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Floral morphology of *Eleaegnus latifolia* L.

H. Rymbai*, N.A. Deshmukh, A.R. Roy, S.S. Roy and A.K. Jha

Division of Horticulture, ICAR Research Complex for NEH Region, Umiam 793 103, Meghalaya

ABSTRACT

***Eleaegnus latifolia* L. is one of the most important life sustaining underutilized fruit crops among the tribes of the eastern Himalaya region. The fruits have several uses and all parts of the fruits including seed are edible at all stages of fruit growth. Despite of great potential, little or no information is available on its flower characteristic, which is a pre-requisite for understanding the reproductive biology of the species. Five genotypes of *Eaeagnus latifolia* L. collected from different locations of Meghalaya were used to study its flowering and fruiting traits. Result revealed that inflorescence is a raceme, appears in clusters from the leaf axils, flowers are light yellow coloured, hermaphrodite, actinomorphic and gives strong aroma. Ovary is inferior surrounded by hypanthium. Significant variations were observed among the genotypes for all the flower characteristics such as dimension of ovary, stigmas and pollen length in the polar region. Maximum bud intensity was recorded in REC-3 (16.0) on current season shoot. Number of flower buds was higher in the current season shoots as compared to previous season. Regardless of genotypes, number of flowers was higher in the middle portion of the shoot. Total number of flowers per shoot was maximum in REC-4 (127.0). Flowering duration per inflorescence varied from 6.0 days (REC-1 and REC-2) to 8.67 days in REC-4. Initial fruit set was recorded maximum in REC-1 (37.95%) and minimum in REC-2 (14.15%).**

Key words: *Eleaegnus latifolia*, floral morphology, *Sohshang.*

INTRODUCTION

Eleaegnus latifolia L., the vernacular name is *Sohshang* in Khasi Hills and *Slangi* in Jaintia Hills of Meghalaya. The fruit has traditionally been used for centuries as one of the most potential underutilized fruit crops among the tribal habitat of North Eastern Himalayan region, India. Geographically, the region stretches between 21°50' and 29°34' N latitudes and 85°34' and 97°50' E longitudes, and altitude varies from near sea-level to over 7,000 m above msl. It is native to the North Eastern Region, India. It is a perennial and semi-deciduous multi-stem shrub, belonging to the family Elaeagnaceae. The family consists of three genera, *viz*., *Elaeagnus Hippophae* and *Shepherdia*. The genus *Elaeagnus* consist about 40 species of shrubs and trees, however, only 3 species are known for planting in other parts of the world, *viz*., Russian olive (*Elaeagnus angustifolia*), silverberry (*Elaeagnus commutate* Bernh. Ex Rydb) and autumn olive (*Elaeagnus umbellate* Thunb). Apart from the fruits, the seeds of most of the species including *Elaeagnus latifolia* are edible. Recently, the genus has become a critical underutilized fruit crops because the trees of the genus *Elaeagnus* have a symbiotic relationship with certain soil bacteria like the genus *Actinomycetes* responsible for producing root nodules and fix atmospheric nitrogen (Follstad Shah, 2). Because of its atmospheric nitrogen fixing abilities, an increase in fruit production up to 10% on intercropping with plum and nuts was reported. The species are quite resistant to high wind velocity and performed well even on nutrient poor acidic soil and soil moisture stress conditions. The fruits are also rich in vitamins, minerals and other bio-active compounds (Rymbai *et al*., 6). More importantly, the fruits are also capable of minimizing the incidence of cancer and reversing the growth of cancer cells (Matthews, 4).

Breeding to develop improved cultivars with high yield and quality is one of the approaches including recent efforts on standardization of agro-techniques (Deka and Rymbai, 1). Thus the development of methods for reliable and efficient breeding techniques in this crop is critical for higher success. However, to achieve success in breeding programme, knowledge of floral morphology and fruiting attributes become pre-requisite. Thus, understanding the reproductive and yield attributes of *Elaeagnus latifolia* is an important issue and indispensable to successful conservation efforts. Flower is an important reproductive organ of any flowering plants. The flower characteristics are unique, expressed consistently and are controlled genetically with very little or no influence of the environmental factors. Therefore, flower structures are very essential component for identification of plants and crop improvement. Till date, very little information is available on flower traits of

