

SHORT COMMUNICATION

## Morphological Characterization of Sweet Orange (*Citrus sinensis* Osbeck) Germplasm under Subtropical Conditions

Sunaiana<sup>1,\*</sup>, M Gupta<sup>1</sup>, HS Rattanpal<sup>1</sup>, GS Sidhu<sup>2</sup> and G Singh<sup>1</sup>

<sup>1</sup>Department of Fruit Science, Punjab Agricultural University, Ludhiana-141004, Punjab, India

<sup>2</sup>School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, Punjab, India

(Received: 11 June, 2019; Revised: 21 September, 2019; Accepted: 06 March, 2020)

Characterization and evaluation of cultivars is basic key for better identification and breeding programmes. Experiment was carried out on eight cultivars in randomized block design with eight replications (one plant per replication) on five year old plants. Rootstock used was rough lemon, planted at a spacing of 3 x 6m. Plants were evaluated for their vegetative and floral characters. Vegetative characters like branch angle, shoot tip surface, shoot tip colour, vegetative life cycle, intensity of green colour, leaf lamina attachment were similar for all the genotypes depicting very less variability in qualitative characters. Significant variability was recorded among quantitative characters like rootstock diameter, leaf lamina length and width, floral characters and pollen viability.

**Key Words:** Floral characters, Morphological characterization, Phule mosambi, Sweet orange, Vegetative characters

### Introduction

Citrus belongs to family *Rutaceae*, falls under subfamily *Aurantioideae*. There are mainly six species of the genus *Citrus* namely *C. sinensis*, *C. limon*, *C. aurantifolia*, *C. paradisi*, *C. reticulata* and *C. maxima*. Citrus covers approximately 976 thousand hectares area, with an annual production of 11717 thousand MT (NHB, 2017). Sweet orange has been reported to be originated in Southern China and, it was introduced to India during 13<sup>th</sup> century (Swingle and Reece, 1967). In India, North Eastern India is claimed as centre of origin for citrus species (Singh *et al.*, 2010).

Sweet orange (*C. sinensis*) is a hybrid between pummelo and mandarin (Wu *et al.*, 2014). The genetic diversity of *C. sinensis* is diminishing rapidly because of a number of factors, such as displacement of the natural gene pool due to selection and introduction of genotypes suitable for intensive horticulture resulting in a limited gene pool. Though, the study on different varieties of sweet oranges had already been done (Kumatkar *et al.*, 2016), still morphological characterization is initial step to check how different varieties behave differently in varied environmental conditions. Study of floral morphology is important for understanding self-

incompatibility and pollen sterility, which have major role in fruit breeding and fruit set are essential pre-requisite for initiating any breeding programme. Besides, such information would also be useful in taxonomical studies (Randhawa *et al.*, 1961). As sweet orange is the most recognizable species among the citrus group (Novelli *et al.*, 2006). Within, sweet oranges there exists a large variability in characters, but much work had not been done on selections *i.e.* Kodour Sathgudi, M3, M4, M8, Phule mosambi and Shamouti. These genotypes were compared with Blood Red and Mosambi under north Indian conditions. So, the present investigation is attempted to study morphological characters of sweet orange germplasm, available at Punjab Agricultural University, Ludhiana, for assessing their genetic diversity and to find out the suitability of varieties Kodour Sathgudi, M-3, M-4, M-8, Shamouti, Phule mosambi, Blood Red and Mosambi for cultivation under Punjab conditions.

### Materials and Methods

The present investigation was carried out at Punjab Agricultural University, Ludhiana to evaluate morphological characters of eight sweet orange (*C. sinensis* Osbeck) genotypes viz., Kodour Sathgudi, M-3, M-4,

\*Author for Correspondence: Email- sunaianasandhu94@gmail.com

M-8, Phule mosambi, Shamouti, Blood Red and Mosambi. Source for these genotypes were Central Citrus Research Institute (CCRI), Nagpur. Age of trees was four years as it is plantation of September 2012 and they were planted at spacing of 6X3m using rough lemon as rootstock. Morphological characterization of sweet orange was done using descriptors based on International Plant Genetic Resources Institute, Italy (Anonymous, 1999).

