



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(1): 2200-2206

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Received: 15-11-2018

Accepted: 19-12-2018

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International Journal of Chemical Studies

Floral biology of wild raspberry (*Rubus macilentus* C.) species of Garhwal Himalaya, Uttarakhand, India

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Abstract

To observe the floral biology of *Rubus macilentus* C., a wild Raspberry species of Garhwal Himalaya, an experiment was conducted at College of Horticulture, VCSG, Uttarakhand University of Horticulture and Forestry, Bharsar during the years 2016 – 2017. *Rubus macilentus* C. produced primocane and floricanes, the secondary shoots arrived in primocane during 1st year growth produces flowers in the next year and termed as floricanes. Floricanes produced secondary and tertiary shoots also flowered. Flower bearing shoots were observed during 2016, April to August and in 2017, January to April. Each inflorescence had strictly three buds with an average 166.6 number of flowers per bearing shoot. Eight stages of flower bud development were observed and took on an average 45.4 days for flower bud emergence to anthesis. The time of flowering and duration was recorded from March to June with the peak period of 45 to 49 days of first flower opening. The flowers were hermaphrodite with white corolla. The peak anthesis and dehiscence was found between 10am to 12 noon.

Keywords: Raspberry, bearing habit, flower bud development, anthesis, dehiscence

Introduction

The genus *Rubus* belongs to family Rosaceae, which contains a large number of highly variable and heterogeneous species, throughout the world except desert regions. The genus has been divided into 12 subgenera of which only a few species have been domesticated (Jennings *et al.*, 1990 and Romoleroux, 1992) [6, 10]. All raspberries are included in the subgenus *Idaeobatus*. *Rubus* is the Latin name for the bramble, which is derived from *Ruber* means red, referring to the colour of the fruit in some species of *Rubus*. It includes the brambles, blackberry and raspberry (Collett, 1921) [3]. Many raspberry species found growing as wild forms throughout the Garhwal Himalaya between altitudes of 600 to 2200 meters above mean sea level (msl). The wild fruit of raspberry species chosen for their high vitamin C content and mineral elements and therefore could be of interest for fruit processing industries. For their economic potential, wild, semi domesticated and less utilized fruits provide better economic return by making a variety of edible products such as jam, jelly, juice, squash and sauce (Maikhuri *et al.*, 1994) [9].

For most of the efficient breeding programmes, the knowledge of floral biology plays a key role. Hence, the present paper pertaining to floral biology is critically studied which comprise of bearing habit of raspberry canes, time and duration of flowering, phenology of floral buds, measurements of flowers and floral abnormalities, anthesis and dehiscence are presented.

Materials and Methods

A field experiment was conducted at College of Horticulture, VCSG, UHF, Bharsar, Pauri Garhwal, Uttarakhand, India during 2016-2017. The experimental site is located at an altitude of 1900 meters above mean sea level at a Longitude of 78.99° E and Latitude of 30.056° N. The climate of Bharsar is typically temperate type with mild summer, higher precipitation during rainy season and severe cold prolonged winter with occasional snowfall. The South-east monsoon commences at the end of June while the North-east monsoon sometimes causes winter showers during November-February. The 25 experimental plants were selected and tagged for the study of floral biology of Raspberry wild species *Rubus macilentus* C. Vernier Callipers were used for the measurement of bearing shoots, hand lens used for floral parts observation, For the study of different stages of anthesis, fifty buds were selected in all four

directions on each experimental plant and buds were closely watched for the entire duration (from petal opening up to the appearance of stamens and pistils) on each experimental plant. In order to study time of anthesis in raspberry, the flower buds (at balloon stage) likely to open on the next day were tagged which is present on all the four side of the plant. Next morning, the numbers of flowers opened were recorded at two hours interval starting from 6 am to 6 pm. The open flowers were removed after each observation to avoid recounting. Percentage of opened flowers was calculated. The mode of anther dehiscence in raspberry was recorded by picking the stamens from freshly opened flowers and examining them with hand lens and with light microscope under lab conditions. Ten flowers located on different parts of each experimental plant were tagged during peak flowering period to record time of anther dehiscence. After anthesis, each day the number of anthers dehiscence were recorded, at every two hours interval with the help of simple hand lens, between 6am to 6pm for a week. The dehiscence anthers were removed from the flowers to avoid recounting. Under laboratory condition Light microscope, glasswares and dissection box were used to study floral parts, anthesis and anther dehiscence. All the experimental plants were kept under similar set of cultural practices during the course of studies.

