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Diversity in leaf morphology and physiological characteristics among mango (*Mangifera indica*) cultivars popular in different agro-climatic regions of India

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ABSTRACT

The present study was aimed to characterize eight different mango cultivars, *viz*., 'Alphonso', 'Borsha', 'Himsagar', 'Fazli', 'Langra', 'Dashehari', 'Totapuri' and 'Neelum' popularly grown in different agro-climatic regions in India. Significant differences in gas exchange and leaf morphological characters were observed among cultivars. Higher photosynthesis rate (P_N) was recorded in 'Borsha', 'Himsagar', 'Langra' and 'Neelum'. Across the cultivars P_N and CE (carboxylation capacity) were highest in 'Neelum' followed by 'Borsha'. 'Langra recorded highest WUE and WUEi. The SLA and SLW were maximum in 'Totapuri' and 'Dashehari' respectively. Maximum epicuticular wax content (ECW) was observed in 'Totapuri'. The content of Chl a, Chl b, total chlorophyll and carotenoids was highest in 'Alphonso'. Adaxial and abaxial stomatal numbers were highest in 'Totapuri' and 'Langra', respectively. The variations in leaf morphology, gas exchange and related traits may be linked to genotypic variation suitable for different agro-climatic regions in India.

1. Introduction

Mango (*Mangifera indica* L.) is one of the choicest fruit crops of tropical and sub-tropical regions of the world. Its popularity and importance can easily be realized by the fact that it is often referred to as 'King of Fruits' in the tropical world. India is the largest producer of mango in the world, contributing 40.48% of the total world mango production (Anonymous, 2013). Mango has been under cultivation in India since 4000 years and over 1200 varieties are said to exist in the country. The cultivated mango varieties in India, exhibit an unusual diversity of fruit forms, flavours and tastes (Mukherjee, 1948). At present, 20–30 land races are cultivated commercially, the majority of which are area-specific. Northern, eastern, western and southern regions of India are recognized as distinct mango growing regions, though some cultivars are grown over wider areas (Yadav and Rajan, 1993).

Photosynthesis is the basis for growth, development and yield in plants, but perennial trees like mango have a very low orchard efficiency (Chacko and Randhawa, 1971). 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). Leaf morphological characters are important to support photosynthesis and both can influence the plant growth strategies of different tree species (Takayoshi et al., 2001). In mango, Kalyan et al. (2012) observed a variation in foliage density, shape of lamina, leaf nature, leaf apex, the colour of new and matured leaves and arrangement of major veins which can be helpful in differentiation among cultivars. The photosynthetic potential of leaves is reported to be inherited (Ojima et al., 1969). 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; Guru et al., 1999; Singh and Rajan, 2009). At the same time, leaf epicuticular wax is one of the important factors, which influence the energy balance of leaves by preventing the overheating of leaves and thereby affect photosynthesis (Armando et al., 2012). The amount of epicuticular wax is positively correlated with tolerance to a variety of abiotic stresses due to its role in regulating gas exchange, leaf temperature and light reflectance properties (David and James, 1978; Mansour et al., 2007).

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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 morphological and 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 morphological and physiological characteristics across the selected mango cultivars, popularly grown in different agro-climatic regions of India.

2. Materials and methods

2.1. Experimental site and plants

The present investigation was carried out during the month of July 2013 on eight indigenous mango cultivars of 25 years age maintained by the Division of Fruit Crops, Indian Institute Horticultural Research (IIHR), Bangalore, India located at 13.58° N and 78° E and elevation of 890 m. The average maximum temperature in summer is about ~32.8 °C and minimum temperature about ~21.7 °C and the average annual relative humidity of this area is about 64% with rainfall of about 760 mm. The trees were maintained under uniform cultural practices. The cultivars selected for the study show different tree habits (Table 1) and are popularly grown in different agro-climatic regions of India. The cultivars chosen for the study are 'Neelum' and 'Totapuri' (Southern India), 'Dasheheri' and 'Langra' (Northern India), 'Fazli' and 'Himsagar' (Eastern India), and 'Borsha' and 'Alphonso' (Western India). Data for all the qualitative and quantitative leaf characteristics was recorded from 3rd or 4th fully expanded mature leaves collected between 09:00 h and 09:30 h. The leaves were brought to the laboratory in polythene bags with minimum loss of time.

