Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya

Thesis

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By

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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Place: Bharsar
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Hemavati Hiregoudar

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus species*) of Garhwal Himalaya"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Horticulture** with major in **Fruit Science,** of the College of Horticulture, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal (UK), is a record of ***bona fide*** research carried out by **Ms. Hemavati Hiregoudar, Id. No. 15209**, under my supervision and no part of the thesis has been submitted for other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Prof. B. P. Nautiyal
Chairman
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CERTIFICATE

We, the undersigned, members of the Advisory Committee, of **Ms. Hemavati Hiregoudar, Id. No. 15209**, a candidate for the degree of **Master of Science in Horticulture** with major in **Fruit Science,** agree that the thesis entitled "**Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya"** may be submitted in partial fulfillment of the requirements for the degree.

Prof. B. P. Nautiyal
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ABBREVIATIONS

am	:	Ante meridiem
cm	:	Centimeter
CRD	:	Completely randomized design
et al.	:	Co-workers
CD	:	Critical difference
^{o}C	:	Degree Celsius
etc.	:	Etcetera
g	:	Gram
l	:	Length
µ	:	Micron
m	:	Meter
mm	:	Millimeter
MT	:	Metric tones
no**.**	:	Number
/	:	Per
%	:	Per cent
pm	:	Post meridiem
±	:	Positive or negative
i.e.	:	That is
viz.	:	Videlicet (namely)
W	:	Width

Chapter-1

INTRODUCTION

CHAPTER 1

INTRODUCTION

The genus *Rubus* belongs to family Rosaceae, which contains a large number of highly variable and heterogeneous species, which occur in all parts of the world except dessert 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). All raspberries are included in the subgenus *Idaeobatus* whereas blackberries are placed in the sub-genus *Eubatus* (Bailey, 1947; Zielinski, 1955). *Rubus* is the Latin name for the bramble, derived from Ruber, red, referring to the colour of the fruit in some species. It includes the brambles, blackberry and raspberry (Collett, 1921). The taxonomy of the genus is confused because of insufficient and inadequate herbarium specimens, as a result, the taxonomic custom has developed of calling the strange forms as hybrids until the species of the parents are known (Bailey 1958). Different species of the *Rubus* genus are native to six continents and have been found from the tops of mountains to coastal locations at sea level (Daubeny, 1996; Thompson, 1995). The management and utilization of genetic resources of wild raspberry and that represents high level of genetic variation among accessions for morphological traits (Patamsyte *et al.,* 2008). They grow especially well as cool climate plants, but will also produce worthwhile crops in the subtropics. They are chiefly natives of cold and temperate regions of the Northern Hemisphere.

Raspberries are produced in 37 countries worldwide on approximately 184,000 acres. The top ten countries in decreasing order of raspberry production are Russia (24%), Siberia and Montenegro (23%), United States (13%), Poland (11%), Germany (7%), Ukraine (5%), Hungry (4%), Canada (3%), UK (2%), and France (2%) (Trivedi *et al.*, 2014).

In India, small quantities of raspberries are grown in Mahabaleshwar (Maharashtra) as well as Bengaluru and Mysuru (Karnataka) mainly for utilization in the fruit processing units located in Bombay and Bengaluru. The species grown in Mahabaleshwar is presumably *R. niveus* while the specific identity of the one grown in Bengaluru is not known, though it might also probably be a form of *R. niveus*. An area of about 8 ha is reported to be used for this fruit in Bengaluru, producing annually 75,000 Kg of fruit which is consumed by the Kissan Products Ltd. for manufacture of jam. The cultivation of Bengaluru raspberry was found to be a good business proposition in the Nilgiris. Some

types of raspberries imported from Australia were also tried, but proved to be unsuitable for this tract (cf. Wealth of India 1972). A good number of exotic species of *Rubus* are planted for the showy flowers and ornamental foliage. A little attempt has been made to study the Indian species of *Rubus* in their natural habitats. In India, there are about 57 species of *Rubus*, out of which 6 are found in Himachal Pradesh (Collett 1921). In Shimla and its surrounding areas, it is commonly eaten raw or dried and preserved. It is certainly one of the best wild fruits of Himachal Pradesh, Uttarakhand and other parts of the Himalaya. It is offered for sale at most hill stations and is regularly exhibited at the horticultural shows. In Kashmir, *Rubus* is represented by 14 species and in view of the great diversity presented by the genus in the valley.

Some of the *Rubus* species are found in all types of forests, but is typical of open Chir (*Pinus* Spp.) and Ban (*Quercus* Spp.) forests as undergrowth. It is a tall, sub erect bush met within the temperate and subtropical Himalaya, from the Indus to Sikkim and Bhutan, at altitude between 600 to 2200 m above mean sea level. It is also found on Khasia hills, Manipur, Burma, in the western Peninsula of India, from Kanara southwards and in the central parts of Sri Lanka (Duthie 1906). Raspberry grows in abundance throughout the mid-hill region of Himachal Pradesh.

Rubus is the most variable genus, the lines of demarcation being often uncertain and indefinite. The botanical characters may differ widely on old and young canes and even on spring and autumn foliage of the same cane. The plants may respond readily to environmental conditions so that sometimes there are marked shade, sun, moist and dryland forms of the same prevailing types. *Rubus* species are annual, biennial or perennial shrubs and vary in size from small prostrate plants to large shrubs of 5m or more in height. Most species are biennial, usually short-lived stems, growing new canes each year which bear fruit in the following year (floricane fruiting). Raspberries have also been selected which bear flowers and fruit on the current years canes without any chilling requirement or dormancy period (primocane fruiting) (Jennings, 1988; Clark *et al*., 2007). Some raspberry varieties also produce fruit on the growing tips of the primocanes. These types of raspberries are called fall fruiting, primocane fruiting or ever bearing raspberries. When the primocanes of these varieties attain a certain number of nodes, the growing tip of the cane switches to a reproductive mode and floral buds are initiated, followed by flowering and fruiting in summer and fall. If not removed during the winter, these canes will also become

floricanes in the following spring and will produce fruit on shoots that develop from lateral buds that did not grow and fruit in the previous year.

During the first spring, the plants develop new shoots from basal buds of a previous year canes or from buds on the rhizomes. In autumn, flower buds develop on the shoots and thereafter produce flower or fruit the following summer. Thus floricane fruiting raspberry produce flowers or fruits only on 2-year-old canes. After the fruiting period, the floricanes are cut down to the ground, removed and discarded. During growing season, the new emerging primocanes compete for nutrition and light with floricanes. The primocanes must always be kept intact and are overwintered without any pruning to produce fruit the following growing season. Progress through these phases depends partially on internal factors and partially on the effects of the environment. For instance, plant in juvenile stage cannot induce flowering until they reach a certain amount of vegetative growth or a certain number of nodes (Williams, 1960).

In autumn, leaves turn yellow or yellowish-red, dry up and fall from the primocanes. Before the falling of the leaves, some nutrients and biochemicals move from the leaves to the canes and roots, where they are stored for next year's growth. The primocane stems and buds remain alive and enter a condition called dormancy or rest. Once plants enter dormancy, a certain number of chilling hours are required before the plant can resume normal growth and development (Jennings *et al.,* 1972). *Rubus* flowers vary from small and inconspicuous with white petals to large and bright pink or reddish in colour. Most species have spines which can be small and hair-like or even large and ferocious, similar to thorns in woody plant and they may be curved or hooked. Some species are smooth-stemmed and there are a few selections of raspberry which are spiny only at the base or spines are completely absent. These have been used extensively in breeding and many new cultivars are genetically spineless (Jennings, 1988).

Wild plants yielding edible fruits are important in the three-dimensional forest farming producing food, fodder and fuel, besides providing environmental conservation. Besides, valuable traits of the wild fruits such as winter hardiness, drought tolerance and superior vigour can be incorporated into their cultivated relatives with a view to improve them.

Raspberry is a rich source of food components vitamins, minerals and bioactive compounds like phenolic flavonoid phytochemicals such as anthocyanins, ellagic acid (tannin), quercetin, gallic acid, cyanidins, pelargonidins, catechins, kaempferol and salicylic

acid (Shahidi and Naczk, 2004). The wild fruit species chosen for their high vitamin C content and mineral elements could be of interest for fruit processing industries. For their economic potential, wild fruits, 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).

