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Floral biology studies of Egyptian clover, *Trifolium alexandrinum* L.

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Floral biology studies of Egyptian clover, *Trifolium alexandrinum* was studied at Forage Section, Department of Genetics and Plant Breeding, CCS, Haryana Agricultural University, Hisar during 2012 and 2013. *T. alexandrinum* flower head of cultivar HB-2 consisted of numerous yellowish-white flowers that measured 22.7±1.3 mm in length and consists of a mean of 70.5±1.70 florets. The mean length of a floret, sepals, petals and stamen were 12.3±1.9, 4.4±0.6, 4.1±2.2 and 3.4±0.5 mm, respectively. On an average a floret, remained in bud stage (A+B+C) for a longer period of 80.4±1.2 hours during 2012 than in 2013 (69.7±7.7 h). The overall mean duration of bud stage A (petals concealed in bud) was the longest (35.6±5.4 h) as compared to stage B when petals became visible but not expended (26.2±3.0 h) and C (24.8±2.0 h) when petals expended but anthers were not visible. The bud stage (A+B+C) lasted for a mean duration of 75.1±3.9 hours. The overall mean duration of the anthesis stage (D_1+D_2) was 63.1±5.6 hours (range 45-87 hours) and that of post anthesis at 37.8±6.9 (range 30-51) hours. The life cycle of *T. alexandrinum* flowers on an average was completed in 186.8±7.0 hours.

Key words: floral visitors, Egyptian clover, biology

Egyptian clover, *Trifolium alexandrinum* L. vernacularly called *berseem* (Family Leguminaceae, sub-family Papilionaceae). *T. alexandrinum* has played a key role in the development of milk, meat, poultry, beekeeping and allied industries, supplying not only the essential nutrients but also being the source of protein, calcium, phosphorus and other minerals to the animals. It has thus, played a dominant role in the development of human health and vitality by providing animal protein and other nutrients. Egyptian clover plant is 18-36 inches in height, erect, non-reseeding, cool season, hollow stem annual recognizable by its typical clover like appearance. The inflorescence is round to oblong with yellowish heads resembling white clover. Heads appear in January, bear 50-200 flowers that open in ascending order from base to top and each floret produce one seed each. Most of the present day Indian cultivars are the derivatives of biotype Mescavi and important varieties are HB-2 (Anonymous, 2012), Wardan, BL-1, BL-10, BL-22, JB-2, BL-2, UPB-110, Bundel Berseem -2 (JHB 146), Bundel Berseem -3 (JHTB 96-4), HFB 478, etc. (Roy *et al*., 2009). Insect pollination in crops is the most critical ecosystem service fulfilling plant's ultimate requirement of pollination, fertilization, seed setting and seed production (Chaudhary, 1998), a fact corroborated by Klein *et al*. (2007) who reported that 87 of the 115 leading global food crops depend upon animal pollination and forage crops are absolutely dependent on insect pollination. Egyptian clover is reported to be highly self-incompatible in its place of origin but in India it is believed to be self fertile. However, the crop has shown wide diversity for self fertility and population with self compatible and self pollinating, self compatible requiring tripping, self incompatible types with broad genetic base and self incompatible requiring tripping is reported to be essential for seed setting (Chowdhury *et al*., 1966). The studies on floral biology were essential to know the floral characters such as length and width of floral parts, location of nectarines, time of blooming, stigma receptivity that ultimately decide the efficiency of pollinators in bringing about the pollination as well as the suitability of the pollinator species for a particular crop.

Materials and Methods

The present investigations were carried out at Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University and Apiculture Laboratory, All India Coordinated Research Project on Honey Bees and Pollinators and Apiculture Laboratory of the Department of Entomology, Hisar Haryana for two years during 2011-12 and 2012-13. To carry out these studies Egyptian clover cultivar "HB-2", the most widely adapted and important cultivar in North India was used.

Floral morphology and dimensions

The detailed floral structure *T. alexandrinum* cultivar HB-2 was studied by observing 25 flowers (three replications) that were brought from the field were dissected and each floral character that included general floral morphology like number, position, colour and size of floral parts including those florets, sepals, petals and stamens were measured by using calibrated ocular micrometer under stereo-zoom microscope.

Floral biology

The detailed floral biology of *T. alexandrinum* flowers was studied by observing 25 flowers (three replications) that were randomly selected and tagged on the flower heads. Various enfolding floral events like bud stage, flower opening, expansion of petals, visibility of anthers, dehiscence, shedding of petals, colour change of floral parts, etc. were recorded at 2-hourly intervals during the period of study. For this, the events with a bearing on the pollination were selected and classified as stages A, B, C, D_1, D_2 and D_3 as suggested by Dafni (1992):

Stage	**Description**
A	Bud stage, petals not visible
B	Bud stage, petals visible but not expanded
C	Petals expanded
D_1	Anthers became visible but did not dehisce
D_2	Anther dehisce
D_3	Pollination/ fertilization occurred, anthers dried up

The duration of individual floral event was converted into hourly values and further duration of bud, anthesis and post-anthesis stage was calculated.