Table 1: Characteristics of bearing shoots of *Rubus macilentus* C.

Average number of shoots observed	Length of bearing shoot (cm)	Number of inflorescence per bearing shoots	Number of buds per inflorescence	Number of flowers per inflorescence	Number of flowers per bearing shoots
5	143.39±3.73	55.66±7.21	3±0.0	3±0.0	166.6±9.83



Fig 1: Bearing habit of *Rubus macilentus* C. The inflorescence appeared both on terminal and axillary position of shoots

Table 2: Time and duration of flowering in *Rubus macilentus* C.

Date of commencement of flowering	Full bloom (about 75% flowers opened)	Date required to attain full bloom	Date of end of flowering	Duration of flowering (average days)
18/3/2017	26/4/2017 (40 days)	30/4/2017 (44 days)	15/6/2017 (59 days)	90

Development of flower bud

Stages of flower bud development

When buds arose, they were sessile and bracteates. On the maturity, however, they developed pedicels which were 1 to 1.5cm long and hairy. Floral buds were arisen along with leaf-buds on lateral as well as on main shoots. The *Rubus macilentus* C. took nearly 45 days from bud initiation to anthesis.

The whole period of bud development, from its appearance upto the full bloom stage (Figure 2) is divided into 8 stages. The observations on the time required for development of

Result and Discussion

Bearing Habit

Initiation of floral buds of *Rubus macilentus* C. was noticed on 1 year old shoots of floricanes in the first week of February, the inflorescence was Corymbose raceme which appeared both on terminal and axillary position of shoots (Figure 1). Flowers were born in the axils of the leaves on the main as well as on lateral shoots. Terminal corymbose raceme arose from the axils of different numbers of leaflets with 1, 2 or 3 leaflets. The opening of flowers was observed from top to bottom or from top to middle. In inflorescence, the upper most bud of terminal cluster opened first, while the uppermost bud of lower clusters opened in the second place rather than other buds in the terminal cluster. On an average, 55.6 numbers of inflorescence per bearing shoot were recorded with an average of 166.6 flowers per bearing shoots with strictly 3 flowers per inflorescence. The average length of bearing shoot was 143.39 (Table 1). Sonstebly and Heide (2009) [12] also reported that cyme inflorescence of red raspberries in which the terminal flower developed first, followed by the sequential development of flowers further down the inflorescence axis. Free (1970) [5] reported that the American red raspberry, *Rubus idaeus* L. produced flowers in spring on the lateral shoots formed in the leaf axils of the previous year canes.

Time and Duration of Flowering

Flowering season of *Rubus macilentus* C. was quite longer (90 days). The flowering started from last week of March and continued upto second week of June. Peak flowering was recorded between 44-49 days from first flower appearance (Table 2). Daniels (1922) [4] reported that the raspberry cultivars grown in America had their fruit bud development between November and March although the date was not clear. Morphological development on the other hand took place at the end of September. The variation in date of start of flowering may be due to agroclimatic conditions. Carew *et al.* (2003) [2] found that both rate of vegetative growth and progress to flowering increased with temperature, with a relatively broad optimum in the low-to-mid 20 °C range.

each stage (Table 3) and the physical characteristics are briefly described below:

Stage I: In the beginning, a compact cluster of tiny buds appeared. Individual bud was rounded below and tapering upwards; light green in colour with pink tinge at the tips and covered with silky hair. Average length and diameter of the bud was 0.1cm. In this stage the pedicel was not prominent and bracts were not clear.

Stage II: The buds were loosened in the cluster and became oval in shape. Size increased and two to three sepal tips

appeared. Calyx lobes became distinct and calyx tips appeared to be compact with reddish brown colour. The final average length and diameter, of the bud was 0.35cm and 0.2cm, respectively. There was an appearance of pair of new lateral buds on pedicel of the old bud. Pedicel was prominent and distinguishable.

Stage III: Upper portion of buds became shorter and a protrusion from the base appeared. The buds were covered with white silky hair and calyx tips were pinkish purple in colour. Average length of the bud was 0.48cm with an average diameter of 0.35cm. In this stage pedicel is prominent, the flower bud is still oval in shape with the four prominent calyx tip.

