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FLORAL BIOLOGY AND PHENOLOGY IN SWEET ORANGE (BARI Malta-1) UNDER SUBTROPICAL CONDITION OF BANGLADESH

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ABSTRACT

Knowledge of the reproductive biology of a species is fundamental in order to develop an efficient program of genetic improvement by hybridization. Floral morphology and biology of BARI Malta-1 was studied to develop a cross-breeding program. In BARI Malta-1, flowers mainly bloom in February to March in a year. Flower type was generally bisexual. Flower structure of this variety was observed with five sepals, five petals and twenty stamens with prominent ovary. Anther and pollen diameter of this variety was measured as 1.83 mm and 27.75 µm, respectively. Average filament length was 6.02 mm. The floral bud development was divided into eight distinct stages starting from the initiation of the detectable floral buds which required 24.4 days for its completion. Anthesis of flower start at 8 am in the morning. Maximum anthesis (44.62%) occurred from 10 am-12 pm. Anther, petal and style start to abscessed after 48.05, 73.1, 252.95 hrs and completed 109.8, 100.8 and 302.2 hrs of anthesis, respectively. Pollen viability decreased drastically after 4 hrs of anthesis.

***Keywords*:** Sweet orange, floral morphology, bud development.

INTRODUCTION

Citrus belongs to the family *Rutaceae* and is the most important fruit crops in the world (Roussos, 2015). It is one of the most horticultural and economically important fruit crops globally (Wu *et al*., 2018), extensively cultivated in more than 140 countries and regions in the tropics and subtropics (Cuenca *et al*., 2016). Citrus fruits represent a source of macro and micronutrients and dietary fiber (Salonia *et al*., 2020). They are also rich in antioxidants compounds (Liu *et al*., 2012), reveal anticancer, and anti-inflammatory properties (Ma *et al*., 2020), and are effective at reducing the risk of cardiovascular disease and type-2 diabetes (Sugiura *et al*., 2016). Among citrus production, oranges account for 2.3%, followed by lemon and lime (47.04%) and pummelo (50.57%) as of year 2018-2019 (BBS, 2020). The lower production of sweet orange might be due to the lack of high yielding variety and appropriate production technology. So far, Bangladesh Agricultural Research Institute (BARI) has released two varieties of sweet orange (BARI Malta-1 & BARI Malta-2) and these varieties are getting popularity day by day. However, problems regarding premature fruit dropping, pest and diseases are being faced by the sweet orange growers. There is an urgent need to develop variety with high yield potentials and tolerant to pest and diseases.

The study of floral biology is a basic condition for the analysis of the interaction between pollen and stigma, flowers and pollination associated with the reproductive success of plant species (Dafni *et al*., 2005). Size, morphology, color, and anthesis are floral data were used in the study, as they not only help to understand the plant pollination but also how the reproductive success occurs (Kevan *et al*., 2007). The knowledge of reproductive biology is based on the floral structure of a species which determines the nature of its reproductive process. The most important advances obtained in the genetic improvement of plants are associated with the knowledge of their reproductive system, through studies relating to the anthesis, the viability of the pollen and the receptivity of the stigma, among others (Peña-Yam *et al*., 2019; Zhang *et al*., 2018). Moreover, abundant heterozygosis is present in most citrus species. Breeding in citrus is much complicated due to heterozygosity, hence information on pollen viability and floral morphology are the

ideal tools to select a superior genotype with better cross combination for hybridization work (Baswal *et al*., 2015). Till date, however, very limited work on assessment of pollen viability and floral morphology among sweet orange has been carried out under sub-tropics of Bangladesh. Hybridization program is very much essential to develop variety with desirable traits. But in Bangladesh, any hybridization program has not been undertaken for the improvement of sweet orange.

Given the importance of the sweet orange, a better knowledge of its floral biology would facilitate both commercial cultivation and hybridization programs of genetic improvement. The lack of information regarding the floral biology of *C. sinensis*, this research aimed to know the morphological characters, different stages of floral bud development and to find out the time of anthesis and suitable time of pollination for sweet orange (BARI Malta-1) flower. The outcome of the study will help to fill the knowledge gap regarding the reproductive biology of *C. sinensis* and will be useful for the plant breeder.