2.2. Leaf morphological characters

The observations on leaf length, leaf width, petiole length, and pelvinus thickness were recorded. The leaf length was measured from apex to base of leaf lamina, leaf width was taken from the broad area of leaf lamina and petiole length was measured from base of leaf lamina to pelvinus using measuring scale. At the same time, the diameter of the pelvinus was measured using vernier calliper (Mitutoya, Japan). The leaf length to breadth ratio was calculated from leaf length and leaf width values. Leaf area of 20 leaves was measured using a portable leaf area metre (LI-3000, LI-COR Biosciences, USA) and leaf fresh weight was recorded immediately after bringing to the laboratory. Subsequently, leaves were kept in hot air oven at 80 °C till they attained constant weight and dry weight was recorded. Specific leaf weight (SLW) was calculated by taking the ratio of leaf dry weight to the total leaf area (leaf dry weight/leaf area) by using the method of Pearce et al. (1968).

Table 1
Tree habit of mango cultivars from different agro-climatic regions.

Cultivars	Crown shape	Tree growth habit	Foliage density
Western India			
Alphonso	Semi-circular	Spreading	Intermediate
Borsha	Semi-circular	Erect	Intermediate
Eastern India			
Himsagar	Semi-circular	Erect	Intermediate
Fazli	Semi-circular	Spreading	Intermediate
Northern India			
Langra	Semi-circular	Spreading	Sparse
Dasheri	Semi-circular	Spreading	Sparse
Southern India			
Totapuri	Semi-circular	Spreading	Sparse
Neelum	Semi-circular	Spreading	Sparse

2.3. Gas exchange characters and related traits

A minimum of three measurements were recorded during 0930–1130 h in fully expanded mature leaves from three trees of each cultivar under ambient light and CO_2 level. The mean CO_2 concentration during the measurement was 388 μmol mol^{-1} and photosynthetic active radiation (PAR) was 1385 μmol m^{-2} s^{-1}. Photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and leaf temperature were measured using portable photosynthesis system (LCpro+, ADC BioScientific limited, UK). Instantaneous water use efficiency (iWUE) was calculated by taking the ratio of photosynthetic rate and transpiration rate (P_N/E) and carboxylation capacity (CE) was calculated by taking the ratio of photosynthetic rate and internal CO_2 concentration of the leaf (P_N/C_i).

2.4. Light harvesting pigments

The chlorophyll and total carotenoids were extracted using dimethyl sulphoxide (DMSO) and acetone. The leaf sample of 0.1 g was immersed in DMSO reagent (10 mL; 1:1 ratio) and incubated in dark for 72 h. The supernatant was collected and absorbance was recorded at 663 nm, 645 nm and 470 nm for estimation of Chl a, Chl b, total chlorophyll and carotenoids using UV-VIS spectrophotometer (T80+ UV/VIS spectrometer, PG Instrument Ltd., UK). During the assay, samples were protected against light to prevent pigment degradation and pigment contents were calculated from the equations proposed by Lichtenthaler and Buschmann (2001).

2.5. Epicuticular wax content

The epicuticular wax content (ECW) of the leaf was estimated using modified and standardized method of Ebercon et al. (1977) for mango. Five leaf segments (3 cm^2 area) from fully opened matured mango leaves were immersed in chloroform (10 mL) and then vigorously shaken for 30 s and chloroform was immediately transferred to glass vial. Chloroform was evaporated till vial was completely dry and to the vial, potassium dichromate reagent (5 mL) was added and then kept in a boiling water bath for 30 min. Final volume was made up to 12 mL using distilled water and an optical density was measured at 590 nm using UV-VIS spectrophotometer (T80+ UV/VIS spectrometer, PG Instrument Ltd., UK). For the extraction of leaf wax, mango leaves were cleaned using cotton and immersed in chloroform (200 mL) for 30 s. Chloroform was completely evaporated and ECW residues were collected using spatula.