Stage IV: Size of the bud further increased and pedicels became elongated. The corolla were visible, the calyx started splitting from top to base. At this stage, sepal lobes became dark green on lower and light green on upper portion and pinkish purple colour of calyx tip reduced and restricted to tips of calyx edges. Hair lost their silkiness. Average length of the bud was 0.54cm, diameter of the bud was 0.42cm. Pedicel was prominent attached to the base of the leaf. The lateral buds were half of the size of the axillary bud.

Stage V: This was the opening stage of bud, surrounded by 5 acute sepal tips, showing white petals inside but below the level of sepal-tips. Stigmas and stamens remained enclosed within the membranous petals. There was a slight appearance

of the petal at the top of flower bud. Average length of the bud was 0.66cm and diameter of the bud was 0.53cm.

Stage VI: In this stage pedicel is longer than fifth stage. The buds were completely green in colour. The sepals were started to open slightly at the top of the flower bud and still tightly attached to the petals. There was a prominent appearance of the petal at the top of flower bud. The flower buds were ready to enter the balloon stage. Average length of the bud was 0.71cm and diameter of the bud was 0.62cm.

Stage VII: The bud was partially opened at this stage. Stamens and stigmas came out of the petals and became clear. In this stage pedicel is longer than sixth stage. The buds were complete with greenish colour calyx and white petals, average length of the bud was 0.85cm and diameter of the bud was 0.72cm.

Stage VIII: Average length of the bud was 0.94cm and diameter of the bud was 0.80cm. The buds were completely green in colour. The sepals were open at the top of the flower bud and still tightly attached to the petals. There was an opening of petal at the top of flower bud have appeared. The flower buds were at the balloon stage.

The total time required for flower bud to reach the anthesis was 30 to 52 days in raspberry plants. Tamura *et al.* (1987)^[13] noticed that the flower bud development period was determined on length/diameter ratio basis found to be a good indicator for observing flower bud development and also reported 7 flower bud development stages in apple.

Table 3: Chronology of bud development stages from emergence to full bloom in *Rubus macilentus* C.

Number of buds observed	Days required for transition from one stage to next stage							Total days required
	I to II	II to III	III to IV	IV to V	V to VI	VI to VII	VII to VIII	
10	12	10	9	5	4	2	1	43
10	12	11	9	6	5	2	1	46
10	11	11	10	6	4	2	1	45
10	12	11	9	6	6	2	1	47
10	11	10	10	7	5	2	1	46
Average	11.6±0.24	10.6±0.24	9.4±0.24	6±0.31	4.8±0.37	2±0.00	1±0.00	45.4±0.67

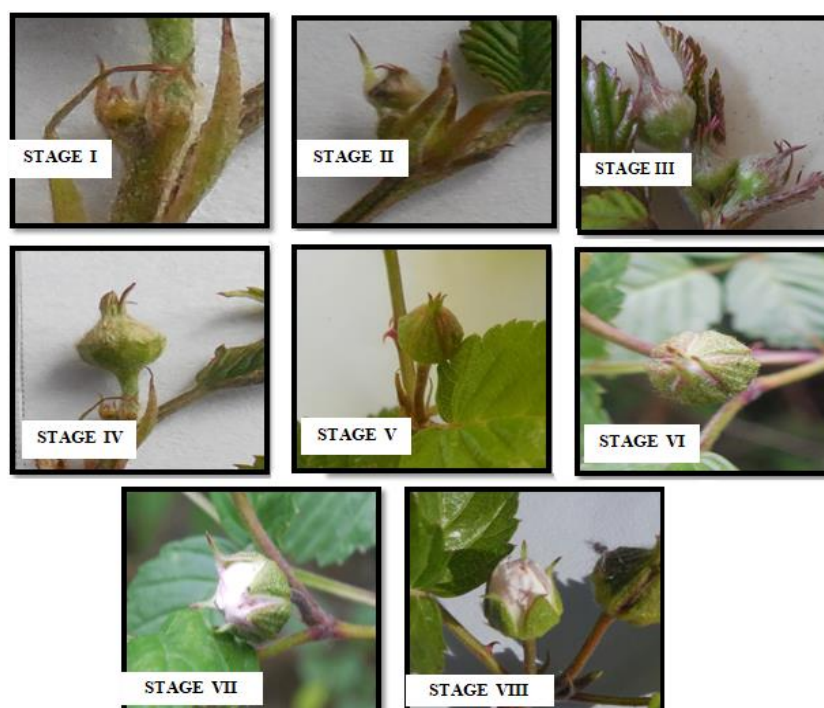


Fig 2: Developmental stages of flower buds in *Rubus macilentus* C.