Although the fruits were undoubtedly popular for food, medicinal uses remained important. Raspberry leaf tea is an ancient, but still popular, herbal infusion. Modern research has shown that a water-soluble extract from raspberry leaves relaxes the uterine muscles. Raspberries have long been valued for their medicinal and nutritional benefits. It is a rich source of antioxidants with potential health benefits (Rao and Snyder, 2010). With today's interest in natural foods and healthy diets, raspberries popularity remains strong. Recent research supports long-held beliefs that raspberries are a particularly healthy part of the human diet. This fruit has been successfully introduced into Florida (United States) for edible as well as ornamental purposes and in Australia for breeding (Jennings, 1988). Although the raspberry has captured the interest of botanists and herbalists, it remains a neglected and unexploited fruit crop in India (Singh and Kumar, 2001). Only few studies concerning morphological, chemical and nutritional properties of wild raspberry has been performed yet (Han *et al.* 2008; Celik and Ercisli, 2009; HanPing *et al.*, 2009).

The Garhwal Himalaya of Uttarakhand region lies in the central part of the western Himalaya that represents a great variation in topography and slope aspect and also well known for its wide diversity of wild raspberry species such as *Rubus foliolosis* D. *Rubus niveus* T. *Rubus paniculatus* S. and other species of *Rubus*. These raspberry species are individually precious and their genetic diversity should be conserved and enhanced. The raspberry plant species, especially in the Himalaya, that have edible parts and might be used more extensively for food and perhaps could be cultivated if they are better known. Many of these are used locally, but are not yet popular and not commercially grown by fruit growers in India. This is partly because of socioeconomic constrains and no attention has been paid to their improvement either in terms of their improved cultivation practices and quality or to make them more attractive for human consumption.

Available variability of raspberry in Uttarakhand might be utilized for selection, commercialization and popularization of raspberries in the Himalayan states of India. The existing wild populations of raspberries are comprised of shrubs of unknown origin that exhibit tremendous variability in growth, flowering, yield and fruit quality attributes,

thereby providing a platform for exploitation. No systematic research work has been conducted from the standpoint of developing this as a new crop, in particular, through the collection and selection of superior clones. The proper knowledge about habitat and habit for proper plant identification, bearing habit, flowering and pollination behaviour of raspberry species will contribute in its crop improvement programmes through the identification and selection of superior potential wild species for domestication or as suitable parents in future hybridization programs.

The aim of present study is to study morphological characters, flowering and pollination behavior of selected wild species of raspberry. Also having an intent to protect the species from endangered population.

OBJECTIVE OF RESEARCH

1. To study morphological characters of different wild species of Raspberry.
2. To study the various components of floral biology for efficient breeding programs by breeders in the future under Garhwal Himalaya.
3. To study the pollination behaviour of selected species.

Chapter-2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

The morphology, floral biology, pollen and pollination studies in raspberry have been conducted by various workers. The available relevant literature on these aspects in raspberry along with some other fruits is reviewed under the following headings.

2.1 CANE MORPHOLOGY

Brainerd and Peitersen (1920) working with *Rubus* species showed that the vegetative parts of the plant can be modified much by the environment than the reproductive parts, thus confirming experimentally the common belief of taxonomists that the latter organs are generally more reliable as diagnostic criteria to separate different species within the genus.

Bailey (1932) reported that flower primordia are initiated in the axillary buds usually in the autumn, develop in the spring and finally primary laterals grow out, bear their crop after which the second year branch or floricane dies down to ground level.

Wright (1962) proposed a hypothesis that the magnitude of morphological variations amongst the individuals of different populations is greater than that of the same populations.

Becking (1979) observed that raspberry plants were generally 1-3 m high and though it may exceed 4 m. Stem were stout purplish brown, young canes ascending, covered with long stiff purplish brown hairs and a few elongate prickles.

Gaur (1999) reported that *Rubus macilentus* C. is an evergreen suberect or rambling shrub, grows to a height of 2.5m. Stem of the plant was glabrous, shinning with red-brown colour. Prickles were curved or straight with 0.5-1cm long and young twigs were pubescent. Whereas *Rubus niveus* T. is a rambling prickly shrub grows to a height of 6m. Stem is glabrous with purple-brown colour and pendulous in nature. The stem is covered with white transparent bloom and often rooting at the tips, prickles on the stem were small and curved. He also reported that the *Rubus paniculatus* S. is an evergreen rambling climber with 20m height. Prickles on the stem were small and curved, branchlets were covered with white tomentum.

Matzke and Weber (1999) reported that stem of *Rubus bollei* F. were high-arching up to 3 m or climbing up to 8 m with 10-25 mm diameter which were strongly branched, angled with flat or slightly furrowed sides and having dark purple, glabrous or glabrescent with sparse simple hairs. Prickles were 6-15 per 5 cm on the angles and subequal, with very broad triangular red base, declining (straight or) slightly curved, 4-8 mm in measurement they also observed that the stem of *Rubus palmensis* A. were high-arching and angled with 8- 20 mm diameter. There were long simple hairs which were sparse to numerous with sessile glands (glabrous or) especially on the angles and few to many yellowish-green to red brown gland-tipped bristles. Declining and curved, yellow or light brown colored slender or broad based pricklets of 2.5-4(6) mm were noticed on stem.

Thompson *et al.* (2008) observed that Prime-Jan and Prime-Jim cultivars of raspberry had on an average floricane branch number four and two and branch length of 43 and 35 cm, respectively.

2.2 LEAF MORPHOLOGY

Bailey (1959) recorded that the floricanes which bear flowers and fruits in the second year may have different foliage than the primocane foliage.

Becking (1979) observed three leaflets in raspberry plants which were elliptic with 4-8 cm long and 3-6 cm wide. Leaves were thick, more or less persistent with terminal or central one was the largest with pointed leaf apex and obtuse base had hollow veins on the upper sides and prominent veins on the back with reddish thorny hairs. Petioles were 2 to 6 cm, where the terminal one were 2 to 3 cm and almost no petioles on the lateral leaflets covered with reddish thorny hairs.

Gaur (1999) reported that leaves of *Rubus macilentus* C. were trifoliate measuring about 1.5 to 4.2cm long, rachis were pubescent, prickles present on the lower side, terminal leaflets usually ovate-lanceolate, acuminate, petioles were 2-2.5cm long, lateral leaves were smaller and sessile, margins of leaf is serrated, glabrous on both surfaces. Whereas the leaves of *Rubus niveus* T. were pinnate with 4-10cm length, leaflets are 5-7 rarely upto11, leaves were ovate-lanceolate in shape. The upper surface is green and glabrous but lower surface is white and tomentose. The terminal leaflets were often large and lobed, lateral ones smalles and sessile. Petioles and midribs were prickly beneath. He also reported that the *Rubus paniculatus* S. had simple leaves with 7.5-15X6-11.5cm in length

and width and petiole with 2-5cm long, hairy leaves. Lamina of leaf is broadly ovate, cordate and acuminate in shape, more or less serrated. Upper surface of leaf is green with coriaceous and lower surface densely white, tomentose and nerves were prominent beneath.

Matzke and Weber (1999) reported that in *Rubus bollei* F. leaves had five leaflets which becomes leathery and dark green, glabrous and shining above, grey or greyish-white-feltet beneath with contrasting light-brown nerves. Terminal leaflet 8-12 x 5-9 cm (in moist, moderately shady sites rich in nutrient even up to 15 x 12 cm), broadly ovate or obovate to nearly round with an acuminate apex measured up to 8-22 mm. Petiolules of basal leaflets 4-18 mm, petioles usually as long as or longer than basal leaflets, coloured like the stem with scattered simple and stellate hairs and 8-25 broad-based, often strongly curved prickles 3-5 mm. whereas, in *Rubus palmensis* A. had five leaflets. Terminal leaflet of 14-19 x 10-15 cm, either ovate with an acuminate apex. The roundish leaflets more or less evenly serrate, the ovate ones more shallowly serrate-dentate with the principal teeth slightly prominent. Leaflet flat or more often convex. Petiolules of basal leaflets was 8-30 mm, petiole longer than the basal leaflets, yellow-green and 25-40 curved prickles 2-3.5 mm.

Hummer (2012) reported that the floral and vegetative morphology of *Fragaria* was also relatively uniform. The leaves were usually evergreen in *Fragaria iinumae* and generally trifolioliate. Five leaflets were typical of some Chinese species, while 4 to 5 leaflets were rarely observed in *Fragaria cascadensis*.

Trivedi *et al.* (2014) studied vegetative characters of raspberry (*Rubus ellipticus* S.) for three consecutive years in Uttarakhand and found that the variability was found in terminal leaf length and width which ranged from 3.32 cm to10.58 cm and 2.17cm to 9.80 cm respectively.

2.3 FLORAL BIOLOGY

Flowering, the first step of sexual reproduction of plants, it is of paramount importance to agriculture, horticulture and plant breeding as it is a major factor determining yield.