*Corresponding author's E-mail: rymbaihort@gmail.com

other species of the genus *Eleaegnus,* such as Russian olive (*Elaeagnus angustifolia*), silverberry (*Elaeagnus commutate* Bernh. Ex Rydb), autumn olive (*Elaeagnus umbellata* Thunb) and *Elaeagnus commutate*. However, there is no report available on floral biology of *Elaeagnus latifolia*. Therefore, the present study on floral morphology of this species was undertaken to provide greater insights on floral characters and fruiting attributes, which would facilitate efficient breeding programme for higher crop productivity.

MATERIALS AND METHODS

The present study was conducted during 2013-15 on five genotypes of *Eleaegnus latifolia* L., *viz*., REC-1, -2, -3, -4, -5 collected from different locations of Meghalaya and maintained at the ICAR Research Complex for NEH Region, Umiam. *Elaeagnus latifolia* is a perennial semi-deciduous tree. Flowering appears during October-November. Three fully grown healthy and well maintained trees were selected from the orchards. All the trees received uniform recommended cultural management practices during the course of study. Qualitative flower characteristics were critically observed in the field. Characters observed were flower bearing habit, colour on sides, *i.e*., abaxial and adaxial, sex, symmetry and aroma. Number of lobe per flower, number of perianth per flower and nature of trichomes were examined under dissecting microscope (Leica EZ4D) at 8X magnification. Bud intensity was observed when the buds appeared on the shoot (30 cm shoot length) separately for current season and on shoots of previous season. Number of flower buds per inflorescence was noted by counting on tagged inflorescences (n = 10) in four directions of each tree. In order to avoid error, the counted flowers on each inflorescence were removed and fresh open flowers were counted on daily basis. Numbers of flowers at apical, middle and base portion of shoot was also recorded as and when they opened. Total number of flowers per shoot was recorded by addition of all the flowers recorded from the apical, middle and base portion of the shoot. Flowering duration per inflorescence was assessed by noting the date of first flowers visible to last flower appeared in an inflorescence on a daily basis. Initial fruit set per inflorescence was recorded at grain stage of fruit development in all four directions of the tree. During full bloom, the newly opened flowers were collected in the morning hours and were directly put in the fixative FAA for microscopic studies. The fixed flowers were mounted on the stage of microscope and flower parts were measured accordingly at different magnifications depending upon the floral organs. Observations on 50 flowers were taken from each genotype for all the parameters. Flower size, petal, ovary, style, anthers, filaments and stigma characters were observed with the help of dissecting microscope (Leica EZ4D) at specified magnification. Flower size and petal dimensions were measured at 8X magnification. Flower length was taken from the base of the ovary to the top of the flower, stamens or stigma. The flower width was observed at the maximum width in two directions of the flower. The average of these two directions was taken as flower width. Petal length was noted from tip to base and width was measured at the maximum portion of the petal. Ovary, stigma and style dimension were determined at 35X magnification. The length was taken from tip to base of the ovary. Ovary width was taken at three portions, *i.e*., apical, middle and at the base. The style length was recorded from tip to base of the style. The style width was recorded at three portions, *i.e.* tip, middle and top. Stigma length was taken from tip to base and diameter was measured at the maximum width portion (middle) of the stigma. Anther and filament dimensions were observed (n = 50) at 25X magnification. The anther length was recorded longitudinally and width was noted in the central portion where the maximum width was observed. The filament length was taken from tip to base, whereas, width was assessed at the base, middle and tip portion of the filament. Pollen dimension was assessed under the light microscope (Olympus BX53) at 40X. Flowers near anthesis period were collected in the morning hours (8:30 am to 9:30 am) and the anthers were dusted on the slide and covered with a cover slip after applying one to two drops of the staining agent fluorescein diacetate (FDA). The slides were then placed under the microscope to measure the length along the polar and the equatorial regions. The data on different parameters were analyzed using analysis of variance (ANOVA) based on randomised block design (RBD) using SPSS (version 18.0). Valid conclusions were drawn only on significant differences between the genotype mean at 0.05 level of probability.