Bud Growth Behaviour

The data on the bud growth behaviour (figure 3) was taken on alternative days from bud emergence to final size of buds just before opening, indicated the buds showed a slow growth rate

during initial stage. After 4th stage of development, the bud growth gradually increased and the maxing growth of buds was seen after 6th stage of bud development with maximum bud size of 0.96cm x 0.80cm (length X width).

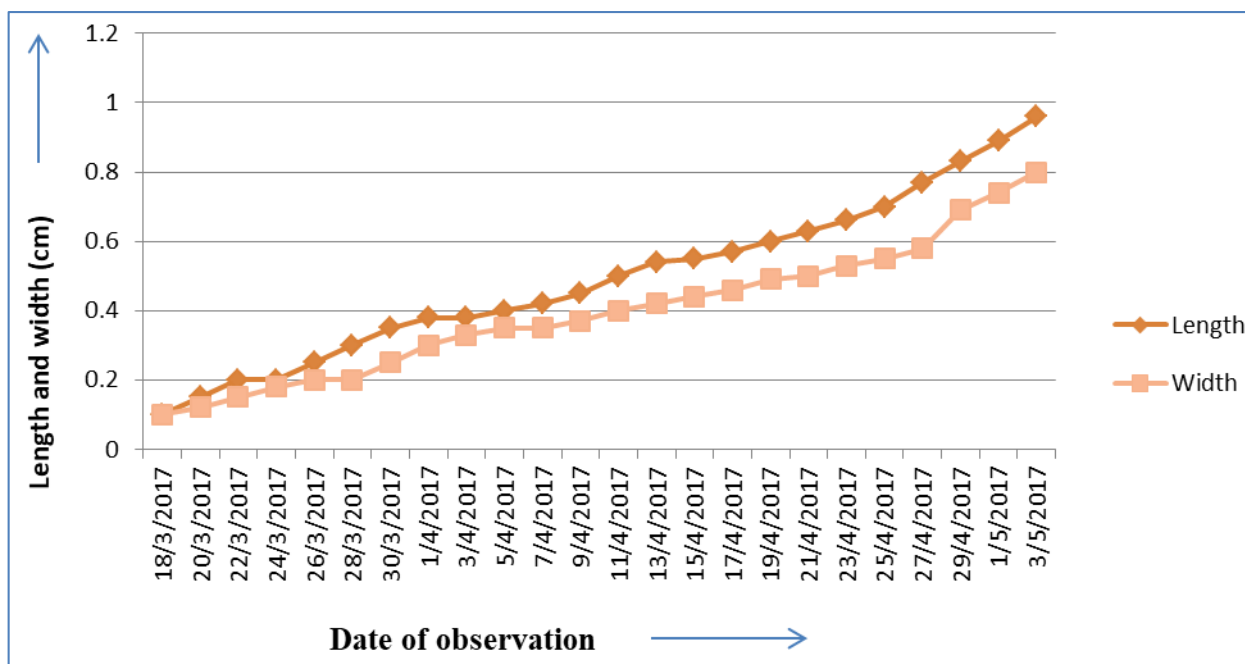


Fig 3: Bud growth behavior of *Rubus macilentus* C.