Result and Discussion

Floral morphology and dimensions

Flower head or raceme of *T. alexandrinum* cv. HB-2 measured 22.7±1.3 mm and consisted of 70.5±1.70 yellowish-white florets (range 67-74) each measured 12.3±1.9 mm (range of 9.7-14.3 mm) (Table 1, Figure 1 & 2). Each true floret like a typical leguminous flower consisted of a five-lobed calyx and a corolla consisting of a standard, two wings, a keel petal which is convex, obtuse at apex, slightly lobed at base and longer than the inferior tooth, ten stamens and a stigma. Mean dimensions of calyx/sepals were 4.4±0.6 mm (range 3.1-5.0 mm), petals 4.1±2.2 mm (3.1-4.9 mm) and stamen measured 3.4±0.5 mm (range 2.6-4.4). The present findings on floral dimensions are in corroboration with observations those made by Abdalla *et al.* (2012) who recorded average size of florets was 11.83 mm, sepals 5.57 mm, petals 4.01 and stamen was 3.71 mm, while Akerberg and Lesins (1949) and Dennis and Haas (1967) reported that the floret size varied from 7.5 to 12.4 mm. However, Malaviya *et al.* (1999) recorded almost similar dimensions for diploid flowers but higher for sepals, petals and stamen (11.5, 6.0, 8.0 and 7.0 mm, respectively) while these values for tetraploid *T. alexandrinum* were still higher at 15.0, 0.7, 10.0 and 8.0 mm, respectively. The reproductive parts remain enclosed in the standard and wing petals and stigma was surrounded by stamens that produce anthers and finally pollen grains. . Stamen is ovate-apiculate, whitish in colour, bear anthers terminally and measure 3.4±0.5 mm in length (range 2.6-4.4 mm). The anthers on dehiscence release ample pollen grains. Florets contain a single ovary that is sessile, and with very long style, which is slightly enlarged toward the middle. The stigma is cristate, with prominent campanulate tube nerves and its throat is completely closed by the membranous operculum. The slightly curved pistil is longer than stigma. The reproductive parts remain enclosed in the standard and wing petals while the stigma is surrounded by stamen. *T. alexandrinum* fruit is formed by the enlarged calyx at maturity. Calyx enlarges at maturity to form a box capsule that contains single seed. Nectar is secreted by the nectarines that open at the base of the stamens and gets collected in the corolla tube. The mean corolla length was 4.1±2.2 mm (3.1-4.9 mm). Similar observations on the floral arrangement were also made by McGregor (1976) and Kennedy and Mackie (1925).

Figure 1: Typical flower of *T. alexandrinum*

Table 1: Dimension of *T. alexandrinum* cv. HB-2 flower

No. of observations	Number of florets	Size of floral parts of *T. alexandrinum* (mm)				
		Flower	Florets	Sepals / calyx	Petals / corolla	Stamen
1	72.3*	23.7	9.7	3.9	4.1	3.3
2	70.7**	20.3	14.0	3.1	3.2	4.4
3	69.3	23.3	14.3	5.0	3.1	2.6
4	69.7	24.0	11.0	4.9	4.1	4.1
5	70.0	21.7	12.0	4.1	4.5	3.1
6	70.7	22.7	13.7	4.4	4.7	3.3
7	70.7	22.0	13.7	4.7	4.4	3.5
8	69.7	24.7	10.7	4.5	4.8	3.0
9	71.3	23.3	9.7	4.3	4.9	3.1
10	70.7	21.0	14.3	4.6	3.4	3.2
Mean	**70.5**	**22.7**	**12.3**	**4.4**	**4.1**	**3.4**
SD	**1.70**	**1.3**	**1.9**	**0.6**	**2.2**	**0.5**

* Observations are mean of two years data; ** Each observation is a mean of 6 replications (n=3 x 2 year)

