar half sib population, maximum stomata size recorded was 347.01 µm and minimum stomata size recorded was 163.73 µm. In case of Mandoor khatta half sib population, maximum and minimum stomata size recorded were 192.22 µm, 36.73 µm respectively. Mean stomata size varies from 129.53 µm to 252.16 µm among the half sib populations as shown in Fig 2. Highest mean stomata size was observed by Malanji half sib group (252.16 µm) followed by Lazzat Baksh (230.99 µm), Himsagar (198.8 µm) and Dashehari half sib population (186.29 µm). Lowest mean stomata size was observed by Mandoor khatta half sib group (129.53 µm) followed by Kesar (135.41 µm), Sensation (144.1 µm) and Goa mankurad half sib group (148.22 µm). Highest coefficient of variation for stomata size was recorded by Goa mankurad half sib group (41.99%) followed by Kesar half sib population (30.18%). In mango the stomatal density is inversely proportional to the size of the stomata which means a leaf with higher stomata density will has smaller stomata. In dry condition, much smaller stomata will provide rapid response to change in water regime in plant while at the same time its high density maximise diffusion of CO2 for optimal photosynthesis (Aasamaa *et al*., 2001) [1]. High stomatal

density is associated to higher stomata conductance and transpiration which is necessary for water and nutrient in xylem, while the smaller stomata allow better stomatal resistance and control during water stress. By this study it is clearly evident that stomata size significantly varied with respect to 8 half sib populations. Selection of cultivars having small stomata size for improving the trait specific character will be followed in future.

Persual of data from table 3 revealed that the chlorophyll content varied from 0.24 mg to 8.12 mg among the half sib populations. In Lazzat Baksh half sib group maximum and minimum chlorophyll contents recorded were 4.44 (mg /100 g), 1.48 (mg/100 g) respectively. In Kesar half sib group maximum chlorophyll content recorded was 4.17 (mg /100 g) and minimum chlorophyll content recorded was 0.24 (mg /100 g). In Goa mankurad half sib group maximum and minimum chlorophyll contents observed were 4.27 (mg /100 g), 0.56 (mg /100 g) respectively. In case of Malanji half sib group, maximum chlorophyll content recorded was 6.22 (mg /100 g) and minimum chlorophyll content recorded was 0.58 (mg /100 g). Dashehari half sib group had maximum chlorophyll content of 5.25 (mg /100 g) and minimum of chlorophyll content of 0.90 (mg /100 g). In Sensation half sib population, maximum and minimum chlorophyll contents recorded were 4.23 (mg /100 g) & 2.45 (mg /100 g) respectively. In Himsagar half sib population, maximum chlorophyll content recorded was 4.43 (mg /100 g) and minimum chlorophyll content recorded was 0.62 (mg /100 g). In case of Mandoor khatta half sib population, maximum and minimum chlorophyll content recorded were 8.12 (mg /100 g), 2.30 (mg /100 g) respectively. Among eight half sib populations, mean chlorophyll content varied from 1.78 (mg/100 g) to 6.69 (mg/100 g) as illustrated in Fig 3. Highest mean total chlorophyll content was recorded by Mandoor khatta half sib group (6.69 mg/100g F.W) followed by Sensation (3.51 mg/100g F.W), Himsagar (3.46 mg/100g F.W), Lazzat Baksh (2.93 mg/100g F.W) and Malanji half sib group (2.91 mg/100g F.W). Lowest mean total chlorophyll content was recorded by Goa mankurad half sib population (1.78 mg/100g F.W) followed by Dashehari (2.22 mg/100g F.W) and Kesar half sib population (2.60 mg/100g F.W) as shown in fig 3. Highest coefficient of variation for total chlorophyll content was recorded by Goa mankurad half sib population (52.24%) followed by Dashehari half sib group (42.34%) among the eight half sib populations. Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. The chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant. Variation in leaf chlorophyll content can provide information about the physiological condition of a leaf or plant. Pramod *et al.* (2015) [16] reported that the age of leaves was an important factor for chlorophyll content. In his study A total of ten plant species were selected namely Mango (*Magnifera indica*), Hibiscus (*Hibiscus rosa sinensis*), Gauva (*Psidium guajava*), Almond (*Prunus dulcis*), Bryophyllum (*Bryophyllum pinnatum*), Sapodilla (*Manikara zapota*), Neem (*Azadiracta indica*), Ashoka (*Polyalthia longifolia*), Ficus (*Ficus benjamina*) and Datura (*Datura metal*) and were used for analysis of chlorophyll content. In all the cases the adult leaves showed higher chlorophyll content in comparison to young leaves. Pandey and Tyagi (1999) [15] reported that total chlorophyll content changes with tree size, age and reproductive periods in selected mango cultivars.