Floral Morphology

Flowers of *Rubus macilentus* C. were regular, complete, perfect, actinomorphic, hermaphrodite or bisexual, hypogynous, Petal white, bracteate and pedicellate; pedicel was hairy. The size of flower was 0.85cm (length) and 2.35cm (width) (Figure 4.a), In addition to normal flower, the abnormal flowers were (Figure 5.a.b.c) also found. Sepals were 5 with green colour, but they also developed light reddish-purple colour at early stages of bud development. Small white, silky hair had covered the calyx (Figure 4.b). Average sepal length was 1.05cm and width 0.30cm and longer than petals (Figure 4.a). Sepal abnormality was noticed in the *Rubus macilentus* C. flowers. The flowers with 4 sepals were considered as abnormal and smaller in size (Figure 5.a.). Abnormal sepal or sepals thus increased the distance between adjacent sepals. They were shorter and less broad in size than the normal ones. The abnormal sepals were unequal in size and also changed shapes. Corolla were five, white in colour sometimes pink tinge was also seen at the edges of the petals, they were broad on the upper portion and tapered downwardly. Average petal length was 1.02cm and width was 0.63cm (Figure 4.c). They were caducous and fell down 1-2 days after anthesis. The petals were alternatively arranged with the sepals and petals were smaller than the sepals (Figure 4.a). Abnormal petals were arisen from the same point from where normal one arises and were smaller than the normal ones or of the size and shape of the normal petal. Sometimes abnormality disturbed the arrangement of petals in a flower, the petals originated so near to each other that they were lying one above the other and also covering one or two sepals (Figure 5.b.c.). Abnormal petals also shortened the distance between adjacent petals and disturbed their alternate

arrangement with sepals. It was further observed that the flower with 4 petals were associated with 4 sepals (Figure 5.a.) having alternate arrangement, while 6 and 7 petals were associated with 5 sepals (Figure 5.b.c).

Stamens were numerous, free and arranged in 4 whorls i.e. one above the other. Inner most whorl with shortest stamens and outer most with longest stamens (Figure 4.d.). Anthers were creamish or light yellow in colour, bitheous, dorsifixed and average length of stamens was 0.32cm (Figure 4.d.e). Androecium surrounded the gynoecium in a circle (Figure 4.a.d). Stamens were observed to be in three different positions (Figure 4.d) with respect to stigmas: (i) lying above the stigmas (ii) lying below the stigmas and (iii) lying at the same level with stigmas. Ovary was polycarpellary, superior and crowded on a convex receptacle (Figure 4.d). Stigmas and styles were creamish white with green coloured ovary (Figure 4.f). Average length of pistil was 0.22cm. Ashman *et al.* (2012) [1] found that strawberry flowers were always actinomorphic, white, sometimes tinged with pink and usually 5-petalled. In some species, staminate and pistillate flowers were readily distinguished, but in others e.g. gynodioecious *Fragaria vesca* subsp. *bracteata* the pistillate flowers had anthers and were very similar to the bisexual ones. Jennings (1988) [7] observed that raspberry flowers were white to pink in color, small about 0.5 to 1.5cm and were initiated in the second year of planting. There were 60 to 90 stamens and 60 to 80 ovaries, each ovary develops into a drupelet. The flowers of *Rubus* were rather structurally similar to those of strawberries with five sepals, five petals, a very short hypanthium, many stamens and an apocarpous gynoecium of many carpels on a cone like receptacle.