RESULTS AND DISCUSSION

Reproductive structures are known to reveal evolutionary relationships more clearly among plants. The present finding divulged that flowers of all the genotypes appears in clusters in the leaf axils and are short (Table 1; Fig. 1a). Flowers are hermaphrodite, actinomorphic, four-lobed and having single perianth (Fig. 1b). It produces strong aroma, which might have been released from the conical papillae of the adaxial epidermis of the perianth. The presence of essential oils in the epidermal cells of the petals might have also been one of the reasons for this strong aroma. The presence of essential oils have also been reported in

Table 1. Qualitative flowering characteristics of different *Elaeagnus latifolia* L. genotypes.

Genotype	Bearing habit	Colour		Sex	Symmetry	Aroma	No. of lobe	No. of perianth	Nature of trichome
		Adaxial	Abaxial						
REC-1	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-2	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-3	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-4	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-5	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate

many plant species (Stpiczynska, 8). Apart from the strong aroma, another attractive feature of the flowers is colouration which is light yellow from the adaxial side and light green in the abaxial side, exhibiting its ability to attract high number of pollinators. Attractive and shining peltate hairs of silvery star shape were found on adaxial surface of sepals. Silvery star shape hairs were observed at the apical portion of the perianth lobes and on the style (Fig. 1g). Peltate hairs are known for their function in reducing transpiration from organs. All these features together might be responsible for attracting high numbers of certain dipteran insects resulting in heavy fruit-set.

Significant differences were detected for flowers bud intensity, number and duration of flower among the genotypes (Table 2). Maximum bud intensity was recorded in REC-3 (16.00) for current season shoot and REC-2 (6.33) on previous season shoot. REC-4 showed the maximum number of flower buds per inflorescence in both the current season (21.67) and previous season shoot (14.33). Result suggests that irrespective of genotypes, bud intensity and number of flower bud per inflorescence were higher in the current season shoot as compared to the previous season shoot. Number of flowers at apical, middle and base portion of shoot revealed a significant variation among genotypes. REC-1 recorded the maximum number of flowers at apical portion (23.33), whereas, REC-5 recorded maximum at the middle (64.00) and base (40.67) portion of the shoot. This indicates that number of flowers varies with position at various length of the shoot. Regardless of genotypes, numbers of flower were higher in the middle portion of the shoot. Therefore, if any hybridization programme is to be carried out successfully, emphasis may be given to the current season and middle portion of the shoot for greater option on flowers availability.

Total number of flowers per shoot was maximum in REC-4 (127.00) and minimum in REC-2 (69.67). Duration of flowering per inflorescence varies from 6.00 (REC-1 and REC-2) to 8.67 days in REC-4. Result indicates that for efficient hybridization, operation must be taken within this period. Initial fruit set was recorded maximum in REC-1 (37.95%)

Table 2. Flower bud intensity, number and duration of flowers and fruitset characteristics of *E. latifolia* L.

Genotype	Bud intensity at 30 cm length		No. of flower buds per inflorescence		No. of flowers			No. of flowers per shoot	Flowering duration (days)	Fruit set (%)
	Current season shoot	Previous season shoot	Current season shoot	Previous season shoot	Apical portion of shoot	Middle portion of shoot	Base portion of shoot			
REC-1	8.33	1.33	11.33	9.33	23.33	40.67	24.67	88.67	6.00	37.95
REC-2	13.67	6.33	17.67	7.00	16.00	32.00	21.67	69.67	6.00	14.15
REC-3	16.00	1.33	19.33	1.00	15.33	58.00	39.67	113.00	7.00	35.18
REC-4	8.33	2.33	21.67	14.33	22.00	34.00	36.67	92.67	8.67	19.38
REC-5	14.00	2.33	19.66	6.33	22.33	64.00	40.67	127.00	7.67	28.48
$CD_{0.05}$	2.61	1.67	2.12	4.29	3.06	5.37	5.16	13.45	1.04	1.41

and minimum in REC-2 (14.15%). This suggests that there is variation among genotypes, which might be due to genetic constitution.