MATERIALS AND METHODS

The study was conducted at the sweet orange orchard of the Pomology Division, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for the time period from February 2016 to March 2017. Seven years old plantation of BARI Malta-1 was selected to study the different aspects of floral biology. Floral morphological characters was determined for the characters such as flower diameter, flower length, petal length, petal width, calyx diameter, length of filament, length of style, pedicel length by using Varnier Calipers (Mituyoto Inc., Japan) following the orientation of Ribeiro *et al*. (2016). All the data were collected based on International Board for Plant Genetic Resources descriptor for citrus (IPGRI, 1999). To study the flowering habit, 100 numbers of inflorescence were monitored, and occurrence of flower opening was recorded from 8 am to 6 pm. With reference to flower biology like chronology of bud development from the visible appearance of flower buds to anthesis, time of anthesis, dehiscence of anthers, and withering time of different floral parts were studied. In order to determine the pollen viability, well grown 20 flowers were picked and put in paper bags and were brought to the laboratory. To determine the viable capacity of pollen, 1% aceto carmine stain solution was put on a clear microscope slide and kept for 5-10 minutes at ambient conditions (Baswal *et al*., 2015). Pollen viability was scored based on the staining level as pollen with red color viable, with light red color semi-viable and with colorless nonviable.

RESULTS AND DISCUSSION

Characterization of flowering

Date of flower initiation, flowering completion, flower arrangement and type of flower in sweet orange are presented in Table 1.

Table 1: Flowering date, arrangement and type of flower in BARI Malta-1

Flowering initiation	Flowering completion	Flower arrangement	Flower type
10 Feb 2016	31 March 2016	Solitary	Bisexual

Flowers were borne singly in the leaf axils of previous season's shoots i.e. solitary arrangement. Flower initiation ranged between 10 February 2016 and 31 March 2016. Flowering in India usually occurs mainly from February to April in most of the *Citrus* species (Jadhav *et al*., 2020). The flowers of BARI Malta-1 was bisexual which was similar to the findings of Ibrahim *et al*. (2011) who reported that flowers of sweet orange are perfect.

Floral morphology

Pedicel and calyx characters of BARI Malta-1 flowers before anthesis are presented in Table 2.

Average pedicel length of BARI Malta-1 was 5.8 mm while its diameter was 0.94 mm. The calyx is a cup like structure surrounding the base of the petals. It is green in color. The number of sepals is usually five, which are united (Gamosepalous). Diameter of sepal in calyx was 4.47 µm. Petals characteristics of BARI Malta-1 flowers are presented in Table 3.

The aestivation of petals is imbricate type. Petal color of BARI Malta-1 flowers was white and are not united (Polypetalous). The corolla has usually five petals. Average length of petal was 12.5 mm, whereas petal width was 5.50 mm. Dorji & Yapwattanaphun (2011) reported that the petal length ranging from 11.3 mm to 10.9 mm in Samtse mandarin and Dagana mandarin, respectively. Baswal *et al*. (2015) also reported that the width of petal was maximum (9.34 mm) from sweet orange cultivar. Etebu & Nwauzoma (2014) reported that the sweet orange flowers are white and perfect while Kumatkar *et al*. (2016) observed 5 petals in the corolla of sweet orange. Stamen and anther characteristics in BARI Malta-1 are presented in Table 4.

Anthers in sweet orange are basified and consist of two lobes. Average number of stamen was 20 while the length of filament in the flowers of sweet orange was 8.80 mm. The results of this findings was similar to the finding of Baswal *et al*. (2015), who reported that the number of stamen per flower ranged from 20-23 depending on the cultivar. Flower structure of sweet orange observed with five sepals, five petals and twenty stamens with superior ovary (Kumatkar *et al*., 2016). Filament and pollen characteristics of BARI Malta-1 are shown in Table 5.

The color of the filament was white. The length and diameter of filament was 6.02 and 0.72 mm, respectively, which corroborated the findings of Baswal *et al*. (2015), who observed 6.18 to 11.36 mm filament length in sweet orange cultivar. The color of the pollen was yellow which was strongly supported by the findings of Hoyt (n.d.); Wee (2014) and Karmakar (2013). The shape of pollen in studied sweet orange variety was round-elliptical which was supported by the findings of Inyama *et al*. (2015). The diameter of pollen was 27.75 µm which was within the range of other study by Al-anbari *et al*. (2015) who reported that pollen diameter range from 25-30 µm in sweet orange varieties. Style characteristics in sweet orange are presented in Table 6.