The experiment was laid out in RBD with eight replications for each genotype and one plant per replication (Gomez and Gomez, 2010). Critical difference at 5% level of probability was computed to compare the statistical significance among different treatments. Analysis of variance was conducted for various quantitative traits using 9.3 version of SAS (Statistical analysis system) software.

The genetic diversity among sweet orange genotypes was computed on the basis of their morphological characters using computer software programme DARwin. The data were subjected to unweighted pair group's method with arithmetic mean (UPGMA) analysis to generate dendrogram (Perrier and Jacquemoud-Collet 2006).

## Results and Discussion

### Tree Characters

Smooth scion trunk surface and spheroid tree shape was recorded in all sweet orange genotypes. Tree growth habit was found erect in M-3, M-4, M-8 and Blood Red whereas tree growth was spreading in all other genotypes. Medium branching habit was observed in Shamouti, Blood Red and Mosambi whereas branch density was observed to be dense in all other genotypes. Branch angle was wide (more than 30°) in all genotypes. Another study also corroborated with findings of present studies as results showed wide branch angle in all strains of Rangpur lime (Singh *et al.*, 2010). Spine density was medium in Blood Red and low in rest of the genotypes. Spine shape was recorded as straight in all genotypes. Similar results with straight shape of spine were recorded in six Rangpur lime strains (Singh *et al.*, 2010). Shoot tip colour was green and shoot tip surface was glabrous in all genotypes. Rootstock diameter was significantly higher in Kodour Sathgudi (126.29 mm) which was statistically at par with M-4 (121.50 mm) and Mosambi (120.91 mm). The minimum rootstock diameter was recorded

in Blood Red (99.36 mm) which was significantly at par with Phule mosambi (102.22 mm). Ratio of scion and rootstock diameter do not vary significantly among all the genotypes. All genotypes were statistically at par with each other for scion: rootstock diameter ratio. Significantly higher spine length was recorded in Kodour Sathgudi (6.32 mm) followed by Blood Red (5.62 mm) and least was recorded in Mosambi (4.23 mm). Phule mosambi and M-3 were statistically at par with each other for spine length. However, there were no spines in Shamouti (Table 1).

### Leaf Characters

Leaf lamina shape was ovate in Kodour Sathgudi, M-8, Mosambi and Phule mosambi whereas shape was elliptic in Blood Red, M-3, M-4 and Shamouti. Kodour Sathgudi, Blood Red and Shamouti had crenate shape of leaf lamina while sinuate shape was reported in all other genotypes. Leaf apex was acute in case of Blood Red and was obtuse in rest of the genotypes. Petiole wing was obdeltate in shape in case of Kodour Sathgudi and Blood Red where as it was obovate in rest of the genotypes. Fused petiole and lamina junction was observed in all genotypes. In similar studies, leaf petiole wing was present in all three varieties *viz.*, bitter sweet orange, Yuma citrange and Sour orange (Jaskani *et al.*, 2006).

Leaf lamina length was significantly high in Shamouti (124.51 mm) followed by M-3 (113.34mm) and M-4 (105.61mm), while it was significantly lower in Kodour Sathgudi (94.16mm). M-4, M-8 and Phule mosambi were statistically at par with each other for leaf lamina length. Similarly, significant variation was recorded in leaf lamina width. Maximum leaf lamina width was observed in Shamouti (77.71mm) followed by M-3 (71.66 mm) whereas least leaf lamina width was observed in Mosambi (60.24mm). M-8 and Phule mosambi were statistically at par with each other for leaf lamina width. Non-significant difference for leaf lamina length: width ratio was observed in all cultivars except in M-3 and Phule mosambi which were at par with each other, while lowest ratio was observed in Blood Red (1.45) (Table 1). Similar studies were conducted on six Rangpur lime strains and maximum leaf lamina length: width ratio was recorded in Noreo (2.4), while minimum was in Marmalade (1.8) (Singh *et al.*, 2010). Significantly higher leaf lamina thickness was observed in Shamouti (0.49 mm) followed by M-4 (0.45 mm),