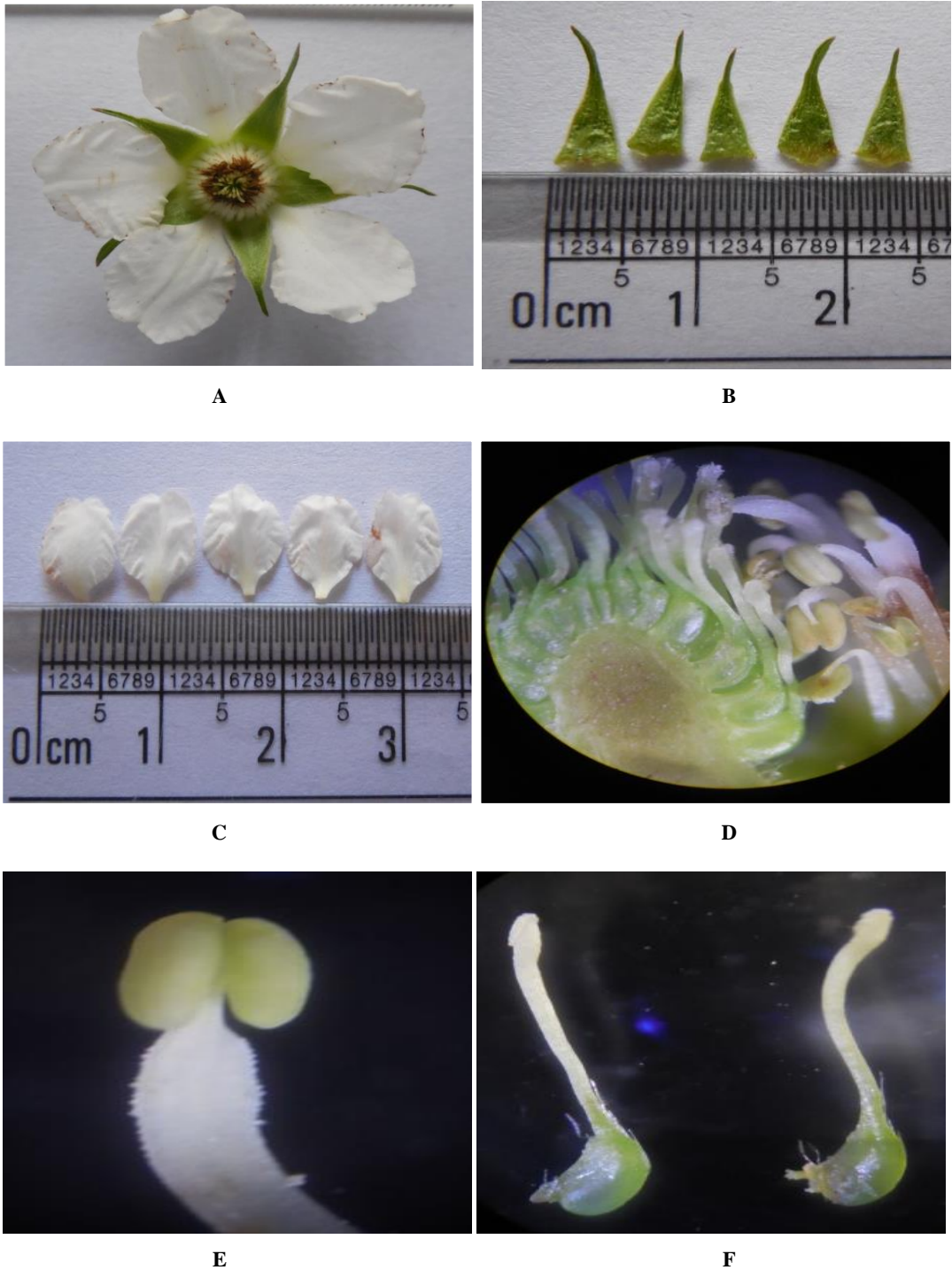


Fig 4: Flower organization of *Rubus macilentus*
 a. Flower b. Calyx c. Corolla d. Arrangement of stamens and pistils e. Stamen f. Pistils

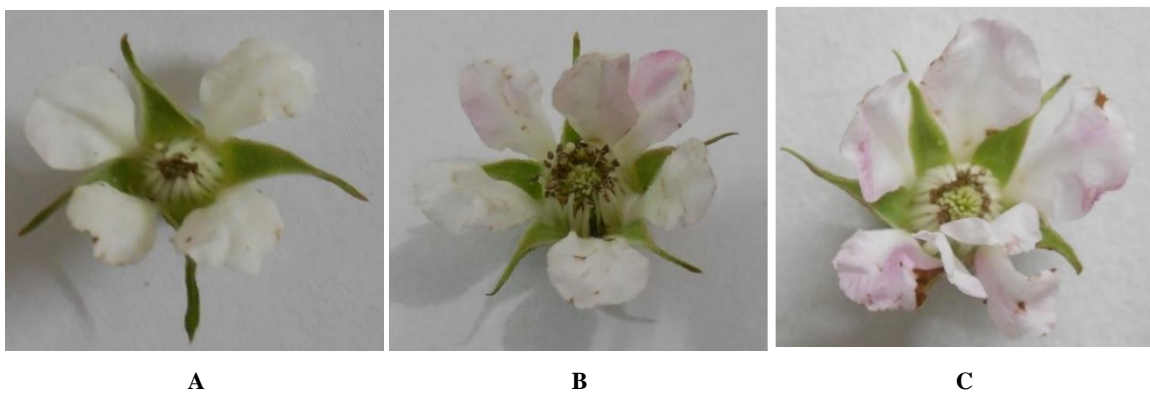


Fig 5: Floral abnormalities
Rubus macilentus C. a. Flower with 4 sepals and 4 petals b. Flower with 5 se pals and 6 petals c. Flower with 5 sepals and 7 petals