Flower size and density determine the food resources for pollinators, which in turn lead to the reproductive success of plants. Genotypes under present study showed varied flower size and were significantly different ($p \leq 0.05$) for flower length and width (Table 3). Maximum flower length was noted in REC-1 (12.23 mm), which was *at par* with REC-3 (11.46 mm); whereas, minimum flower length was recorded in REC-5 (7.88 mm). Similarly, maximum flower width was recorded in REC-1 (8.65 mm), which was *at par* with REC-3 (8.25 mm), while minimum width was recorded in REC-2 (6.95). These variation in flower size among the genotypes might be genotype dependent.

Sepals are important parts of the flower which protect the inner parts of the flower and prevent desiccation during its development. It was observed that sepals were small and greenish in colour irrespective of the genotypes. Sepals were four angled, fused together to form tubular and bell-shaped structure (Fig. 1b & c). It was observed that sepals and stamens are linked by a tissue of long tube extending below ovary. A hypanthium is a floral cup or calyx tube developing from common zonal growth at the base of perianth (congenitally fused and androecium) parts. Furthermore, any tubular structure bearing the calyx, corolla lobes and the stamens are considered to be a hypanthium.

The petals vary dramatically among species, which have been more commonly used to distinguish and classify the species. Petal characteristic also play a vital role in proper pollination by attracting pollinators. Irrespective of genotypes, *Sohshang* flower has four numbers of petals. Significant differences ($p \leq 0.05$) in petal size were observed within the five *Sohshang* genotypes (Table 3). REC-1 recorded maximum petal length (3.59 mm), which was *at par* with the REC-5 (3.42 mm) and REC-3 (3.36 mm). Shortest petal (2.81 mm) was found in REC-2. Similarly, maximum petal width was also noted in REC-1 (3.01 mm), which was *at par* with REC-5 (3.00 mm) and REC-3 (2.96 mm). These variations among genotypes for petal size could be due to genotypic effects.

The ovary position and number of ovule with respect to the other floral parts is often used as a trait to distinguish taxa, especially families. In all the genotypes, ovary appears to be inferior, although there was no connection observed between the ovary walls with the external wall. However, there was a constriction above the ovary (Fig. 1f). This inferior position of ovary was further stressed due to the presence of a nectary on the hypanthial slope, surrounding the style. Ovary characteristics also exhibited significant variation ($p \leq 0.05$) among genotypes (Table 3). At apical portion, REC-1 recorded highest ovary width (0.57 mm), while lowest (0.44 mm) was observed in REC-2 and REC-4. Ovary width at middle portion was recorded maximum in REC-3 (0.63 mm), while ovary width at the base was highest in REC-1 (0.52 mm). Maximum ovary length (1.25 mm) was recorded in REC-2, as compared to minimum in REC-5 (0.82 mm). These variations in ovary dimension might be a unique trait of each genotype. Irrespective of genotypes, one ovule was recorded, which is erect and inverted in position (Fig. 1d).

Style is the supportive stalk of stigma and the pathway for pollen tubes to grow from pollen grains adhering to the stigma. In all the genotypes, silvery and shining star shaped hairs on the style (Fig. 1g) and a disc located at the base of the style were observed (Fig. 1h). The base of the style was enclosed by the hypanthial slope. Significant variation was also observed in style dimension among different genotypes of *Sohshang* ($p \leq 0.05$; Table 3). Maximum style width at the base was recorded in REC-5 (0.58 mm), while minimum was noted in REC-4 (0.39 mm).

Table 3. Flower parts of different *Elaeagnus latifolia* genotypes.

Genotype	Flower		Petal			Ovary						Style			
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Petal No.	Width (mm)			Length (mm)	Type	Ovule No.	Width (mm)			Length (mm)
						Apical	Middle	Base				Base	Apical	Middle	
REC-1	12.23	8.65	3.59	3.01	4.00	0.57	0.62	0.52	1.01	Inferior	1.00	0.47	0.52	2.05	6.83
REC-2	10.78	6.95	2.81	2.73	4.00	0.44	0.52	0.40	1.25	Inferior	1.00	0.46	0.51	0.59	7.57
REC-3	11.46	8.25	3.36	2.96	4.00	0.51	0.63	0.46	1.16	Inferior	1.00	0.50	0.49	0.64	7.51
REC-4	10.91	7.75	3.14	2.71	4.00	0.44	0.41	0.33	0.65	Inferior	1.00	0.39	0.40	0.59	6.45
REC-5	7.88	8.01	3.42	3.00	4.00	0.50	0.59	0.49	0.82	Inferior	1.00	0.58	0.74	1.19	8.05
$CD_{0.05}$	1.33	0.89	0.42	0.23	-	0.03	0.11	0.07	0.27	-	-	0.20	0.09	0.84	0.78