Phule mosambi (0.42 mm) and M-8 (0.42 mm) while leaf lamina thickness was observed to be significantly lower in Kodour Sathgudi (0.34 mm). However, Blood Red and M-3 were statistically at par with each other for leaf thickness. The data in Table 1 revealed that among all sweet orange genotypes petiole wing width was recorded as significantly higher in Blood Red (4.98 mm) which was statistically at par with Shamouti (4.97 mm). Similar studies were carried out in 30 local pummelo accessions and reported that accession number five had the highest petiole wing width, while accession number 17 had minimum petiole wing width (Rahman *et al.*, 2003).

### Flower Characters

Ambe bahar was observed as main flowering season. Colour of fully opened flowers was white. Similar results were reported in Assam lemons, where all open flowers were white in colour (Nath, 1999). Pale yellow coloured anthers were noted in Shamouti and Kodour Sathgudi whereas yellow colored anthers were recorded in case of Blood Red, M-3, M-4, M-8, Phule mosambi and Mosambi. Earliest flower initiation was recorded in M-3 (2<sup>nd</sup> March 2017) flowering had initiated at last in Shamouti (15<sup>th</sup> March 2017) whereas Shamouti was the first to terminate its flowering by 30<sup>th</sup> March 2017 and last to terminate was Blood Red, Kodour Sathgudi, M-3 and Phule mosambi (2<sup>nd</sup> April 2017). Maximum duration of flowering was reported in Phule mosambi (31 days) and it was minimum in case of Shamouti (15 days) (Table 2).

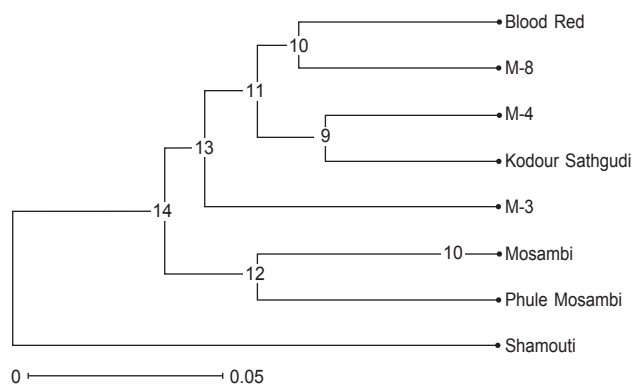
Significantly higher flower length was recorded in M-8 (25.84mm) whereas flower length was low in M-3 (19.10mm) which was statistically at par with Blood Red. Similar studies were carried out on 20 sweet orange genotypes. Flower length was recorded to be maximum in Campbell Valencia (41.21 mm) and minimum flower length was reported in Sanguinelli (25.69 mm) (Baswal *et al.*, 2015). Flower diameter was recorded to be highest in Mosambi (39.05 mm) followed by M-4 (36.22 mm) and Phule mosambi whereas flower diameter was recorded significantly lesser in Shamouti (23.67 mm). Similar variation in 20 sweet orange genotypes were recorded in which, maximum flower diameter was recorded in Campbell Valencia (41.21 mm) and minimum was recorded in Sanguinelli (25.69 mm) (Baswal *et al.*, 2015). Calyx diameter was significantly higher in Mosambi (7.15mm) and least in Shamouti (4.88 mm). Staminate flower percentage varied significantly in different sweet orange genotypes (Table 2). Percentage of staminate flowers was observed to be significantly higher in Phule mosambi (11.97 %) and significantly low percentage was observed in Mosambi (6.62 %). The data pertaining to perfect flower percentage of sweet orange revealed that significantly higher percentage of perfect flowers were recorded in Mosambi (93.37%) while lowest perfect flower percentage was recorded in Phule mosambi (88.02%). Similarly, pollen viability was significantly higher in Mosambi whereas significantly lower pollen viability was recorded in Shamouti (32.47). However, Blood Red and Kodour Sathgudi were statistically at par with each other for pollen viability (Table 2). Similar