Anthesis

The period of anthesis for *Rubus macilentus* C. varied from 6.00 am to 4.00 pm. The maximum average anthesis (41.43%) was recorded between mornings 10 am to 12 noon, followed by 8 am. to 10 am. (23.71%) and the minimum anthesis (0.06%) was observed between 4.00 am to 6.00 pm. Both temperature and relative humidity influenced the time and rate of anthesis (Table 4). The mode of anthesis was studied in 5 stages (Figure 6) as follows:

Stage I: On the day of anthesis, the buds became balloon shaped and a small split in the center of the upper portion of corolla was noticed as the outermost petal slightly stretched out.

Stage II: In the second stage of anthesis sepals were free and upper most petal was slightly opened. The remaining petals were held inside the calyx cup.

Stage III: In the third stage of anthesis the sepals and petals became free. As the outermost petal stretched out wards the second and third petal became free and slightly opened. The

stamens over lapping the outer most pistils were clearly visible.

Stage IV: In this stage the sepals and petals became free, the outermost petal completely opened, three to four petals after the second petals were also open. Complete out ward stretching of sepals were noticed, stamens and pistils were clearly visible.

Stage V: In the fifth stage of anthesis sepals and petals were completely free. In this stage all the petals get open. There was a complete appearance of stamens and pistils in the central part of the flower.

Sharma (1970) [11] observed that the anthesis occurred between 6.00 am to 6.00 pm, which gradually increased up to 12.00 noon and then decreased slowly and ended by 6.00 pm. in apple cultivars. Kumar (1996) [8] also reported that the percentage anthesis increased gradually from 8:00 am to 2:00 pm., after which it decreased.

Table 4: Time of anthesis in *Rubus macilentus* C.

Date of observation	Total number of flowers	Percentage of flowers opened at different time interval						Humidity (%)	Temperature (°C)
		6 am - 8am	8 am - 10am	10 am - 12noon	12noon - 2pm	2 pm - 4pm	4 pm - 6pm		
12/4/2017	50	8.00	20.00	44.00	22.00	6.00	0.00	60	15
13/4/2017	50	6.00	24.00	44.00	20.00	6.00	0.40	60	15
14/4/2017	50	6.00	26.00	42.00	18.00	8.00	0.00	50	15
15/4/2017	50	4.00	22.00	44.00	20.00	8.00	0.00	60	14
16/4/2017	50	10.00	26.00	38.00	22.00	8.00	0.00	50	16
17/4/2017	50	4.00	24.00	38.00	24.00	10.00	0.00	60	15
18/4/2017	50	8.00	24.00	40.00	16.00	10.00	0.00	60	14
Average	50±0.00	6.57±0.84	23.71±0.80	41.43±1.04	20.29±1.01	8.00±0.61	0.05±0.05	57.14±1.84	14.85±0.26