The light harvesting pigments are involved in light capture and photosynthesis in leaves. Hence, changes in pigment content in leaves can affect photosynthesis of plants. In mango, the pigment content is influenced by different seasons, cultivars, growth and maturity stages of leaves. The cultivars with high chlorophyll content can produce higher biomass and increase photosynthesis. In matured mango leaf, increase in photosynthesis with increasing chlorophyll content has been reported (Nii *et al*., 1995) [14]. Whereas. Singh and Bhargava (2011) [19] found that Kesar had the maximum chlorophyll content (a, b, and total). Jyothi *et al.* (1998) [11] reported that Chlorophyll a, b and total chlorophyll contents differed significantly which was higher in Neeleshan, Mallika, Neelum and Totapuri than in Alphanso, Baneshan and Dashehari.

Data in Table 4 and figure 4 illustrated, total phenol content varied from 36.83 mg to 270.90 mg. In Lazzat Baksh half sib group maximum and minimum total phenol content recorded were 193.24 (mg /100 g), 65.41 (mg/100 g) respectively. In Kesar half sib group maximum total phenol content recorded was 164.20 (mg /100 g) and minimum total phenol content recorded was 83.08 (mg /100 g). In Goa mankurad half sib group maximum and minimum total phenol content observed were 270.90 (mg /100 g), 138.02 (mg /100 g) respectively. In case of Malanji half sib group, maximum total phenol content recorded was 213.37 (mg /100 g) and minimum total phenol content recorded was 79.72 (mg /100 g). Dashehari half sib group had maximum total phenol content of 233.99 (mg /100 g) and minimum total phenol content of 57.18 (mg /100 g). In Sensation half sib population, maximum and minimum total phenol content recorded were 193.39 (mg /100 g) & 105.91 (mg /100 g) respectively. In Himsagar half sib population, maximum total phenol content recorded was 213.97 (mg /100 g) and minimum total phenol content recorded was 59.88 (mg /100 g). In case of Mandoor khatta half sib population, maximum and minimum total phenol content recorded were 192.19 (mg /100 g), 36.83 (mg /100 g) respectively. Among eight half sib populations, mean total phenol content varied from 92.01 (mg/100 g) to 197.81 (mg/100 g) as shown in Fig 4. Highest mean total phenol content was recorded by Goa mankurad half sib group (197.81 mg/100 g) followed by Sensation (180.08 mg/100 g), Dashehari (166.6 mg/100 g), Malanji (159.78 mg/100 g) and Himsagar (144.37 mg/100 g) half sib population. Lowest mean total phenol content was recorded by Mandoor khatta (92.01 mg/100 g) half sib population followed by Kesar (108.65 mg/100 g) and Lazzat Baksh half sib group (137.04 mg/100 g). Highest coefficient of variation for total phenols was recorded by Mandoor khatta (30.64%) followed by Lazzat Baksh half sib group (25.04%). It is evident from the data that significant differences were observed for total phenol content among the half sibs. Phenolic compounds are a group of antioxidants that have the function of suppressing cell damage, caused by free radicals, by giving electrons speedily and transforming the free radicals into stable forms, in order to prevent oxidative damage that cause ailments. The presence of antioxidants in plants is primarily as a protective compound from pest and disease, known as bioactive compounds. Grundhofer *et al.* (2001) [10] explains that mango is a rich source of many phytochemical compounds. Main compounds such as phenolic and flavonoid can obtained from various parts such as fruit, kernel, leaves, and bark. Previous research related to the antioxidant potential of wild mango species from Sumatra had been

carried out qualitatively by Fitmawati *et al*. (2020) [6]. In this study, there is a necessity to conduct quantitative phytochemical analysis to determine the level of phenolic compounds. Singh *et al.* (2012) [20] observed that mean phenol content of leaf averaged over different mango varieties was significantly higher at 3–4 months stage (78.48 mg phenols/100g) then at 1–2 months stage (54.66 mg phenols/100g). They further observed the phenol content in 1–2 months old leaves of Amrapali and Langra was 60.6 mg phenols/100g and 59.809 mg phenols/100g. The depletion in sugar level was found to be responsible for the accumulation of total phenols since the sugars are utilized for the synthesis of total phenols. Sharma *et al.* (2001) [21] deciphered the role of polyphenol oxidase and phenolic compounds in imparting resistance against diseases. Similar findings were reported by Fitmawati *et al.* (2020) [6] and Emanuela *et al*. (2019) [5] in mango.