2.3.1 Bearing habit

Keep (1969) reported that there may be secondary and tertiary buds present at a node, depending on genotype. Some raspberry genotypes do not have any secondary buds, while others have secondary buds at almost every node. According to Free (1970) reported that the American red raspberry, *Rubus idaeus* L. produced flowers in spring on the laterals formed in the leaf axils of the previous years canes. Jennings (1988) find out that most raspberry *(Rubus idaeus* L.) cultivars produce a single fruiting lateral at each node. The floricane bearing red raspberries initiate flowering in response to short days at low temperature Wolukau (1992). Moore (1994) reported that development of secondary and tertiary buds is usually suppressed by the primary bud. When the primary bud is damaged, other buds may grow and produce a crop.

Sonsteby and Heide (2008) reported that the number of flowers in each inflorescence varies widely and the number of inflorescences on each fruiting lateral increases steadily from the top to the base of the main shoot of raspberry plant.

Thompson *et al.* (2008) observed that on an average, un-tipped primocanes of Prime-Jan and Prime-Jim cultivars of raspberry developed two lateral branches when these branches were always located on nodes near the base of the cane and these canes maintained apical dominance and were determinate in growth, thus fruiting sites were always found at the tips of the main cane and branches.

Heide and sonsteby (2011) inferred that the biennial-fruiting cultivars of raspberry have an absolute low temperature (≤ approx. 15°C) requirement for floral initiation and were facultative short-day plants with a critical photoperiod of 15 h at intermediate temperatures; flowering is promoted by long photoperiods in at least some annual-fruiting cultivars. However, the essential difference that determines whether the shoot life-cycle becomes annual or biennial is that, in biennial-fruiting genotypes, floral initiation is linked to the induction of bud dormancy. Whereas, in annual-fruiting cultivars, floral initiation is followed by direct flower development.

2.3.2 Flower bud development

Several authors have reported flower bud growth and different stages of flower bud development. Fleckinger (1946) in pear recorded nine stages of flower bud development by

recording phenological notation and graphs of the floral development as a general method for studying biology and ecology. While Soensen (1948) observed ten prebloom stages of bud development in apple and pear, while studying the stages of flower bud development in Jonathan apple.

Elek (1966) recorded eight stages of flower bud development. Whereas, studies on floral biology by Kolesnikov (1966) indicated fourteen stages of bud development in Russian apples. Whereas Bhartiya (1980) undertaken studies on floral biology, pollination, incompatibility and fruit set in various cultivars of apple and reported twelve flower bud development stages.

Asada (1987) studied morphological development of flower bud in early spring with various apple cultivars and observed that L/D ratio (length/diameter) was similar for all the cultivars rising from 0.25 at dormancy to 0.65 at flowering.

Kapil (1988) studied eight stages of bud development and inferred that growth of buds was very slow during early stages but it was accelerated after 6th stage in winter and 4th or 5th stage in spring flowering, however, bud took more time to develop in winter. The speed of flowering (interval between induction and the flower appearance) and the number of flowers formed increase as the number of consecutive inductive photoperiods increases.

Takeda *et al.* (2002) reported that the phenology of flower bud differentiation varied among the cultivars of blackberry and was strongly influenced by prevailing winter temperatures. The results suggested that the shortening day lengths of late summer trigger flower bud development and once initiated, was continuous but the periods of low temperature (<2°C) can arrest development.

Sonsteby and Heide (2008; 2009) reported that **t**he inflorescence of red raspberries is a cyme, in which the terminal flower develops first, followed by the sequential development of flowers further down the inflorescence axis. While the uppermost lateral buds produce only one-to-three inflorescences, the complexity of the flowering laterals increases gradually in buds at lower positions. The number of flowers in each inflorescence varied widely and the number of inflorescences on each fruiting lateral increases steadily from the top to the base of the main shoot.

Selamovska (2013) reported that differentiation of flower buds passed through 4 phases viz., induction, initiation, differentiation and organ development of flower buds. The inner hidden changes which take place inside the bud i.e. III to VII stage were the micro-phenophase of differentiation and development of flower organs outside the bud i.e. VIII to XII stage were phenophases of differentiation. The order of rising and dynamic of growth and development of mixed buds was in correlation with their differentiation. The apical mixed bud was developed first one, after which the side mixed buds were developed. Dynamic and time of the flower bud formation was specific for each plant species.

2.3.3 Time and duration of flowering

Semwal *et al.* (1981) inferred that the time of flowering of *Rubus ellipticus* S. was also related with altitude and occurred earlier in lower altitude than in higher altitude also reported that flowering is earlier in Alpine zone in Garhwal Himalayas (India) and it is gradually delayed with increasing altitude.

Kapil (1988) repored that flowering in *Rubus ellipticus* proceeded from top to lower portions, thereby lengthening the duration of flowering period as well as making best use of the pollinators. Production of large number of inflorescences and flowers at the ends of branches provided greater attraction for insects, the majority of which were flying insects.

Daniel (1922) studied flower evocation is the inductive process whereby a meristem becomes committed to reproductive development through a change in developmental state and the expression of that state leads to the initiation of the floral apex and ultimately the formation of flower.

Thompson *et al.* (2008) inferred that the time required for first to last flower opening within an inflorescence of primocane took on an average 17 days for both Prime-Jim and Prime-Jan cultivars of raspberry.

2.3.4 Flower morphology

Nybom (1986) reported two types of stamen arrangement in senescing flowers of *Rubus* cultivars. He suggests that in the self-pollinating species, stamens curve over the pistils so that the anthers are in contact with the stigmas but in cross-pollinating species, the stamens are at the same level with stigmas and curve outwards away from them.

Kapil (1988) reported that the flower size and shape showed variability which appeared within a population, within the same plant and even within the same branch. This is suggestive of intramorphotypic morphological variation, also found that total abnormality percentage in the sepals in December (winter) flowering was 25.52% and in petals 31.83%. Thus, it is clear that abnormality in petals was more frequent in comparison to sepals. Apomictic flowers looked completely normal but the androecium became functionless while gynoecium remained functional. It increased in size and developed into fruit

Gaur (1999) reported that flowers of *Rubus macilentus* C. were white with 1-1.5cm diameter. Flowers were solitary axillary or found in clusters of 2 to 3. Peduncles were 2-3cm long. Calyxes were 5-10 lobed and pubescent on both sides with ovate shape. Petals were 5 oblong to ovate in shape and slightly larger than sepals. *Rubus niveus* T. were pink or purplish white 0.5 to 1.5cm across, in axillary or terminal panicled corymbos, pedicels tomentose. Calyx 5 –lobed, lobes lanceolate, acute, tomentose. Petels 5, broadly ovate, smaller than sepals *Rubus paniculatus* S. had Calyx 5-lobed, lobes were tomentose, pointed. Petals 5, extremely short, 4-5mm long. Flowers from march to june.

Matzke and Weber (1999) reported that *Rubus bollei* F. had sepals of greyish white-flat, short-pointed and reflexed. Petals were white or rarely pale pink and measured up to 16 x 10 mm which were broad-ovate. Filament was white and exceeding the green styles. Anthers and carpels were glabrous and receptacle was hairy. They also noted that *Rubus palmensis* A. had sepals with green or greyish-green color, narrow with parallel margins and were short-pointed with reflexed. Petals were white measuring up to 12 x 6 mm or even longer. Filaments were white, exeeding (greenish) styles. Anthers and carpels were glabrous, receptacle hairy.

Tianming *et al.* (2000) reported that the main floral characteristics of pear Xiangli (*Pyrus bretschneideri* R.) were complete flower, corymb inflorescence, inferior ovary, rotate corolla, androecium with 16 to 30 stamens, compound pistil with five to six stigmas.

Trivedi *et al.* (2014) studied vegetative characters of raspberry (*Rubus ellipticus Smith*) for three consecutive years in Uttarakhand, India and found that the variability was found in petal length, petal width and flower diameter which ranged from 0.3 cm to 1.36 cm, 0.15 cm –1.00 cm and 0.56 cm –3.26 cm ranged from to cm respectively.

2.3.5 Anthesis

Sharma (1970) studied the floral biology and fruit set in apple and reported that anthesis occurred between 6.00 am to 6.00pm and reaches peak at 12.00 noon and then declines slowly up to 6.00pm.

Bhartiya (1980) described that in apple maximum (27.04%) flower opening in some low chilling cultivars of apple between 12:00 noon to 2:00 p.m.

2.3.6 Dehiscence

Sharma (1970) reported that the dehiscence in apple varieties begin at 6:00am with a progressive increase till 12:00 noon, ending by 6:00pm. The dehiscence in general started almost at 8:00am to12:00 noon.

Bhartiya (1980) found that there was gradual upward trend in dehiscence in apple upto1:00pm and thereafter a gradual fall in dehiscence rate.