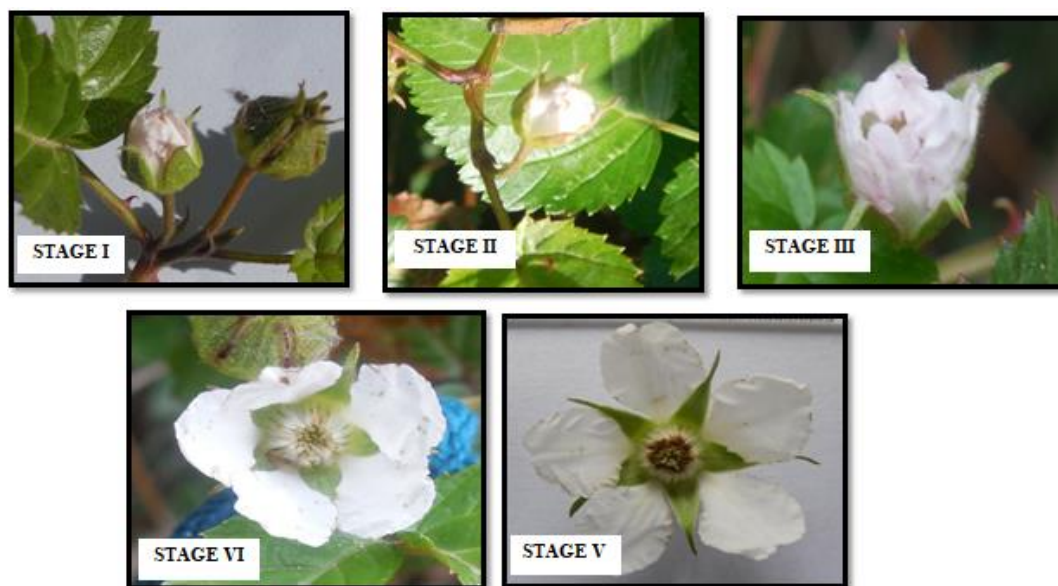


Fig 6: Stages of anthesis of *Rubus macilentus* C.

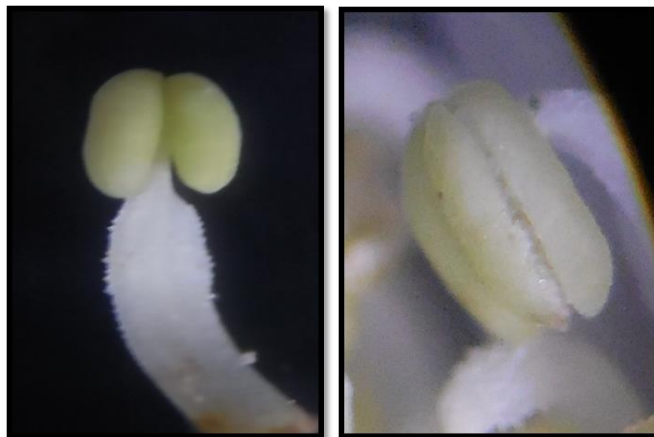
Dehiscence

The anther dehiscence in raspberry plants increased gradually from 10.00am to 12.00 noon, after which it gradually decreased. The peak period of dehiscence was recorded between 10.00 am to 12.00 noon (20.29%), followed by (17.11%) between 12.00 noon to 2.00 pm. The minimum (4.36%) anther dehiscence was observed from 4pm to 6 pm 70.64% on the day of anthesis. Remaining 29.36% was

occurred on next day of anthesis (Table 5). Few flowers also showed anther dehiscence before anthesis. The anthers showed longitudinal mode of dehiscence (Figure 7). Sharma (1970) [11] reported that the dehiscence of anthers begin at 6.00 am with a progressive increase till 12.00 noon, ending by 6.00 pm. In different apple varieties. The dehiscence in general started almost at 8.00 am and ended around 4.00 pm.

Table 5: Time of anther dehiscence

Date of observation	Total number of anthers observed	Percentage of anthers dehised at different time interval						Humidity (%)	Temperature (°C)
		6 am - 8am	8 am - 10am	10 am - 12noon	12noon- 2pm	2pm-4pm	4pm-6pm		
19/4/2017	128	6.25	14.06	21.87	18.75	11.71	4.68	50	15
20/4/2017	135	7.40	11.85	20.00	14.81	9.62	4.44	40	14
21/4/2017	122	5.73	11.47	19.67	20.49	9.83	3.27	50	15
22/4/2017	141	4.25	13.47	20.56	14.18	9.21	4.25	60	14
23/4/2017	152	6.57	11.84	20.39	18.42	11.84	5.26	40	15
24/4/2017	118	5.08	10.16	21.18	16.94	11.01	4.23	50	14
25/4/2017	136	6.61	12.50	18.38	16.17	11.76	4.41	50	15
Average	133.143±4.38	5.98±0.39	12.19±0.49	20.29±0.42	17.11±0.85	10.71±0.42	4.36±0.22	48.57±2.60	14.57±0.20

**Fig 7:** Mode of anther dehiscence in *Rubus macilentus C.*

Conclusion

Raspberry plants produce hermaphrodite or bisexual flowers during the period of November to March. Flowering continued for eighty to ninety days and floral buds takes 39 days for its development. Opening of the flowers starts 8 am in the morning and continue up to 4 pm with the peak period between 10 am to 12 noon. The anthers start dehiscence just after opening of the flowers and complete of a flower takes place within 8 to 9 hours. These findings clearly suggested that the breeding operation in raspberry under study should be done during spring season.

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