- = No analysis was done

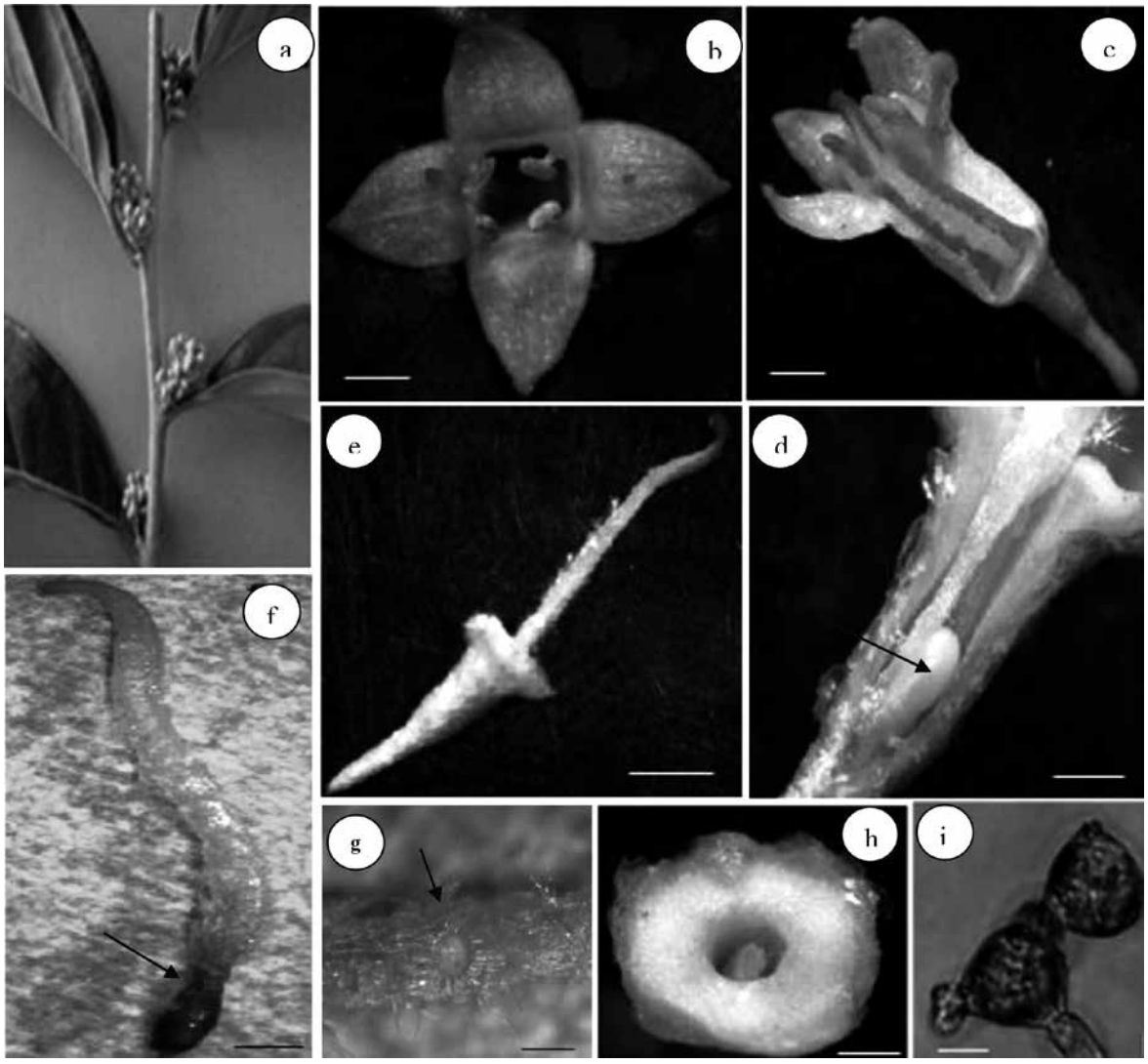

Fig. 1. (a) Cluster of flower buds in leaf axil, (b) Actninomorphic flower, (c) Location of style and stamens, (d) Erect and inverted ovule, (e) Flower stalk and style, (f) Constriction between the ovary and style, (g) Peltate hairs, (h) Disc at the base of the style, (i) Pollens.