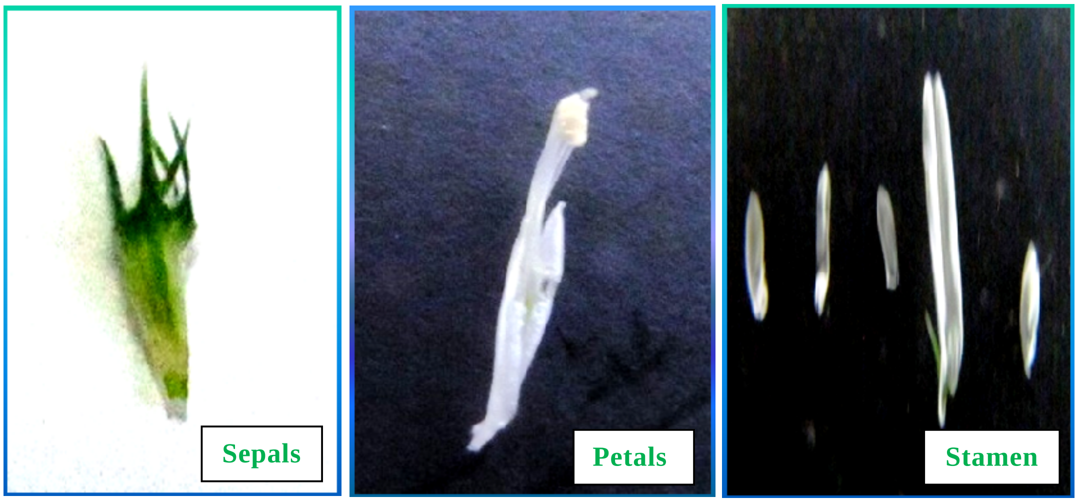

Figure 2: Sepals, petals and stamen of *T. alexandrinum* flower

Table 2: Floral biology of *T. alexandrinum*

Flower Stages	Duration of different stages (hours) in years				Overall	
	2012		2013		Mean	S.D.
A. Bud Stage	**Mean**	**S.D.**	**Mean**	**S.D.**		
A	28.6*	1.5	42.7	10.8	35.6	5.4
	(27-30)**		(27-54)		(27-54)	
B	**26.2**	3.6	26.3	5.1	26.2	3.0
	(24-42)		(24-45)		(24-45)	
C	25.6	3.8	24.0	2.4	24.8	2.0
	(9-30)		(21-27)		(9-30)	
Total Duration	**80.4**	**1.2**	**69.7**	**7.7**	**75.1**	**3.9**
	(78-81)		**(54-75)**		**(54-81)**	
B. Anthesis Stage						
D1	23.9	0.6	32.3	4.7	28.1	2.4
	(21-24)		(21-39)		(21-39)	
D2	31.3	9.7	37.3	3.8	34.3	5.1
	(21-45)		(33-48)		(21-48)	
Total Duration	**56.2**	**10.0**	**70.1**	**6.1**	**63.1**	**5.6**
	(45-69)		**(54-87)**		**(45-87)**	
C. Post Anthesis stage						
D3	**37.8**	**7.0**	**37.8**	**7.1**	**37.8**	**6.9**
	(33-48)		**(33-48)**		**(30-51)**	
Total Duration (Mean + SD)	**173.3**	**13.3**	**200.4**	**2.4**	**186.8**	**7.0**
Range	**(153-201)**		**(198-201)**		**(153-201)**	

*Figures are mean of 25replications **Figures in parentheses are the range values in hours

Floral biology

The floral biology reflects the different events of the flower opening and reproductive receptivity of the flower. The floral biology of the *T. alexandrinum* was classified into different stages.

Bud Stage

The florets remained in the bud stage (A) -when petals were concealed in the bud and not visible from outside- for a mean duration of 35.6 ± 5.4 hours (range 27-54 hours) (Table 2). The second bud stage when petals became visible but were still not expanded (B) lasted for 26.2±3.0 hours (range 24-45 hours). It then entered the third bud stage when petals expanded but anthers were not visible (C) and remained for 24.8± 2.0 (range 9- 30 hours) A floret, on an average remained in bud stage (A+B+C) for a period of 75.1± 3.9 hours.

Anthesis stage

Anthesis stage further divided into D_1 when petals became fully expanded and anthers were visible and D_2 stage (when anthers were fully exposed for pollination). The pooled mean duration of D_1 stage lasted for 28.31±2.4 hours with a range of 21-39 hours and that of D_2 stage was little longer 34.3±5.1 hours (33-48 hours). The overall mean duration of the anthesis stage was 63.1±5.6 hours (range 45-87 hours).

Post-anthesis stage

The florets spent mean post anthesis D3 stage (anthers dried up) of 37.8± 6.90 hours with a range of 30-51 hours in. The mean floral cycle of a *T. alexandrinum* flower lasted for 186.8 hours (range, 153-201 h) which was shorter (173.3 h) during 2012 than 2013 when it took little longer (200.4 hours).

This study forms the first report of detailed investigations on floral biology of *T. alexandrinum*. However, in a related crop Polymorphous clover (*T. polymorphum*), Speroni *et al.* (2009) reported extended life cycle of a flower that lasted for 12 days and staggered anthesis period which started on 6th day with the exposure of wings and keel petals and flowers remained open for another 5-6 days. Anther dehiscence began during pre-anthesis at low level (20%), 1-3 days before anthesis and increased to more than 60 percent a day before anthesis. Such variations may be explained due to differential crop phenology and high temperature prevailed during the crop season which favours the early flower opening, anthesis and short post-anthesis period.

CONCLUSION

These floral biology studies give insight to the receptivity period of the flower that enables the maximum pollination and also efficient utilization of the floral resources for maximization of honey production.

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