**Table 1. Quantitative tree characters of sweet orange genotypes**

Genotypes	RD (mm)	RSRD	SL (mm)	LLL (mm)	LLW (mm)	LLLW	LT (mm)	PWW (mm)
Kodour Sathgudi	126.29 <sup>a</sup>	1.03 <sup>a</sup>	6.32 <sup>a</sup>	94.16 <sup>e</sup>	60.32 <sup>e</sup>	1.56 <sup>ab</sup>	0.34 <sup>e</sup>	3.93 <sup>c</sup>
M-3	109.41 <sup>b</sup>	1.02 <sup>a</sup>	4.55 <sup>ef</sup>	113.34 <sup>b</sup>	71.66 <sup>b</sup>	1.58 <sup>b</sup>	0.44 <sup>bc</sup>	4.35 <sup>b</sup>
M-4	121.50 <sup>a</sup>	1.06 <sup>a</sup>	5.33 <sup>c</sup>	105.61 <sup>c</sup>	64.66 <sup>d</sup>	1.63 <sup>a</sup>	0.45 <sup>b</sup>	3.96 <sup>c</sup>
M-8	105.78 <sup>bc</sup>	1.08 <sup>a</sup>	4.85 <sup>d</sup>	106.75 <sup>c</sup>	67.00 <sup>cd</sup>	1.59 <sup>a</sup>	0.42 <sup>c</sup>	3.77 <sup>cd</sup>
Phule mosambi	102.22 <sup>cd</sup>	1.09 <sup>a</sup>	4.56 <sup>e</sup>	106.67 <sup>c</sup>	66.40 <sup>cd</sup>	1.60 <sup>a</sup>	0.42 <sup>c</sup>	3.63 <sup>d</sup>
Shamouti	109.51 <sup>b</sup>	1.06 <sup>a</sup>	0.0	124.51 <sup>a</sup>	77.71 <sup>a</sup>	1.60 <sup>a</sup>	0.49 <sup>a</sup>	4.97 <sup>a</sup>
Blood Red	99.36 <sup>d</sup>	1.05 <sup>a</sup>	5.62 <sup>b</sup>	100.58 <sup>d</sup>	69.06 <sup>bc</sup>	1.45 <sup>c</sup>	0.43 <sup>bc</sup>	4.98 <sup>a</sup>
Mosambi	120.91 <sup>a</sup>	1.04 <sup>a</sup>	4.23 <sup>f</sup>	100.48 <sup>d</sup>	60.24 <sup>e</sup>	1.66 <sup>a</sup>	0.39 <sup>d</sup>	3.76 <sup>cd</sup>

Abbreviations: RD: Rootstock diameter, RSRD: Ratio of scion: rootstock diameter, SL: Spine length, LLL: Leaf lamina length, LLW: Leaf lamina width, LLLW: Leaf lamina length:width, LT: Leaf thickness, PWW: Petiole wing width

studies were conducted on seven genotypes of lemon, highest pollen viability was reported in Meyer variety (86.74%) followed by Kutdiken (69.22%) and least was in case of BATEM Sarisi (Demir *et al.*, 2015).



**Fig. 1. Dendrogram illustrating genetic relationship among eight sweet orange genotypes generated by UPGMA tree analysis based on morphological traits. Cluster-I (Shamouti) and Cluster-II (Phule Mosambi, Mosambi, M-3, Kodour Sathgudi, M-4, M-8 and Blood Red).**

Non-significant difference was observed in number of petals and sepals in different sweet orange genotypes. Significantly higher petal length was recorded in M-8

(21.26 mm) followed by Phule mosambi (20.05 mm) while least petal length was reported in Blood Red (16.40 mm). However, M-3 and M-4 were statistically at par with each other for petal length. Significantly higher petal width was observed in Mosambi (7.93 mm) and significantly lower petal width was recorded in Shamouti (5.28 mm). Length of style varied significantly and was recorded to be highest in Mosambi (6.69 mm) whereas significantly lesser style length was recorded in Shamouti (4.71 mm) (Table 2).