Bekey and Lawrence (1985) reported that anther dehiscence began in a few of the anthers of a flower almost immediately after the split of the calyx. At field temperatures dehiscence was heaviest on the first three days and nearly ceased by the fourth day of flower opening. Thus pollen is available during the first two days, the time of maximum bee visitation of the flowers. The peak period of receptivity of raspberry flowers lasts from 2-3 days after anthesis.

Kapil (1988) observed that some flowers showed dehiscence before anthesis and position of stamens above the stigmas which might lead to self-pollination and these flowers showed reduced fruit size.

2.3.7 POLLEN STUDY

2.3.7.1 Number of pollens per anther

Tianming *et al.* (2000) reported that the pollen grain mass of pear Xiangli (*Pyrus bretschneideri* R.) was about 4000 in each anther.

2.3.7.2 Pollen morphology

Brewbaker (1957) noted that pollen grains of the Rosaceae are binucleate with gametophytic incompatibility and Reitsma (1966) reported that the pollen grains of Rosaceae are tricolpate and striate. Tricolpate and stephanocolporate grains occur as normal variation. He has organized 13 types of pollen grains within this family.

Sharma (1970) observed that the pollen grain in apple had triangular shape, in a study on pollen germination and pollen tube characteristics in a range of apple cultivars. Whereas Stott (1972) reported that pollen size in apple cultivars varied from 25 to 50 µ (microns). The largest pollen grains up to 55 µ were observed in tetraploid cultivar Giant Wealthy.

Nair (1974) reported that pollen organization is more or less stable within the same species and when there is a deviation from the general type, it brings about the biological change in the species. It is imperative that the pollen also undergoes change in its morphological characters and that change provide an important tool to analyse and interpret the taxonomic relationship and evolutionary trends of the plants concerned.

Bist and Sharma (1986) reported that pollen grains of apple were elliptic and tricolpate in dry condition whereas in acetocarmine solution the pollen grains assumed the triangular shape. The average length and breadth of fresh pollen grain in dry condition ranged from 33.96 to 47.95 microns and 23.97 to 29.97 microns respectively.

Kapil (1988) found a wide size variation in different populations of *Rubus ellipticus* S. which falls between 12-26 µ.

Fang (1990) reported that 13 species and 16 varieties of *Pyrus* L. from China had the common features of morphology such as oblong or elliptic in side view and was obtuse triangle or trilobate circular in polar view. There were 3-colporate, stripe ornamentation and perforation on the surface of pollen grains. The shape, size and ornamentation of pollen vary with species, variety and type.

Gilani *et al*. (2010) studied morphological characters of the pollens of six species of *Prunus* and found that the pollen shape varied from triangular-acute-convex to triangular-obtuse-convex to circular in polar view and elliptic-acuminate-acute to elliptic-acuminate-

obtuse in the equatorial view. All these variations were very useful in the identification of the species with respect to their pollen shape and class.

2.3.7.3 Pollen viability

Ourecky (1975) reported that the blackberry pollen can be stored for several years at -5 °C and 10-20% relative humidity and storage at 5°C prolongs raspberry pollen viability upto 4 weeks.

Seilheimer and Stosser (1982) studied the viability of pollen of 14 diploid and nine triploid apple cultivars *in vitro* and *in vivo.* The findings revealed that except McIntosh, Jamba and Mantet produced a high percentage of viable pollens and triploid cultivars except Mutsu and Junagold had a low percentage of pollen viability.

Otterbacher *et al.* (1983) found that red raspberry pollens stored at room temperature lost their viability in less than a week, whereas pollen stored at 3°C retained some viability for 6 weeks and storage at -4°C extended pollen viability as long as for 4 years.

Chapliev (1985) found that retention of pollen viability was affected not only by the storage method but also by the physiological condition of the pollen grains particularly in their sucrose accumulation which is dependent upon temperature during pollen grain formation.

Perry and Moore (1985) studied longevity of pollen under various storage conditions and conducted in vitro pollen germination studies on each of 14 blackberry and reported that *in vitro* germination decreased linearly with increased duration of storage at 22°C. Time required to reach 50% non-viability varied among cultivars from 2.5 to 10 days and storage at 6°C more than doubled the time required to reach 50% non-viability. Which means blackberry pollen loses viability quickly and is short-lived when stored at room temperature.

Bajwa *et al.* (1991) observed that plum pollen had viability as high as 90 per cent. Lal (1993) reported the pollen fertility of eight plum cultivars ranging between 74.32 to 92.51 per cent and the pollen fertility was worked out in 1.0 per cent acetocarmine and deeply stained normal looking pollen grains were counted as viable.

Gercekcioglu *et al.* (2000) while studying the pollen grains of eight different pome and stone fruit cultivars (plum, peach, sweet cherry and apple cultivars) observed pollen viability in the range of 71.53 to 81.78 per cent.

Sharma (2001) observed that the pollen viability was highest with acetocarmine followed by tetrazolium chloride and lowest with Erythrosine B. This is because in acetocarmine solution even the dead pollen grains were also counted as viable as they also get stained because of the presence of protein.

Petrisor *et al.* (2008) reported that the pollen viability of ten apple cultivars varied from 89.92 per cent to 74.64 per cent, which differed statistically from control.

2.3.7.4 Pollen germination

Kobel (1926) reported that sucrose solution of 5 per cent for quince, 10-15 per cent for pears, 5-15 per cent for apple, 10 per cent for peach and 10-15 per cent for plum were the optimum concentrations for pollen germination tests.

Visser (1955) compared pollen germination to fruit-set in apple, pear and tomato and concluded that at least 50% germination was needed to achieve normal fruit-set. When germination was less than 20%, fruit-set was poor with blackberry. However, a relatively large quantity of pollen may be applied to each stigma and the chance of viable grains affecting fertilization is high, even with pollen which shows poor germination assays.

Larsson (1969) reported that pollens of 2x *Rubus* germinated best on 15% sucrose medium, whereas 20-25% sucrose was best for 4x material.

Boev (1973) observed in apple and pear pollen with low (9.4 – 19.0) percentage germination gave fruit set equal to that of pollen with high (50- 58) percentage germination.

Papunov (1974) observed self fertility and interfertility of introduced apple cultivars in Grimes and found that the pollen germination was not always correlated with pollinating capacity. Red Delicious pollen showed 55 per cent germination in 10 percent sucrose solution, but as a pollinizer gave only 3.8 per cent fruit set, whereas Lord Lambourne shows 16 per cent germination and gave 14.3 percent fruit set.

Bhartiya (1980) observed 79.65 to 86.70 per cent pollen germination in apple and highest germination was observed in 10 per cent sucrose solution.

Perry and Moore (1985) reported that a 20% sucrose-agar medium was satisfactory for germination study of the pollen from tetraploid blackberries but gave poor results on a diploid clone.

Kapil (1988) observed that in both winter and summer seasons, pollen viability was more as compared to pollen gerrnination. Thus, it could be suggested that all the viable pollen grains are not able to germinate, so their germination can be less. But maximum pollen germination took place in 25% sucrose-agar medium.

Junqi (1990) repoted pollen grain of apricot emerged after they attached on stigmas approximately for two hours, pollen tubers entered the ovary 50 hours after pollination and the double fertilization was finished in fourth day.

Shivanna and Rangaswamy (1992) studied *In vitro* pollen germination in different concentration of sucrose solution alone (10, 15, 20 and 25%) and in combination with 0.01% boric acid. The pollen grains collected from the freshly dehisced anthers were suspended in different concentration of sucrose solution. Pollen grains, which had germinated and produced pollen tubes in the medium, were recorded. Length of pollen tubes in different concentration was recorded 45 minutes after the commencement of germination and percentage of pollen germination was calculated and the average length of pollen tube was recorded.

Lal (1993) reported the pollen germination of eight plum cultivars ranged between 44.61 per cent to 63.74 per cent in 15 per cent sucrose solution.

Grauslund (1996) obtained 13-89 per cent pollen germination in different apple cultivars in a solution containing 15 per cent sucrose, 15 ppm boric acid and 150 ppm calcium nitrate.

Eti *et al.*(1998) studied pollen germination of three summer apple cultivars, grown at Pozanti. Turkey and reported that 20 per cent sucrose concentration was the best medium for all three cultivars and pollen germination rates varied between 54.32 to 64.42 per cent.

Abdel (1999) reported 73.30 to 86.10 per cent pollen germination in 15 per cent sucrose solution in three apple cultivars.

Gercekcioglu *et al*. (2000) reported the *in vitro* pollen germination by hanging drop method using sucrose concentration 0, 10, 15, 20 and 25 per cent at 15, 20 and 25°C. Germination ability was 3 to 41.7 per cent. They found that the concentration of sucrose except for control i.e. zero per cent had no effect on germination. The best germination was observed at 20°C temperature.