Colour of style was grey in sweet orange. The average length and diameter of style in BARI Malta-1 was 5.92 and 1.17 mm, respectively. The findings of style length in this study was similar to the findings of Baswal *et al*. (2015), who observed a range of 2.95 to 11.08 mm from different cultivar of sweet orange in Punjab. Sometimes rudimentary style was

observed in sweet orange flowers at the end of flowering season, which had a negative impact on successful fruit setting. Stigma and ovary characteristics in sweet orange are given in Table 7.

Brownish, ovoid shaped of stigma having whitish, ovoid shaped ovary was observed at the time of anthesis. Average diameter of stigma in the flowers of studied variety was 2.14 mm which was slightly differed with other findings of Shiraishi *et al*. (1975) who observed about 3 mm diameter of stigma in Satsuma sweet orange having number of papillary cells. This difference in stigma diameter might be due to differences in genotype. Diameter of ovary was recorded as 2.25 mm. There were 21 numbers of locules in ovary which was similar to the findings of Khan *et al*. (2021) in sweet orange.

Time of anthesis and dehiscence

The opening of the flower or anthesis represents the starting point of the reproductive program of plants (Estornell *et al*., 2016). Time of anthesis and dehiscence is very much important to the breeder for hybridization. Bagging and emasculation for crossing must be done before anthesis and before starting of stigma receptivity. Time of anthesis and dehiscence of anthers in flowers of studied sweet orange variety are presented in Table 8.

A represents anthesis, D represents dehiscence of anthers Counting the number of flower started at 8.00 am, when practically opening of flowers just started and continued till 6.00 pm, when the anthesis was more or less over. The first sign of anthesis is marked by the opening of the outermost petal followed by the adjacent two petals. The remaining two petals are united in a boat-shaped fashion for a considerable time before the flower fully opens. The whole process takes about two hours for completion. It is clear from the data (Table 8) that the time of anthesis is spread from 8.00 am to 6.00 pm with peak anthesis (44.62%) at 10.00 am to 12.00 pm followed by 12.00 pm to 2.00 pm (23.50%) and 2.00 pm to 4.00 pm (13.85%). Similar pattern of anthesis recorded in sweet orange by Manju & Rawat (2010). The rate of anthesis was retarded during 4.00 to 6.00 pm. The dehiscence of anthers started simultaneously with the anthesis. The anther became pale yellow with powdery mass and a longitudinal slit was formed between the lobes. The anther dehiscence of this variety took place just after anthesis started at 8 am and continued up to 6 pm. Maximum anther dehiscence (43.85%) took place between 10 am to 12 pm. In some citrus varieties, reported the time of anthesis between 9 am to 12 noon and dehiscence of anthers between 10 am to 2 pm, thus indicating species and varietal difference in respect of anthesis and anther dehiscence (Kumatkar *et al*., 2016). Similarly, the peak period of dehiscence was observed from 10 am to 12 pm in Kuliana lime (*Citrus aurantifolia*) (Mishra, 2017).

Flower bud Development

The floral bud development was divided into eight distinct stages starting from the initiation of the detectable floral buds which required 24.4 days for its completion (Table 9) (Figure 1). Sharma *et al*. (2017) reported similar result under semi arid condition in sweet orange. Among them Stage-VII and Stage-VIII is very important because stigma become receptive approximately 24 hrs before anthesis and continued up to 60 hrs after anthesis. Kumatkar *et al*. (2016) reported that the duration of floral bud development of citrus was 19-23 days depending on the cultivar. Hoque (2015) also reported a total of 27.7 to 31.2 days were required from a bud initiation to reach its fully developed stage in pummelo.