Table 1: Descriptive statistics for extent of variability in Stomata number / mm^2 belonging to different half sib group of Mango (*Mangifera indica* L.)

S. No	Half sib group	Minimum	Maximum	Mean ± SE	S.D	C.V (%)
1.	Lazzat Baksh	750	1550	1184 ± 41.4	0.91	20.00
2.	Kesar	850	1600	1232 ± 42.61	0.72	16.25
3.	Goa Mankurad	900	1700	1244 ± 34.79	0.76	15.67
4.	Malanji	800	1400	1184 ± 27.34	0.70	15.94
5.	Dashehari	950	1300	1080 ± 39.89	0.62	13.21
6.	Sensation	950	1300	1114 ± 22.4	0.87	20.51
7.	Himsagar	900	1400	1118 ± 27.06	1.22	18.68
8.	Mandoor khatta	950	1350	1126 ± 22.19	0.81	13.79

Table 2: Descriptive statistics for extent of variability in Stomata size (µm) belonging to different half sib groups of Mango (*Mangifera indica* L.)

S. No	Half sib group	Minimum	Maximum	Mean ± SE	S.D	C.V (%)
1.	Lazzat Baksh	146.63	348.90	230.99 ±1.00	66.2	28.68
2.	Kesar	97.33	347.70	135.41 ± 0.90	40.8	30.18
3.	Goa Mankurad	117.30	517.99	148.22 ± 0.80	62.2	41.99
4.	Malanji	110.12	389.64	252.16 ± 0.70	62.2	24.66
5.	Dashehari	145.97	350.53	186.29 ± 0.70	37.2	20.01
6.	Sensation	106.23	185.22	144.1 ± 1.03	19.7	13.67
7.	Himsagar	163.73	347.01	198.8 ± 0.64	33.6	17.01
8.	Mandoor khatta	36.73	192.22	129.53 ± 0.40	26.2	20.24

Table 3: Descriptive statistics for extent of variability in Total Chlorophyll content (mg/ 100 g) belonging to different half sib groups of Mango (*Mangifera indica* L.)

S. No	Half sib group	Minimum	Maximum	Mean ± SE	S.D	C.V (%)
1.	Lazzat Baksh	1.46	4.44	2.93 ± 0.49	1.00	34.12
2.	Kesar	0.24	4.17	2.60 ± 0.47	1.02	39.23
3.	Goa Mankurad	0.56	4.27	1.78 ± 0.38	0.93	52.24
4.	Malanji	0.58	6.22	2.91 ± 0.37	1.07	36.76
5.	Dashehari	0.90	5.25	2.22 ± 0.36	0.94	42.34
6.	Sensation	2.45	4.23	3.51 ± 0.37	0.45	12.82
7.	Himsagar	0.62	4.43	3.46 ± 0.39	0.84	24.27
8.	Mandoor khatta	2.30	8.12	6.69 ± 0.47	1.80	26.90

Table 4: Descriptive statistics for extent of variability in Total Phenol content (mg/ 100 g) belonging to different half sib groups of Mango (*Mangifera indica* L.)

S. No	Half sib group	Minimum	Maximum	Mean ± SE	S.D	C.V (%)
1.	Lazzat Baksh	65.41	193.24	137.04 ±1.30	34.3	25.04
2.	Kesar	83.08	164.20	108.65 ± 0.90	17.1	15.75
3.	Goa Mankurad	138.02	270.90	197.81 ± 7.00	36.2	18.30
4.	Malanji	79.72	213.37	159.78 ± 0.70	28.3	17.75
5.	Dashehari	57.18	233.99	166.6 ± 0.65	31.7	19.06
6.	Sensation	105.91	193.39	180.08 ± 0.40	27.7	15.42
7.	Himsagar	59.88	213.97	144.37 ± 0.30	33.8	23.44
8.	Mandoor khatta	36.83	192.19	92.01 ± 0.39	28.8	30.64