Min *et al.* (2008) concluded that GA_3 at lower mass concentrations stimulated pollen germination and tube growth of Chaohong peach, but it inhibited pollen germination and tube growth at higher mass concentrations and the most suitable mass concentrations that stimulated pollen germination and tube growth was at 25-100 mg/L.

Ping and Bin (2008) observed that the pollen germination rate of 5 cultivars of pear was reduced very fast with the time of being preserved at room temperature, contrasting to its germination rate before being preserved. 60 days later, the viability of pear pollen fell under per cent, the pollen germination rate of 6 pollen cultivars ranged from 3.8 per cent to 5.8per cent.

Xing *et al.* (2008) reported that after 20 hours culture of pollens of *Prunus armeniaca* L.cv. Katy in 20 per cent sugar, 0.04 per cent boric acid and 0.01 per cent $CaCl_2$ the pollen germination rate was 68.76 per cent and its tube length was 1083.53 μm.

2.4 STIGMA RECEPTIVITY

Blasse (1980) obtained maximum fruit set when pollination was done on the day of flower opening and upto two days after anthesis in cultivars of apple.

Bhartiya (1980) in apple recorded high stigma receptivity one day before and on the day of anthesis and receptivity declined one day after anthesis, some stigmas remained receptive for three to four days after anthesis.

Bekey (1985) reported that the peak receptivity period of 8 cultivars of red raspberry lasted from 1 to 4 days. Most cultivars set as well with one day of hand pollination at the peak of the receptivity period as with three days of consecutive pollination. Autogamy was

as effective as allogamy in flowers of raspberry. The development of flowers, pollen tube growth and drupelet set were highly affected by temperature.

Gonzalez *et al*. (1995) reported screening on papilla and the receptivity period of female flower of kiwifruit. They studied different stages of fruit set and observed that the effective stigmatic receptivity period lasted until four days after anthesis and reported fruit set at five days after anthesis which was reduced significantly and was nil at seven days after anthesis.

Kumar *et al.* (1997) while studying flowering behaviour of some scab resistant and susceptible apple cultivars observed that the stigma remained receptive from Two days before anthesis to five days after anthesis. Stigma receptivity was greatest on the day of anthesis.

Hong *et al.* (2001) reported that stigma receptivity of almond lasted for about 4 days, the more pollination early the more set easily. It took about 6 days for the pollen tube to grow through a style and an additional day to grow reach ovule.

2.5 POLLINATION STUDY

Rozanova (1939) made an attempt to the self-sterility found in European and North American wild forms of the red raspberry (*Rubus idaeus vulgates* and *Rubus idaeus strigosus*) in contrast to the self-sterility of cultivated varieties. She stated that Wild strains are as a rule, self-sterile.

Gustafsson (1947) reported that facultative apomixes in *Rubus* may give a pattern of variation and a type of evolution which in some ways is similar to that found in self-fertilized organisms. The potentiality for new variation is independent of the fate of the sexual species.

Fryxell (1957) reported that 39 species of Rosaceae family are self-incompatible or which include some self-incompatible varieties. They comprise one species of *Fragaria*, ten of *Prunu*s, four of *Pyrus*, five of *Malus*, four of *Rosa*, one of Potentialla, six of *Rubus* and a few ornamentals.

Chaudhari (1966) reported that raspberries in India have zero to five percent self-pollination, though these are naturally cross-pollinated plants.

Liwerant (1966) while studying pollination found a decrease in yield by 50 per cent on an average in self pollinated orchards of Golden Delicious during poor weather conditions, whereas in cross pollinated orchards the decrease in yield by 2-8 per cent.

Lange (1967) observed that the in Tydeman's Red apple the percentage of flower cluster which set fruits averaged 6.9 per cent for self pollinated flowers, 20.40 per cent with Red Delicious pollen and 32.80 per cent with Golden Delicious pollen.

Daubeny (1968) reported that the highest percent drupelet set in raspberry cultivar was obtained by at least two or three times hand pollination of individual flowers (69.03 per cent) than open pollination (50.53 per cent) and the drupelet set was found to be lowest in self pollination (39. 80 per cent).

Keep (1968) stated that self incompatibility has been demonstrated in 11 of 23 *Rubus* species and found that the wild clones of *Rubus idaeus* were self incompatible due to stylar inhibition of self-pollen tubes, also reported that nearly all British red raspberry cultivars were fully self-fertile and Daubeny (1969) reported a lower percentage of druplet set when flowers of seven red raspberry cultivars were self pollinated in British columbia.

Shanks (1969) observed that without any additional pollination a berry developed frequently, although it was be relatively small and may had a tuft of unpollinated pistils at its center. Insect activity helped to spread pollen over the pistils and to increase the set of drupelets. The extent of this increase seemed to vary with the cultivar.

Daubeny (1971) found that flowers pollinated only with their own pollen or that from adjacent flowers set nearly as well (57 to 77 %) as open pollinated flowers. Most commercially grown cultivars of red raspberry are self fertile.

Gattschalk and Kaul (1974) reported that Apomixes was caused when the stamens were shorter than pistils, shrunken and non-dehiscent. As a result, there was failure of stamen development or the transformation of male sex-organs into female ones in raspberry.

Bhartiya (1980) inferred lowest fruit set percentage was obtained with selfing by bagging in all the cultivars of apple under taken for study, Winter Banana, Red Gold and Golden Delicious proved more effective pollinizers for Starkrimson in decreasing order. Cultivars varied in self compatibility, Royal Red, Red Delicious and Starking Delicious were completely self incompatible.

Liferova and Pavlov (1983) studied viability of pollen and drupe-setting during inter cultivar crossing of *Rubus idaeus.* He reported that hybrid raspberry was productive in monovarietal plantations.

Nesterov and Shipota (1984) reported that the Golden Delicious spur variants did not show mutual cross compatibility but varieties of Red Delicious group proved cross compatibility with them.

Whitney (1984) reviewed the reproductive biology of raspberries (*Rubus idaeus* and *Rubus pubescens*). *Rubus idaeus* is an abundant species which offers almost unlimited supplies of nectar during part of its extended 3-4 month flowering season.

Nybom (1985) reviewed the active self-pollination in blackberries and observed that most of the species were apomictic and pseudogamous. The stamens of cross pollinating species seldom touch the stigmas while those of self pollinating species arch over the pistils and dry up in this position. All species set seed well after artificial self pollination and were thus self-compatible, also did a study of wild Swedish polyploid blackberry species, it was demonstrated that in some species, the stamen behaviour can lead to self pollination.

Bekey (1985) reported that most raspberry cultivars set as well with one day of hand pollination at the peak of the receptivity period as with three days of consecutive pollination.

Kapil (1988) observed apomixis in some *Rubus ellipticus* S. plants, when the flowers opened, stamens appeared to be very dull and shorter than stigmas. No dehiscence took place, as a result no shedding of pollen grains and thus no fertilization. Even then, ovary appeared to develop normally and after some time changed into a fruit. The fruit looked like a normal one but smaller in size but with deformed shapes, as they were mostly seedless. Stamens after shrinking became dull brown and after some time perished. Crosspollination might be the most efficient among the different modes of pollination as it resulted in highest fruit facundity.

2.6 FRUIT SET

Pasqual *et al.*(1981) reported that eight cultivars of apple under study were compatible with Golden Delicious and fruit set ranged from 75.1 to 61.3 percet and Rejman

(1983) obtained highest fruit set and improved fruit colour when cultivar of apple, Yellow Transparent was used as pollinizer in a pollination study.

Perry and Moore (1985) reported smaller fruits and generally decreased seed number per fruit due to self-pollination in some cultivars of blackberries, also reported that caged plants of blackberry produced larger fruits than uncaged plants which may have been due to lower temperature from shading, whereas the uncaged field plants were exposed to unreasonably high temperature.

Engel and Drescher (1986) findings indicated that hand or bee cross pollination was essential for high seed number and selfing resulted in poor fruit set in apple cultivars.

Kapil (1988) did a very peculiar observation in the study was that the fruit which appeared in December remained unripe till mid April. This may be due to low temperature prevailing during this period. They started ripening with the rise in temperature in April month. The flowers which opened in the first week of April, became unripe fruits after 12-16 days and started ripening in the month of May when the temperature further increased. Fruit-ripening occurs only at higher temperature because heat is required for the maturation of fruit. Thus, it is clear that temperature significantly affected the number of days to fruit ripening. Maximum fruit-ripening was seen in May and June, when maximum temperature was 22 to 24.2°C, but ripening started at the end of March and April in some locations, when maximum temperature was 28-36°C and 38-43°C respectively. There was great variation in fruit-size within the population and even within the same plant and branch, it was concluded that small- sized fruits with few seeds resulted from inadequate pollen dispersal within the flower. These fruits were malformed, indicating a lack of pollination of all pistils in the aggregate flower. Fruit bearing in April-May was healthy as enormous numbers of flowers in the inflorescence were pollinated.