Table 2 : Pedicel and calyx characteristics of BARI Malta-1 flower before anthesis

Pedicel			Calyx		
Color	Length (mm)	Diameter (mm)	Colour	Number of sepal	Diameter (µm)
Green	5.58	0.94	Green	5	4.47

Table 3 : Petal characteristics of BARI Malta-1 flowers

Colour	Number	Tip shape	Margin shape	Length (mm)	Width (mm)
White	5	Round	Smooth	12.5	5.50

Table 4 : Stamen and anther characteristics of BARI Malta-1 flower

Stamen		Anther		
Number	Length of stamen (mm)	Colour	Length (mm)	Diameter (mm)
20	8.80	Brown	2.45	1.83

Table 5 : Filament and pollen characteristics BARI Malta-1 flower

Filament			Pollen		
Colour	Length (mm)	Diameter (mm)	Colour	Shape	Diameter (µm)
White	6.02	0.72	Yellow	Round-elliptical	27.75

Table 6 : Style characteristics of BARI Malta-1 flower

Style			Rudimentary style
Colour	Length (mm)	Diameter (mm)	
Grey	5.92	1.17	Few

Table 7 : Stigma and ovary characteristics of BARI Malta-1 flower

Stigma			Ovary			Number of locules/ovary
Colour	Shape	Diameter (mm)	Colour	Shape	Diameter (mm)	
Brown	Ovoid	2.14	White	Ovoid	2.25	21.00

Table 8 : Time of anthesis and dehiscence of anther in BARI Malta-1 (Flowers opened at two hours interval in percentage)

8 am to 10 am		10 am to 12 pm		12 pm to 2 pm		2 pm to 4 pm		4 pm to 6 pm	
A	D	A	D	A	D	A	D	A	D
11.93	11.65	44.62	43.85	23.50	24.69	13.85	11.80	4.70	3.90

Table 9 : Flower bud development stage of BARI Malta-1

Number of days required for one stage to the other stage								Total days
Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	Stage VIII	
4.5	4.4	3.7	3.2	2.3	2.2	2.1	2.0	24.4

Fig. 1 : Successive bud development stage from stage I to VIII (left to right)

Stage-I: In the first stage, buds just emerged; they are in the leaf axil or terminal end and fully covered with calyx lobes. The pedicel of buds was deep green in color with presence of hairs. At this stage the shape of bud was globose; color of calyx was deep green. Calyx length and diameter was very close to each other. Petals were about to be enclosed with calyx. The buds at stage-I took 4.5 days to reach the second stage.

Stage-II: The second stage commenced when the calyx lobes were observed to have just separated at the apex and the corolla tube was visible. The color of pedicel was green and petal is light green. Shape of bud and color of calyx remained same as observed in the Stage-I. Bud length was 3.95 mm. Bud required on an average 4.4 days to reach the next stage.

Stage-III: At this stage, buds are conical to roundish in shape, length of corolla tube and calyx cup are almost equal, the color of calyx turned to light green and light separation line was observed in the petals. Bud length was 3.86 mm. On an average, 3.7 days required to reach in the next stage.

Stage-IV: When the buds were almost half developed, they were considered to be in the fourth stage of development. The length of corolla is almost double the length of calyx. The color of pedicel and calyx at the fourth stage were observed as green and light green, respectively. Shape of bud was ovate and the petals were cream colored with some green spots on them. Bud length was 6.8 mm. Buds of this variety took 3.2 days to reach the next stage.

Stage-V: At the fifth stage of floral bud development in sweet orange, buds are usually ovate in shape, length of corolla tube being approximately three times the calyx cup. Length of buds increased to 7.47 mm and the buds remained in this stage for 2.3 days.

Stage-VI: In stage-VI, color of pedicel changed to light green having hair in stage six. The shape of bud changed to obovate showing cream color. Petal length in this stage was found to be greater than diameter, which implies that the shape of corolla was changing to obovate from ovate. Buds were 8.32 mm long that took 2.2 days to reach the next stage.

Stage-VII: At the seventh stage, the color of pedicel and calyx were same as observed in stage-VI. Bud shape was obovate and cream colored petals were without any spot. A prominent constriction of splitting was observed in the petals. Length of bud was recorded to be 9.12 mm and the bud took 2.1 days to reach the next stage.

Stage-VIII: All the qualitative characters were same as observed in Stage-VII, except one or two petals were observed to be spitted in Stage-VIII. Average length of bud was 9.93 mm. The bud required 2.0 days to reach its full developed stage (Figure 1). Therefore, it can be said that sweet oranges required a total of 24.4 days from bud initiation to reach its full developed stage.