Similarly, pedicel length was highest in Blood Red (7.45 mm) which was statistically at par with Mosambi (7.38 mm) and lowest pedicel length was recorded in Shamouti (5.36 mm). Similar variation in pedicel length was recorded. The pedicel length of Mosambi was 5.2 mm whereas it was 16.5 mm in case of Red fleshed pummelo (Singh, 2006). Highest filament length was recorded in Phule mosambi (11.16 mm) while significantly lower filament length was recorded in Shamouti (7.63 mm). Similar studies on grapefruit were conducted and variability in length of filament was reported (Baswal *et al.*, 2015). Similarly, pedicel length was highest in Blood Red (7.45 mm) which

**Table 2. Quantitative flowering characters of sweet orange genotypes**

Geno- types	SDF	FBDF	EDF	DF (days)	FL (mm)	FD (mm)	CD (mm)	PF (%)	SF (%)	PV (%)	PL (mm)	PW (mm)	LS (mm)	LF (mm)	PDL (mm)	NS
Kodour Sathgudi	6 <sup>th</sup> March	21 <sup>st</sup> -24 <sup>th</sup> March	2 <sup>nd</sup> April	27	18.66 <sup>d</sup>	27.87 <sup>e</sup>	5.81 <sup>c</sup>	93.30 <sup>a</sup>	6.70 <sup>e</sup>	53.50 <sup>d</sup>	15.68 <sup>f</sup>	6.29 <sup>ef</sup>	6.05 <sup>bc</sup>	8.35 <sup>d</sup>	6.67 <sup>b</sup>	21.62 <sup>c</sup>
M-3	2 <sup>nd</sup> March	18 <sup>th</sup> -22 <sup>rd</sup> March	2 <sup>nd</sup> April	30	19.10 <sup>d</sup>	33.54 <sup>c</sup>	6.05 <sup>bc</sup>	92.01 <sup>cd</sup>	7.99 <sup>bc</sup>	33.50 <sup>f</sup>	17.76 <sup>d</sup>	7.25 <sup>b</sup>	5.76 <sup>cd</sup>	9.06 <sup>c</sup>	5.97 <sup>c</sup>	24.00 <sup>a</sup>
M-4	8 <sup>th</sup> March	20 <sup>th</sup> -24 <sup>th</sup> March	1 <sup>st</sup> April	24	21.46 <sup>c</sup>	36.44 <sup>b</sup>	6.02 <sup>bc</sup>	92.62 <sup>b</sup>	7.38 <sup>d</sup>	58.23 <sup>c</sup>	18.10 <sup>d</sup>	6.67 <sup>de</sup>	5.42 <sup>d</sup>	9.16 <sup>c</sup>	5.65 <sup>cd</sup>	23.00 <sup>b</sup>
M-8	4 <sup>th</sup> March	19 <sup>th</sup> -24 <sup>th</sup> March	1 <sup>st</sup> April	28	25.84 <sup>a</sup>	32.82 <sup>c</sup>	6.26 <sup>b</sup>	91.62 <sup>d</sup>	8.38 <sup>b</sup>	46.37 <sup>e</sup>	21.26 <sup>a</sup>	7.16 <sup>bc</sup>	6.00 <sup>bc</sup>	10.58 <sup>b</sup>	6.84 <sup>b</sup>	22.62 <sup>b</sup>
Phule mosambi	3 <sup>rd</sup> March	21 <sup>st</sup> -26 <sup>th</sup> March	2 <sup>nd</sup> April	31	24.09 <sup>b</sup>	36.18 <sup>b</sup>	5.98 <sup>bc</sup>	88.03 <sup>e</sup>	11.97 <sup>a</sup>	68.94 <sup>b</sup>	20.05 <sup>b</sup>	6.84 <sup>cd</sup>	6.21 <sup>b</sup>	11.16 <sup>a</sup>	5.91 <sup>c</sup>	21.37 <sup>c</sup>
Shamouti	15 <sup>th</sup> March	22 <sup>nd</sup> -26 <sup>th</sup> March	30 <sup>th</sup> March	15	15.56 <sup>e</sup>	23.67 <sup>f</sup>	4.88 <sup>d</sup>	92.05 <sup>cd</sup>	7.95 <sup>bc</sup>	32.47 <sup>g</sup>	14.98 <sup>f</sup>	5.28 <sup>g</sup>	4.71 <sup>e</sup>	7.63 <sup>e</sup>	5.36 <sup>d</sup>	19.75 <sup>d</sup>
Blood Red	8 <sup>th</sup> March	24 <sup>th</sup> -28 <sup>th</sup> March	2 <sup>nd</sup> April	25	19.38 <sup>d</sup>	30.96 <sup>d</sup>	5.74 <sup>c</sup>	92.25 <sup>bc</sup>	7.75 <sup>cd</sup>	53.14 <sup>d</sup>	16.40 <sup>e</sup>	6.25 <sup>f</sup>	5.02 <sup>e</sup>	8.87 <sup>c</sup>	7.45 <sup>a</sup>	20.37 <sup>d</sup>
Mosambi	9 <sup>th</sup> March	16 <sup>th</sup> -22 <sup>nd</sup> March	31 <sup>st</sup> March	22	24.69 <sup>b</sup>	39.05 <sup>a</sup>	7.15 <sup>a</sup>	93.38 <sup>a</sup>	6.62 <sup>e</sup>	77.17 <sup>a</sup>	18.88 <sup>c</sup>	7.93 <sup>a</sup>	6.69 <sup>a</sup>	11.03 <sup>a</sup>	7.38 <sup>a</sup>	22.37 <sup>b</sup>