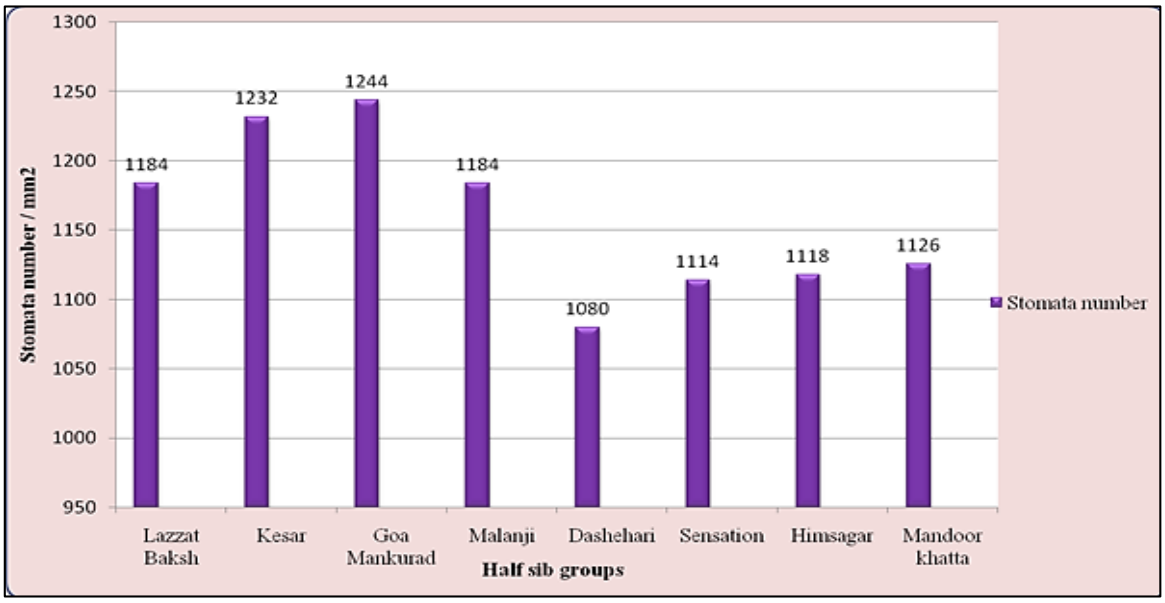

Fig 1: Extent of variability in stomata number / mm^2 among different half sib populations of Mango (*Mangiferaindica* L.)

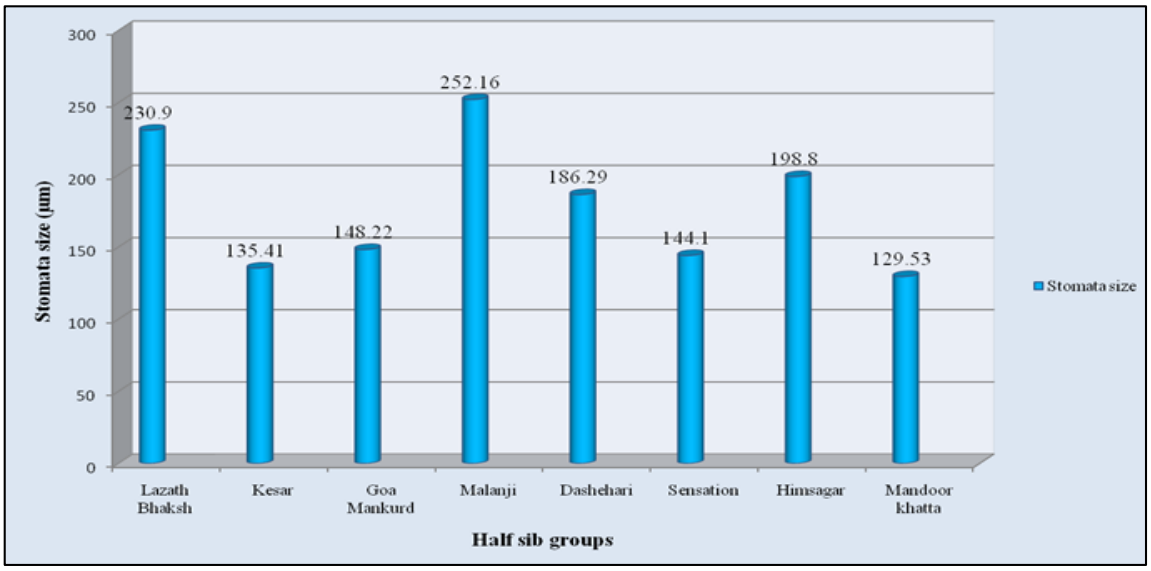

Fig 2: Extent of variability in stomata size (µm) among different half sib populations of Mango (*Mangifera indica* L.)