Abscission time of different floral parts in BARI Malta-1

Among the floral parts, stamen withered and abscised first followed by petal and style (Table.10). Abscission of stamen started after 48.05 hrs of anthesis and completed after 109.8 hrs of anthesis. Petals started to be abscised after 73.1 hrs of anthesis and completed after 100.8 hrs of anthesis.

Table 10 : Abscission time of different floral parts in BARI Malta-1 after anthesis

Stamen		Petal		Style	
Start (hrs)	Complete (hrs)	Start (hrs)	Complete (hrs)	Start (hrs)	Complete (hrs)
48.05	109.8	73.1	100.8	252.9	302.2

Practically, it was observed that petals started to wither and abscise when some stamens are still attached with the flowers. The color of the ovary rind turned light green to green after the abscission of petals and stamens, though the style had not abscised. Style took long time to start abscission and it was 252.9 hrs after anthesis. Abscission of style completed after 302.2 hrs of anthesis. Eti & Stosser (1991) reported the abscission time of style in sweet orange was after 288 hrs of anthesis. This result of abscission of different floral parts differed slightly with the findings

reported by Hoque (2015) in pummelo (*Citrus grandis*). The author reported the abscission time of petals, stamens and style after 77-13, 53-123 and 167-226 hrs of anthesis, respectively. This variation might be attributed to genetic and environmental differences (Estornell *et al*., 2016) as environmental factors (temperature, light, humidity, wind speed etc.) are important for abscission of floral parts.

Pollen viability

Pollen viability is important for obtaining information to support plant breeding programs. Percentage of pollen viability recorded the highest at anthesis (98.3%) and thereafter, decreased gradually up to 4 hrs after anthesis in sweet orange (Figure 2).

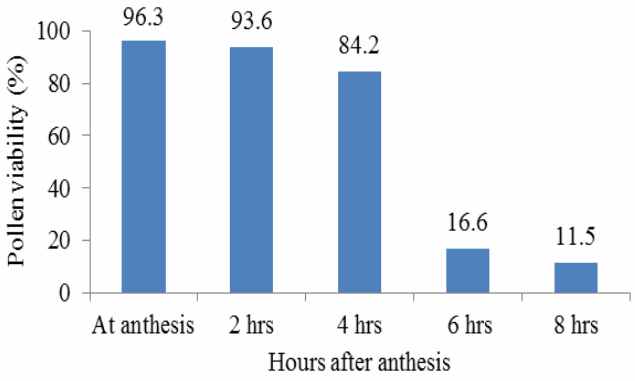

Fig. 2 : Viability of pollen grains in sweet orange at different hours after anthesis

Pollen viability was more than 80% even at 4 hrs after anthesis. After 4 hrs of anthesis, viability of pollens decreased drastically. This result indicated that preferably fresh pollens at anthesis should be used in artificial pollination and if not possible, the time should not exceed in no case from 4 hrs after anthesis. This results corroborated the findings of Cavalcante *et al*. (2000) and Hoque (2015) who found 9% to 98% pollen viability in tangerine and pummelo. This result differed from the findings of other researchers (Brugnara *et al*., 2009; Turgutoglu *et al*., 2015), who reported 42% to 86% pollen viability in Meyer lemon and sweet oranges. However, Ribeiro *et al*. (2016) observed that estimates of pollen viability may vary between species and even between samples of the same species or individual. Moreover, Soost & Roose (1996) reported that pollen viability may vary among species, but not in the same plant or species. It is clear from the above results that BARI Malta-1 can be used as a donor parent in any hybridization program as indicated by Baswal *et al*. (2015).

CONCLUSION

The flowers of BARI Malta-1 are bisexual, white colored, borne singly or in clusters, having 5 sepals and petals, 20 stamens with 8.80 mm long. Stages of floral bud development from initiation to anthesis were divided into 8 distinct stages. In BARI Malta-1, a total of 24.4 days are required from a bud initiation to reach its fully developed stage. Abscission of stamen started after 48.05 hrs of anthesis and completed after 109.8 hrs of anthesis. Petals started to be abscised after 73.1 hrs of anthesis and completed after 100.8 hrs of anthesis. Maximum anthesis occurred between 10:00 PM to 12:00 PM of the day which is about 44.62% of total flower.

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