Kumar (1988) observed maximum fruit set in cross pollination and minimum fruit set was obtained with hand self pollination. Dashad and Sharma (1993) compared the fruit set under various modes of pollination and reported that mean fruit set of seven cultivars was higher with open pollination (21.41 to 23.24%) than with self pollination (0.75% - 0.82%), while Sigh (1998) from two years of study on pollination reported that fruit set through cross pollination in various cross combinations varied from 20.00 to 66.00 per cent in first year, whereas, it varied from 22.00 to 70.00 per cent in following year in apple.

Thompson *et al.* (2008) reported that the earliest observed short structures began blooming in early April, but ovule viability was poor, with fruit set averaging 57 per cent and 47 per cent for Prime-Jan and Prime-Jim cultivars of raspberry respectively. In long floral structures, bloom began in mid-May and fruit set averaged 94 per cent and 87 per cent for Prime-Jan and Prime-Jin, respectively. The time from anthesis to black fruit and floral structure types were on an average 57 days. Short floral structures thus had fruit ripening in early to mid-June while fruit on long floral structures ripened in mid-July.

Chapter-3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The present investigation entitled Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya, at the College of Horticulture, VCSG, Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, during 2016 - 2017. Observations were recorded on the following aspects.

3.1 GENERAL STUDIES

3.1.1 Site

The experimental plants were present in College of Horticulture, VCSG. UUHF. Bharsar, The site is located at an altitude of 1900 meters above mean sea level at a Longitude of 78.99^0 E and Latitude of 30.056^0 N.

3.1.2 Climatic and weather

The climate of Bharsar is mild summer, higher precipitation and colder or severe cold prolonged winter. The South-east monsoon commences towards the end of June while the North-east monsoon causes occasional winter showers during November-February. During winter, snow fall is common in this region.

3.1.3 Experimental material

As per the experiment conducted in Bharsar campus during the period between April 2016 – June 2017 which was entitled as Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry *(Rubus species)* of Garhwal Himalaya, during the investigation following experimental materials were used: The present study has been carried out in three wild species of raspberry *viz.*, *Rubus macilentus* C. *Rubus niveus* T. *Rubus paniculatus* S. present in the College of Horticulture, VCSG. UUHF. Bharsar, Vernier Callipers, hand lens, tags, butter paper bags and dissection box are used for field studies. For lab studies light microscope, glasswares, standardized ocular and stage micrometer and

chemicals like boric acid, acetocaramine and glycerin were used for preparation of different media and other operations. All the experimental plants were kept under similar set of cultural practices during the course of studies.

3.2 CANE MORPHOLOGY

Cane color, stem shape and arrangement of prickles on the stem and other typical characters were studied.

3.2.1 Cane height (cm)

Cane height was measured from the base of cane to the apex during flowering with the help of a measuring tape, data was recorded and expressed as centimeters.

3.2.2 Diameter of stem (cm)

Diameter of the stem was measured at the base of the plant with the help of Vernier Callipers during flowering and that of primocane was taken at its maximum vegetative growth period and expressed in centimeters.

3.2.3 Number of laterals per plant

The number of laterals arising from floricane were recorded during flowering period and expressed in numbers.

3.2.4 Diameter of laterals (cm)

Diameter of laterals were measured at the basal bud with the help of Vernier Callipers during flowering and expressed in centimeters.

3.2.5 Colour and type of stem

Colour and type of stem were studied by visual observations (Datta,2000).

3.2.6 Prickles and bristles on stem

Prickles and bristles present on the stem are studies by close visual observations (Datta, 2000).

3.3 LEAF MORPHOLOGY

Leaf type, color, shape, size, number of leaves and leaf area was recorded (Datta, 2000).

3.3.1Phyllotaxy

Arrangement of leaves on the stem or branches were recorded and type of leaves was studied

3.3.2 Shape and size of leaflets (length and width)

Shape and length and width of the leaf were measured by using measuring scale at the time of flowering and average leaf size is expressed in terms of centimeters.

3.3.3 Leaf area (cm^2)

Leaf area was recorded with the help of leaf area meter and mean value was expressed in cm^2.

3.4 GROWTH BEHAVIOUR

3.4.1 Length of the shoot

To record the time of emergence of vegetative shoots and their growth behavior, on each experimental plant five newly emerging shoots were marked for taking observations on their length at ten days interval.

3.4.2 Diameter of the shoot

To record the time of emergence of vegetative shoots and their growth behavior, on each plant five newly emerging shoots were marked for taking observations on their diameter at ten days interval.

3.4.3 Emergence of the new leaves

To record the time of emergence of phyllotaxy and their growth behavior, on each plant five newly emerging shoots were marked for taking observations on the newly emerging leaves at every two days interval.

3.5 FLORAL BIOLOGY

The following attributes of floral biology were investigated in selected species of wild raspberry.

3.5.1 Bearing behavior

On each plant five healthy branches were marked to record the various observations pertaining to the bearing behavior of raspberry.

3.5.1.4 Emergence of the inflorescence

To record the time of emergence of inflorescence and their growth behavior, on each plant five newly emerging laterals from secondary laterals were marked. Observations had taken on the newly emerging inflorescence at every two days interval.

3.5.2 Flower bud development

For observing the floral bud development, on each plant, five buds were tagged in four different directions, before they had started to show the any sign of growth. The changes in buds such as length and breadth and color were recorded on every alternate day till the buds get completely open.

3.5.2.1 Stages of flower bud development

To observe the stages of bud development, any differences in the flower buds were noted. The stages of flower bud development were differentiated based on the small changes in the structure and the components of the flower buds.

3.5.2.2 Flower bud growth behaviour

It was recorded by noting the difference in the parameter of bud like diameter of the bud and the length of the pedicel.

3.5.3 Time and duration of flowering

The data on time and duration of flowering were recorded from the anthesis of first flower to anthesis of last flower on the experimental plants of raspberry. To study the time and duration of flowering, five representative branches well spread around the periphery of the plants were selected. Numbers of anthesised flowers were noted from the Anthesis of first flower to last flower. After recording the observation anthesised flowers were removed to avoid the recounting. The peak period of flowering (full bloom) was considered when nearly more than 75 per cent flowers get anthesised on the plant.

3.5.4 Floral morphology

The relative size of floral components was determined by measuring the different parts of the flower with the help of a measuring scale. The floral components were measured when the flower was freshly opened and completely expanded. To study the floral morphology primarily ten plants are selected. From each plant ten flowers were selected for the study. The length and breadth of calyx and corolla, stamens and pistils was recorded from the same number of flowers.

3.5.5 Anthesis of flower bud

3.5.5.1 Mode of Anthesis

In order to study the different stages of anthesis, the selected buds are closely watched for the entire duration (from petal opening up to the appearance of stamens and pistils) on each experimental plant.

3.5.5.2 Time of Anthesis

With the view to record the time of anthesis in raspberry under investigation, 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.

3.5.6 Anther Dehiscence

3.5.6.1 Mode of Dehiscence

The observations for the mode of dehiscence under present study were recorded by picking the stamens from freshly opened flowers and examining them with hand lens.

3.5.6.2 Time of Dehisence

Ten flowers located on different parts of each experimental plant were tagged. On each day, after anthesis, the number of anthers dehisced were recorded at every two hours interval with the help of hand lens, between 6am to 6pm. If anther dehiscence time is too short, then the time interval for the recording of observation was reduced as par the requirement. The dehisced anthers were removed from the flowers to avoid recounting.

3.6 POLLEN STUDIES

3.6.1 Pollen collection

During the peak period of flowering, floral twigs bearing copious number flower buds which were at the balloon stage (likely to open next day) were gathered from the experimental plants on the previous evening of anthesis day. Cut ends of the flower buds were immersed in distilled water in beakers and maintained under laboratory conditions. On the succeeding days as the dehiscence of anthers was ensured, a single gentle tap on the flowers released the pollen grains easily on the petridish.

3.6.2 Number of pollens per anther

The anthers were obtained from closed flowers prior to anthesis, placed in a small vial containing 1 ml of glycerine 1%, anthers were smacked and the pollen grains were suspended. From this concentrate, five (10 µl) droplets were taken out in the slides and pollen grains were counted under the microscope.

3.6.3 Number of pollens per flower

Production of pollen grains per flower was estimated by multiplying the number of pollen grains per anther with the number of anthers per flower.

3.6.4 Pollen morphology

The fresh pollen grains as collected from flowers of species under study were used for making size measurements in different medium, viz., water, acetocarmine, glycerin jelly and without any medium (dry condition). The size of pollen grains was measured with the help of ocular micrometer indexed against stage micrometer. The average size (length and breadth) of 50 pollen grains under each medium was considered to arrive the final value.