2.6. Stomatal number

The stomatal imprints of the adaxial and abaxial leaf surface were taken according to the methodology of Hamill et al. (1992) and the average number of stomata on both the surfaces was recorded from 10 fields under 25/0.55 magnification factor using a microscope (Leitz Neo-PROMAR, Germany).

2.7. Statistical analysis

The experimental data were analysed statistically using the AGRES V 3.06 software. General linear model univariate ANOVA was run for each data set.

Table 2
Qualitative leaf morphological characters of mango cultivars from different agro-climatic regions.

Cultivars	Blade shape	Apex shape	Base shape	Margin	Twisting	Pubescence
Western India						
Alphonso	Lanceolate	Acuminate	Acute	Entire	Absent	Absent
Borsha	Elliptic	Acuminate	Acute	Wavy	Present	Absent
Eastern India						
Himsagar	Oblong	Acuminate	Acute	Wavy	Absent	Absent
Fazli	Elliptic	Acute	Acute	Entire	Absent	Absent
Northern India						
Langra	Lanceolate	Acuminate	Obtuse	Entire	Absent	Absent
Dasheri	Lanceolate	Acuminate	Acute	Wavy	Absent	Absent
Southern India						
Totapuri	Elliptic	Acuminate	Obtuse	Entire	Absent	Absent
Neelum	Elliptic	Acuminate	Acute	Entire	Absent	Absent

3. Results and discussion

3.1. Leaf morphological characters

Variations in leaf characteristics are reported to be due to genetic divergence of mango cultivars (Srivastava et al., 1987; Sharma et al., 1999; Reddy et al., 2000) and the current study showed considerable variations in leaf morphological characters among the eight cultivars (Tables 2 and 3). Maximum leaf length and leaf ratio were observed in 'Alphonso' and minimum in 'Langra' and Fazli. Maximum and minimum leaf width were observed in 'Totapuri' and 'Dashehari' respectively. Maximum leaf area, leaf fresh and dry weights were recorded in 'Alphonso'. Lowest leaf area and leaf dry weight in 'Borsha' and leaf fresh weight in 'Totapuri' were observed. The pelvinus thickness was maximum in 'Borsha' and minimum in 'Totapuri'. At the same time petiole length was longest in 'Alphonso' as compared to other cultivars, while 'Dashehari' recorded the minimum. In 'Alphonso', maximum number of secondary veins was observed, while 'Borsha' recorded the minimum number of secondary veins.

The higher SLW indicates the higher photosynthate production, translocation, and accumulation in leaves due to higher photosynthetic rate (Singh and Rajan, 2009) and its influence on the development of reproductive organs and yield (Surendar et al., 2013). In the present study, SLW showed significant variation among cultivars and 'Totapuri' recorded the maximum and 'Dashehari' recording the minimum (Table 3). On the contrary, in our experiment P_N was not influenced by SLW and our results are not matching with earlier studies in mango (Singh and Rajan, 2009). At the same time our current results are corresponding with the results reported by Chen et al. (2010) who observed no relationship between P_N and SLW in mature leaves of mango.

3.2. Gas exchange characters and related traits

Gas exchange characters and related traits are highly important for the growth and yield in plants. Variations in leaf morphological characters and content of light pigments can directly influence the leaf gas exchange (Takayoshi et al., 2001). In our study, significant variations were noticed in gas exchange characteristics, WUE, iWUE and CE among various cultivars (Table 4). Results showed that P_N and CE were highest in 'Neelum' followed by 'Borsha', while the least value was recorded in 'Fazli'. There was a strong positive correlation reported between P_N and CE in mango cultivars (Shivashankara and Mathai, 2000) as CE represents the ratio between P_N and C_i. In our study, maximum C_i was found in 'Fazli' and minimum in 'Alphonso' followed by 'Borsha'. The C_i can be limited because of stomatal and non-stomatal mechanisms under normal and stressed conditions (Laurent et al., 2006). Transpiration rate (E) and leaf temperature were higher in 'Himsagar', while minimum E and leaf temperature were noted in 'Langra'. A negative correlation between E and leaf temperature has been reported in mango cultivar and increased transpiration can help in maintaining leaf temperature in normal conditions (Luvaha et al., 2007). Significantly high g_s was observed in 'Neelum' and least was in 'Langra'. The g_s recorded in the present study was in accordance with the findings of Urban and Jannoyer (2004). The iWUE was highest in 'Langra', while it was lowest in 'Fazli'. The water use efficiency provides information regarding the carbon fixed by photosynthesis in relation to the water lost by stomatal conductance and transpiration or capacity of plants to preserve water and maximizing carbon fixing (Laurent et al., 2006). The variation among cultivars for these characters might be due to unique genetic features of individual cultivars under the same environmental condition.