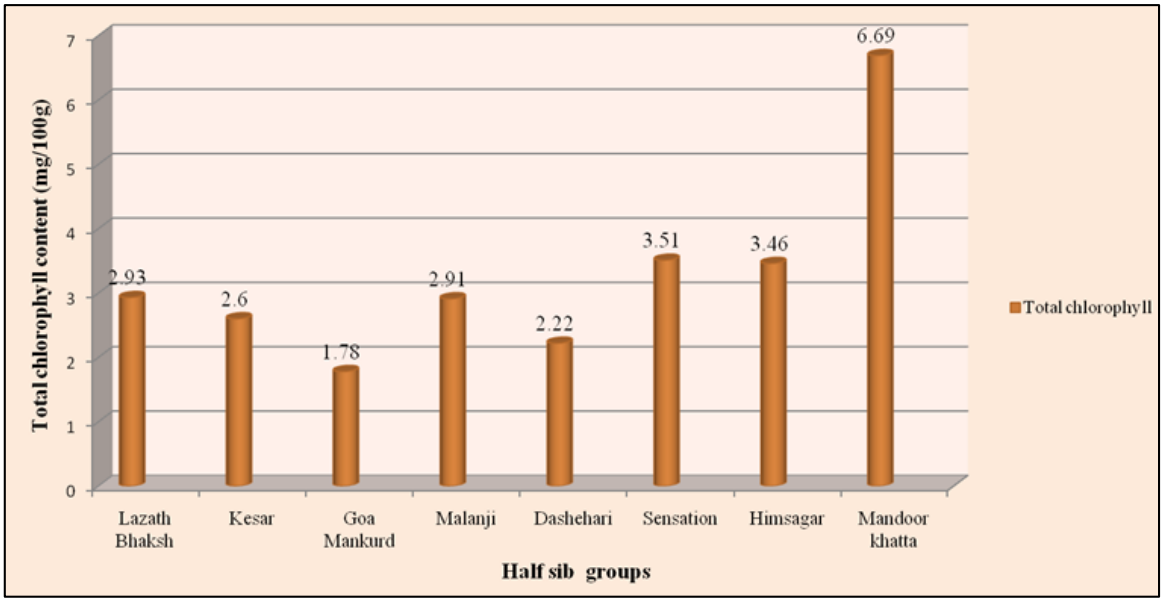

Fig 3: Extent of variability in total chlorophyll content among different half sib populations of Mango (*Mangifera indica* L.)

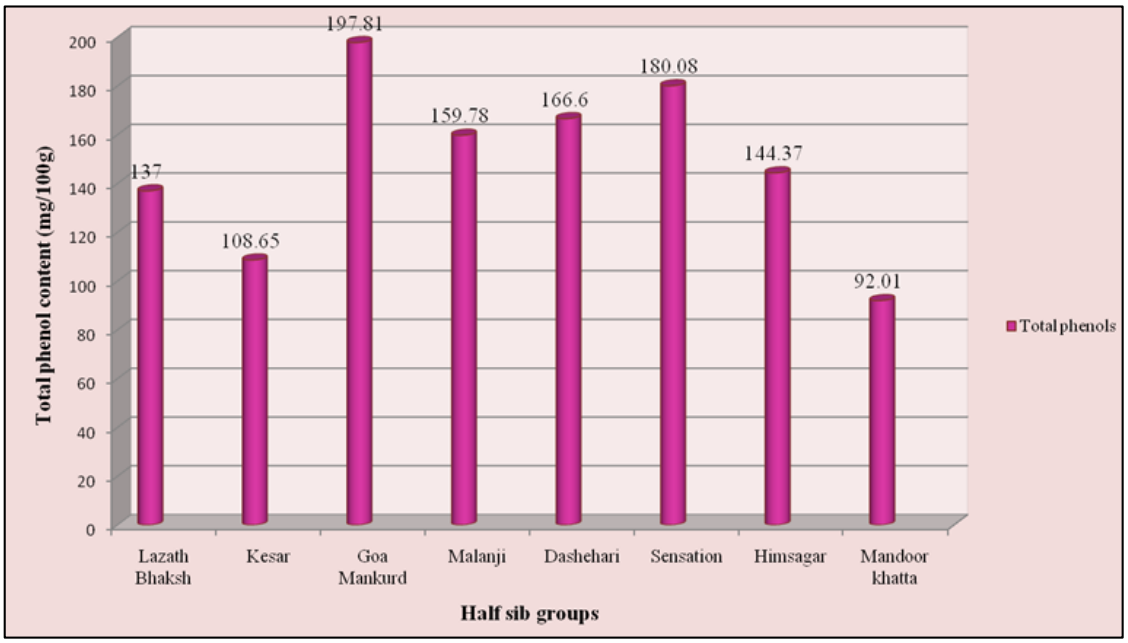

Fig 4: Extent of variability in total phenol content among different half sib populations of Mango (*Mangifera indica* L.)

Conclusion

In conclusion, the study indicated that the eight mango halfsib groups have shown significant differences in gas exchange parameters, total chlorophyll content and total phenols. The variations in stomatal density and size may be related to genotype differences evolved due to a wide range of environmental conditions. Variations in physiological and biochemical parameters also depend on genetic make-up of the tree.

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