Data was analyzed with completely randomized design having 4 treatments and 6 replications which is mentioned in the Table – 3.1

Table 3.1 Treatment details for study of pollen morphology

Treatment	Treatments details
T_1	Water
T_2	Glycerine
T_3	Acetocarmine
T_4	Dry condition

3.6.5 Pollen viability

The viability of fresh pollen grains of flowers was estimated separately by acetocarmine test. Acetocarmine solution was freshly prepared by taking 55 ml of water, 45 ml of glacial acetic acid and 1 gm of carmine boiled for some time and then filtered. One to two drops of acetocarmine solution was placed on the slide and then the pollen grains were dusted, cover with a cover slip examine under the microscope. The grains staining deeply and looking normal under microscope were counted as viable, whereas shriveled and poorly stained were

considered as nonviable. Care was taken to include data from several microscopic fields to cover the pollen grains laying both at the peripheral and central regions of the cover slips.

For longevity study, the fresh pollen grains of raspberry species under investigation were stored in dry specimen tubes, covered with cotton plugs and maintained at room temperature and pollen viability was calculated with the following formula.

$$\text{Pollen viability} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

3.6.6 Pollen germination

The fresh pollen grains of the raspberry species under study were planted in artificial media of sucrose, boric acid and gibberellic acid at different concentration combinations and water served as control. The "Sitting Drop" culture method (Shivanna and Rangaswamy, 1992) was employed for pollen germination studies. Slides were examined at 6, 18, 24 and 48 hours(if required number of hours can be increased upto 72 hours), after planting the pollen grains in different media, the observations on germination of pollen grains and pollen tube length were recorded at least under ten different microscopic fields under both the methods for calculating the average values.

Data was analyzed with completely randomized design which have 16 treatments and 3 replications, as mentioned in the Table 3.2 and pollen germination percentage of each treatment was calculated with the following formula.

$$\text{Pollen percentage germination} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

Table 3.2 Treatment details for study of pollen germination

Treatments	Treatments detail
T_1	Water
T_2	0.1% Boric acid + 5% Sucrose
T_3	0.2% Boric acid + 10% Sucrose
T_4	0.3% Boric acid + 15% Sucrose
T_5	0.4% Boric acid + 20% Sucrose
T_6	0.5% Boric acid + 25% Sucrose
T_7	0.1% Boric acid + 1ppm Gibberellic acid
T_8	0.2% Boric acid + 2ppm Gibberellic acid
T_9	0.3% Boric acid + 3ppm Gibberellic acid
T_{10}	0.4% Boric acid + 4ppm Gibberellic acid
T_{11}	0.5% Boric acid + 5ppm Gibberellic acid
T_{12}	5% Sucrose + 1ppm Gibberellic acid
T_{13}	10% Sucrose + 2ppm Gibberellic acid
T_{14}	15% Sucrose + 3ppm Gibberellic acid
T_{15}	20% Sucrose + 4ppm Gibberellic acid
T_{16}	25% Sucrose + 5ppm Gibberellic acid

3.7 STIGMA RECEPTIVITY

The stigma receptivity of the raspberry species under study were studied by the two methods, viz., visual observations and fruit-set method.

3.7.1 Visual observation method

In order to find out the extent and nature of receptivity by visual observations, the stigmas of different age groups of flower buds, varying from three days prior up to three days after anthesis, were examined daily in the morning with the help of hand lens for observing their general features such as freshness, shininess, secretion, stickiness and color etc. The stigmas looking shiny, sticky, fresh and attractive were considered as receptive while dull, faded, non sticky and brownish stigmas were considered as non-receptive.

3.7.2 Fruit set method

To study the receptivity of stigma by fruit set method, 10 emasculated flower buds were hand self pollinated at different ages varying as three, two and one day prior anthesis, on the day of anthesis and one, two and three days after anthesis. The pollinated buds were covered with paper bags and tagged as usual. The fruit set may observe 21 days after pollination which may be indicated by initiation of swelling of ovaries, calyx cup covering back the ovaries, weathering of stamens and the fruit set percentage was calculated with the formula given below.

$$\text{Fruit-set (\%)} = \frac{\text{Number of fruits set}}{\text{Total number of pollinated flowers}} \times 100$$

3.8 POLLINATION STUDIES

The following methods were employed to study the mode of pollination in raspberry wild species. These pollination studies were conducted as follows.

3.8.1 Allogamy

Mature flower buds, ready to open next day were emasculated and pollination was done on next day (at anthesis) with the pollens collected from the freshly dehisced anthers of another plant or same species, these pollinated flowers were bagged again and allowed to remain on the plants for fruit set. Interspecific crosses between *Rubus macilentus* C. X *Rubus niveus* T., *Rubus macilentus* C. X *Rubus paniculatus* S., *Rubus niveus* T.X *Rubus macilentus* C., *Rubus niveus* T. X *Rubus paniculatus* S., *Rubus paniculatus* S. X *Rubus macilentus* C. and *Rubus paniculatus* S.X *Rubus niveus* T. were carried out.

3.8.2 Autogamy

The shoots bearing flower buds were tagged and bagged with all necessary care, a day before anthesis and left for self-pollination and fruit-set inside the bags.

3.8.3 Geitonogamy

Mature flower buds, ready to open next day were emasculated and pollination was done on next day (at anthesis) with the pollens collected from the freshly dehisced anthers of same plant. These pollinated flowers were bagged again and allowed to remain on the plants for fruit set.

3.8.4 Natural or open pollination

Perfect flower buds on ten plants of each raspberry wild species were tagged before anthesis and allowed to remain on the trees for recording various observations with respect to fruit-set.

3.8.5 Self incompatibility

Self incompatibility was obtained by dividing the average fruit set after self pollination by the average fruit set after cross pollination (Lloyd and Schoen, 1992). The value of one indicates complete self incompatibility

3.8.6 Apomixes

Anthers and stigma of buds were clipped a day prior to anthesis and bagged. If there is fruit set then it was considered as an apomictic fruit.

3.9 FRUIT SET

3.9.1 Time of fruit set

The time of fruit set in the raspberry wild species under study was carried out by marking five branches on the periphery of a plant during flowering season. The flowers on each branch were counted. The observation on fruit set was started after one week of natural pollination. The date on which percentage of fruit set recorded is 75per cent or above was considered as the time of fruit set.

3.9.2 Percentage of fruit set

After three weeks of open pollination, fruit set in each branch was recorded and the fruit set per 100 flowers was calculated in order to get percentage of fruit set in all the raspberry wild species under study and Percentage of fruit set was calculated with the following formula.

$$\text{Percentage of fruit set} = \frac{\text{Number of fruits set}}{\text{Total number of flower counted}} \times 100$$

3.10 Stastical analysis

The mean values of data were subjected to analysis of variance as per Statistical Procedure for Agricultural Research. Gomez and Gomez (1983) for Randomized Block Design.

ANOVA TABLE

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	K-1	BSS	BSS/K-1(V_1)	V_1/ V_3
Error	n- K	ESS	ESS/n-K(V_2)	V_2/V_3
Total	n- 1	TSS	TSS/n-K(V_3)	

Where,

df = degree freedom

k = treatment

n = Total no. of observation

BSS = Treatment sum of square

ESS = Error sum of square

TSS = Total sum of square

Chapter-4

EXPERIMENTAL RESULTS

4.1.5 Colour and type of the stem

Young stem was green and soft but its colour mostly changed on maturity. The young shoots were soft but woody at maturity. Primary branches arose from the main stem, which in turn gave rise to larger secondary and smaller tertiary branches, which hanged downwards. So, the stem was said to be trailing. It was noticed that *Rubus paniculatus* S. showed larger branches than other two species.

The young stem or primocane of *Rubus macilentus* C. was five angled, light green colour with white minute spots and soft in texture (Plate 4.1 a.). The young shoots of floricane were four angled with reddish purple colour, while the mature stem of floricane had larger diameter as compered to young shoots of floricane, soft textured, round shaped with deep purple coloured (Plate 4.1 b.). *Rubus niveus* T. showed light green coloured young shoots of primocane with deep purple blotches on stem (Plate 4.1 c.) and shoots whereas the floricane had deep purple colour of primary as well as secondary shoots (Plate 4.1 d.), often rooting at the tip indicating tip layering as a natural mode of propagation. The new shoots of *Rubus paniculatus* S. arose from the previous year shoots since the species is perennial in growth behavior. The young shoots were soft and covered with white tomentum (Plate 4.1 e.), the mature stems were woody and light brown to light black in colour (Plate 4.1 f.).