3.3. Light harvesting pigments

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 (Takayoshi et al., 2001). In mango, the pigment content is influenced by different seasons, cultivars, growth and maturity stages of leaves (Pandey and Tyagi, 1999; Nii et al., 1995). Chl a, Chl b, total chlorophyll and carotenoid content was highest in 'Alphonso' followed by Neelam and 'Langra'. The lowest total chlorophyll and carotenoid content was found in 'Fazli'. The mean Chl a/b ratio was highest in 'Dashehari' and least was found in 'Totapuri'. Concurrently, Chl/Car ratio was maximum in 'Borsha' and minimum in 'Dashehari' (Table 5). The cultivars with high chlorophyll content can produce higher biomass and increase photosynthesis (Hassan et al., 2009). In matured mango leaf, increase in P_N with increasing chlorophyll content has been reported (Nii et al., 1995). In contrast to the above conclusions, in our study there was no direct relationship found between P_N and pigment concentrations. The variation in P_N in mango cultivars indicates that, CE has an important role in enhancing P_N than the concentration of chlorophyll pigments as reported in mango (Shivashankara and Mathai, 2000) and there was no relationship found between total chlorophyll and photosynthesis in matured leaves of mango cultivars (Chen et al., 2010).

3.4. Epicuticular wax content

Maximum leaf epicuticular wax content was observed in 'Totapuri' and minimum in 'Dashehari' (Table 6). The variation in ECW might be due to genetic factors, as observed by Dragon et al. (2008) and in addition it was observed that ECW acts as a mechanical barrier against insect pests. Similarly, excessive deposition of ECW

Table 3
Quantitative leaf morphological characters of mango cultivars from different agro-climatic regions.

Cultivars/agro-climatic region	Leaf length (cm)	Leaf width (cm)	Leaf ratio (L:B)	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dry weight (g)	Specific leaf weight (g/cm^2)	Pelvinus thickness (mm)	Petiole length (cm)	Number of secondary vein
Western India										
Alphonso	19.72	4.74	4.21	78.71	24.16	11.00	0.140	3.40	3.88	45.25
Borsha	16.28	4.52	3.62	42.62	17.27	5.13	0.120	4.06	2.74	34.75
Eastern India										
Himsagar	17.10	4.70	3.64	54.51	17.32	8.10	0.149	3.52	2.92	40.50
Fazli	14.44	4.46	3.25	57.92	16.30	7.68	0.133	3.40	3.00	40.25
Northern India										
Langra	14.62	4.54	3.22	55.18	16.20	7.70	0.140	3.36	2.28	44.00
Dasheri	14.98	4.16	3.60	69.02	21.27	5.80	0.084	3.98	2.08	39.75
Southern India										
Totapuri	16.08	4.86	3.32	51.97	14.43	7.80	0.150	3.34	2.22	41.75
Neelum	15.12	4.34	3.65	56.80	18.18	5.44	0.096	3.92	2.26	42.25
SE±	1.11	0.28	0.17	6.93	0.31	0.77	0.003	0.19	0.36	2.16
C.D. (0.05)	2.29	0.57	0.35	14.27	0.67	1.52	0.007	0.38	0.73	4.07

Table 4
Variation in gas exchange and related parameters of mango cultivars from different agro-climatic regions.

