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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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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.