Abbreviations: SDF: start date of flowering, FBDF: full bloom date of flowering, EDF: end date of flowering, DF: duration of flowering, FL: flower length, FD: flower diameter, CD: calyx diameter, PF: perfect flower, SF: staminate flower, PV: pollen viability, PL: petal length, PW: petal width, LS: length of style, LF: length of filament, PDL: Pedicel length, NS: number of stamens.



**Table 3. Distinguish characters**

Genotypes	Flower length	Pollen viability	Length of filament
Phule mosambi	24.09 mm	68.94 %	11.16mm

was statistically at par with Mosambi (7.38 mm) and lowest pedicel length was recorded in Shamouti (5.36 mm) (Table 2). Similar variation in pedicel length was recorded by Singh (2006), he found pedicel length of Mosambi was 5.2 mm whereas it was 16.5 mm in case of Red fleshed pummelo. Significantly higher number of stamens was recorded in M-3 (24.00) and least was in Shamouti (19.75) and Blood red (20.37). Similar studies on floral morphology of mandarin genotypes was conducted and highest number of stamens were reported in Tsirang and Dagana (14.9) and lowest were (14.6) in Samste (Dorji and Yapwattanaphun, 2011).

### Diversity Analysis

All the eight sweet orange genotypes were divided into two clusters. Cluster-I comprised of only one genotype (Shamouti) and in Cluster-II seven genotypes were clustered (Phule mosambi, Mosambi, M-3, Kodour Sathgudi, M-4, M-8 and Blood Red).

On the basis of this cluster analysis study, all the eight genotypes were grouped into different clusters, irrespective of their geographical origin. Similar studies on morphological variation indicated that the mandarin genotypes under study were consisted of phenotypically different individuals (Singh *et al.*, 2016). The difference in individuals could be attributed to cross pollination and mutation.

### Conclusion

The study concluded that there is not much variation in qualitative parameters of all the sweet orange genotypes. However, existence of wide variations in quantitative characters was observed during the investigation. The variations are indicative of the underlined genetic diversity in sweet orange germplasm which will be very useful in citrus breeding and crop improvement programme; evergreen vegetative cycle, longer flowering period and higher pollen viability are some of the useful characters for further breeding programmes.

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