Cultivars/agro-climatic region	P_N (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	C_i (ppm)	LT (°C)	iWUE	CE
Western India							
Alphonso	8.64	0.10	1.73	201.00	30.53	5.48	0.043
Borsha	9.59	0.13	2.15	200.00	30.40	5.30	0.048
Eastern India							
Himsagar	8.57	0.12	2.43	212.67	32.67	3.59	0.040
Fazli	6.94	0.12	2.13	247.67	31.67	3.32	0.028
Northern India							
Langra	7.93	0.09	1.19	214.00	27.90	6.76	0.037
Dashehari	7.44	0.12	2.35	234.67	31.57	3.46	0.032
Southern India							
Totapuri	8.84	0.10	1.83	204.33	30.40	5.45	0.043
Neelum	11.02	0.15	2.29	220.67	29.87	4.84	0.050
SE±	0.27	0.01	0.14	5.67	0.40	0.34	0.002
C.D. (0.05)	1.09	0.02	0.23	23.13	2.30	1.79	0.007

P_N, photosynthetic rate; g_s, stomatal conductance; E, transpiration rate; C_i, internal leaf CO_2; LT, internal leaf temperature; iWUE, instantaneous water use efficiency; CE, carboxylation capacity.

increases leaf reflectance of visible and near infra-red, decreases net radiation in the field and decreases cuticular transpiration. Therefore, ECW content is an effective component of avoidance mechanism for drought, temperature, diseases and insect-pest stress (David and James, 1978; Mansour et al., 2007; Laxman et al., 2013; Wilkinson and Cummins, 1981). Therefore, higher ECW content might impart stress tolerance in the mango cultivars.

3.5. Stomatal number

The adaxial and abaxial stomatal numbers were highest in 'Totapuri' and 'Langra', respectively (Table 6). 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

Table 5
Variation in light harvesting pigments and related parameters of mango cultivars from different agro-climatic regions.

Cultivars/agro-climatic region	Chlorophyll (mg g^{-1} FW)			Total carotenoids (mg g^{-1} FW)	Chl a/b	Chl/Car
	a	b	Total (a + b)			
Western India						
Alphonso	1.76	0.51	2.27	0.33	3.43	6.95
Borsha	1.27	0.37	1.63	0.23	3.48	7.08
Eastern India						
Himsagar	1.17	0.31	1.49	0.23	3.74	6.46
Fazli	1.10	0.32	1.42	0.22	3.45	6.61
Northern India						
Langra	1.47	0.39	1.86	0.29	3.83	6.43
Dashehari	1.45	0.37	1.82	0.30	3.86	6.13
Southern India						
Totapuri	1.24	0.39	1.64	0.25	3.19	6.53
Neelum	1.62	0.45	2.08	0.32	3.60	6.47
[5pt] SE±	0.11	0.04	0.15	0.02	0.04	0.19
C.D. (0.05)	0.24	0.08	0.32	0.05	0.11	ns

ns; nonsignificant.

Table 6
Variation in stomatal number and epicuticular wax content of mango cultivars from different agro-climatic regions.

Cultivars/agro-climatic region	Stomatal number		Epicuticular wax content (mg cm^{-2} leaf area)
	Abaxial	Adaxial	
Western India			
Alphonso	2.06	0.28	0.396
Borsha	4.22	0.22	0.382
Eastern India			
Himsagar	6.00	0.39	0.345
Fazli	4.39	0.33	0.344
Northern India			
Langra	4.20	0.80	0.347
Dasheri	4.44	0.67	0.242
Southern India			
Totapuri	7.17	0.22	0.462
Neelum	5.33	0.22	0.351
SE±	0.50	0.29	0.06
C.D. (0.05)	1.09	ns	ns

ns; nonsignificant.

with photosynthesis. But stomata have a direct relationship with water use efficiency and transpiration in plants (Zhenzhu and Guangsheng, 2008).

4. Conclusion

In conclusion, the study indicated that the eight mango cultivars have shown significant differences in gas exchange parameters, leaf morphological characters and related traits. Cultivars such as 'Borsha', 'Himsagar', 'Langra' and 'Neelum' grown in different agro-climatic regions recorded higher photosynthesis. The variations in leaf morphology, gas exchange and related traits may be related to genotype differences evolved due to a wide range of environmental conditions prevailing in this country.

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