REC-1 showed the highest style width in the middle (2.05 mm), while minimum (0.59 mm) was recorded in REC-2 and REC-4. Maximum style width at apical region was recorded in REC-5 (0.74 mm), and the minimum was recorded in REC-4 (0.40 mm). Style length ranged from 6.45 mm in REC-4 to 8.05 mm in REC-5. The present finding on style length range was similar to *Elaeagnus umbellate* (Murraya, 5).

It was noted that number of stamens in all the genotypes were four and no variations were observed (Table 4). Anthers and filaments dimension of different genotypes showed significant variations ($p \leq 0.05$). Anther length was maximum in REC-5 (1.35 mm), and least in REC-2 (1.09 mm). Similarly, maximum anther width was also recorded in REC-5 (0.58 mm), while minimum was recorded in REC-3 (0.30 mm). Similarly, variations were also observed within the filaments. REC-4 recorded the maximum filament width at the base (1.38 mm) and in the middle portion (0.90 mm) and least in REC-2 both in the base (0.37 mm) and in the middle portion (0.32 mm), respectively. Filament width at the apical portion showed non-significant variations. REC-5 recorded maximum filament length (1.84 mm), while minimum was observed in REC-2 (1.24 mm). These variations among genotypes could be attributed to genetic factors.

Stigma is the receptive part of the female reproductive system which binds pollen and mediates tube migration into the style. Irrespective of genotypes,

Table 4. Stigma, androecium and pollen characteristics of different *Elaeagnus latifolia* L. genotypes.

Genotype	Stigma		Androceium							Pollen size (µm)	
			Anther		Filament width (mm)			Filament length (mm)	No. of stamen		
	Width (mm)	Length (mm)	Length (mm)	Width (mm)	Base	Middle	Apical			Polar region	Equatorial region
REC-1	0.53	1.87	1.18	0.39	0.66	0.41	0.36	1.47	4.00	2.53	2.15
REC-2	0.29	1.23	1.09	0.41	0.37	0.32	0.22	1.24	4.00	2.05	1.72
REC-3	0.36	1.23	0.97	0.30	0.54	0.35	0.30	1.35	4.00	2.07	2.00
REC-4	0.54	1.50	1.15	0.36	1.38	0.90	0.39	1.43	4.00	2.03	1.95
REC-5	0.47	0.37	1.35	0.58	0.77	0.46	0.36	1.84	4.00	2.41	2.00
$CD_{0.05}$	0.14	0.39	0.19	0.03	0.27	0.08	NS	0.33	-	0.03	NS

NS = Non significant difference; - = No analysis was done

it was observed that the stigmas were elongated; curved and brown dotted (Fig. 1e & f). Stigma characteristics exhibited significant variation among different genotypes of *Sohshang* ($p \leq 0.05$; Table 4). Stigma width ranged from 0.29 (REC-2) to 0.54 mm (REC-4). Longest stigma was recorded in REC-1 (1.87 mm), while shortest was found in REC-5 (0.37 mm). Stigma dimension showed variation among genotypes might be due to genetic influences.

Pollen dimension may contribute to the systematic and evolutionary arrangement in the family. Pollen dimension in different genotypes of *Sohshang* was also significantly different ($p \leq 0.05$; Table 4; Fig. 1i). REC-1 recorded significantly highest pollen length in the polar region, which was *at par* with REC-5 (2.41 µm). However, minimum pollen length was observed in REC-2 (2.04 µm) in the polar region. The pollen size along equatorial region showed non-significant variations among the genotypes, although the size ranged between 1.72 (REC-2) to 2.15 µm (REC-1). The results confirmed the findings of Sarkissian and Harder (7) that pollen size normally varies little within a species. However, there are also reports of intervarietal variation in pollen grain size (Franchi *et al.*, 3).

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