4.1.6 Prickles and bristles on stem and leaf

Stem and branches were covered with curved or hooked prickles. They also differed in their colour and size on young and mature stems. Bristles were light yellow or purple on young stems and dark-brown or purplish-brown on mature stems. Bristles were straight and pointed outwards.

The prickles were light green with yellow tinge on young stems and thick, large, purplish-brown on mature stems of *Rubus macilentus* C. and it was noticed that there were strictly three prickles between each node in whole plant (Plate 4.2 B.c.). Curved spines were not found on the upper surface but present on the midrib and petiole of leaf on the lower surface. Sometimes these curved spines could be seen on the lateral nerves. These were yellowish-green or purple coloured depending upon the colour of the midrib (Plate 4.3 B.c.).

Prickles of *Rubus niveus* T. were lager on primocane than the floricane with deep purplish colour on mature stems (Plate 4.1 d.), whereas light green to light purple colour on primocane. Even some times the prickles appeared in pair. The prickles on the stem and shoots of *Rubus paniculatus* S. were small and curved (Plate 4.1 e.).

Table 4.1 Variations in morphological characters of *Rubus* species.

Characters	*Rubus macilentus* C.	*Rubus niveus* T.	*Rubus paniculatus* S.
1. Habitat	Open, grassy, sunny area with moist soil.	open, dry, sunny area on slopes.	Shady, slopy area, as undergrowth in a thin forest.
2. Growth behavior	Bi-annual growth behavior.	Bi-annual growth habit,	Perennial in nature
3. Cane height (cm)	2.5 to 3 meter	4 to 6 meter	12 to 20 meter
4. Diameter of stem (cm)	1.5	1.9	2.2 to 2.4
5.Number of laterals per plant	105	32	432
6. Diameter of laterals (cm)	0.56	0.74	0.93
7. Colour of the stem	Primocane – soft, five angled, light green colour with white minute spots Floricane-Young shoots: woody with four angles, green shoots with reddish purple streaks. Mature stem: reddish purple with round	Primocane- soft and green with purple streaks. Floricane- Young and mature shoots – woody with deep purplish red colour.	Young –dull white to light green. Mature – brownish green.

	shape.		
8.Prickles on the stem	Young- light green with yellow tinge. Mature- dark purple with light brown tip. Strictly 3 prickles were present between each node.	Young- light green with purple tinge. Mature- dark purple with light brown tip.	Young and mature stem had the minute brownish white prickles with brown or black tips.
9. Branching	Smallest of all and less shrubby.	Small and less shrubby.	Largest and more shrubby.
10. Colour of leaflets	Upper surface - dark green. Lower surface - light green.	Upper surface - dark green. Lower surface – white tamentose.	Upper surface – less darker (green). Lower surface – White tamentose.
11.Type of leaf	Compound leaf	Compound leaf.	Simple leaf
12. Number of Leaflets	3 leaflets	5-7, some times up to 11 leaflets	Single leaf
13. Shape of Leaflets	Pinnate leaves	Pinnate leaves	Heart shaped leaves
14. Number of Nodal Leaves	Two leaf	Nodal leaves absent	Nodal leaves absent
15. Stipules	Two, thin, two thinner and smaller at the base of each nodal leaf.	Present at the base of leaf arising from each node	absent

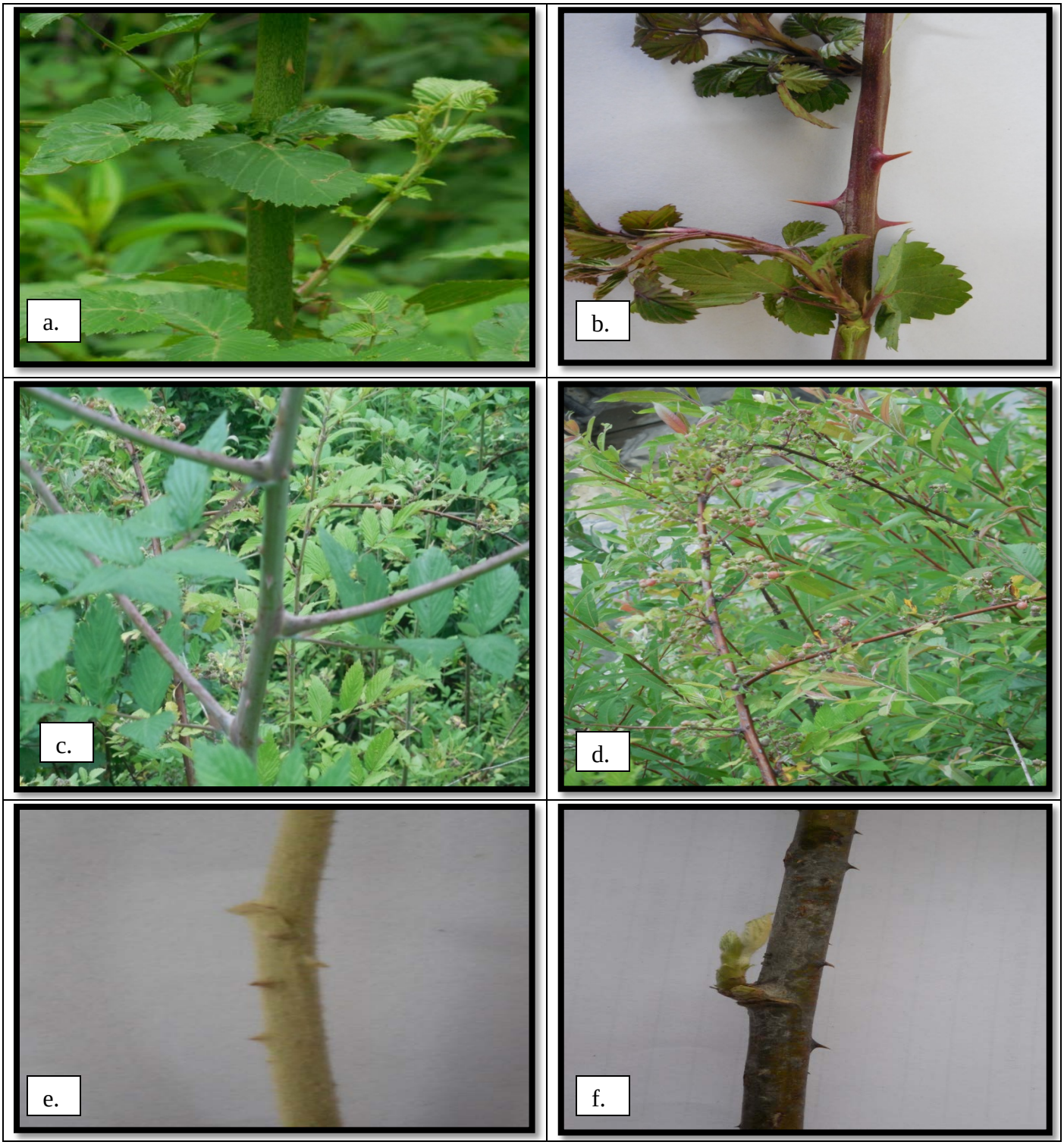


Plate 4.1 Characteristics of shoots

1. *Rubus macilentus* C. **a.** Stem of primocane **b.** Stem of floricane

2. *Rubus niveus* T. **c.** Stem of primocane **d**. Stem of floricane

3. *Rubus paniculatus* S. **e.** New shoots **f.** Old shoots

4.2 LEAF MORPHOLOGY

Leaves were compound with variable number of leaflets in each leaf. Leaf venation was reticulate with prominent veins. Nerves were sunk on the upper surface and prominent beneath, which bifurcated at the end of leaf margin and were alternately arranged. It had been observed in young leaves that nerves were light green in colour on the lower surface and their colour changed into very light purple on maturity probably due to the accumulation of anthocyanin pigments. The mid-ribs and lateral nerves were of the same colour as that of the petiole and leaf stalk. Size of the leaves differed in each three wild species of raspberry as given in Table 4.2.

4.2.1 Phyllotaxy

Phyllotaxy is found to be same in both primocanes and floricanes of the species under study. Many nodes appeared on each branch which varied in number depending upon the length of the branch. Each branch showed an alternate arrangement of leaves.

In *Rubus macilentus* C. from each node, arose two small basal or nodal leaves along with a petiolated compound leaf having 3 leaflets (Plate 4.2 A.a,and A.b.). Out of these three leaflets, terminal one matured first and the laterals later on. When the leaflets were on the way to maturity, 2-3 leaf buds together began to arise in the middle of nodal leaves at the same node (Plate 4.2 A.b.). The leaf which appeared first became longer and gave rise to a tertiary branch having nodes and internodes, from where further arose nodal leaves and leaf-buds from each node at the opposite side (Plate 4.2 A.a, and A.b.). These were sessile but rarely short stalked when mature. Both the nodal leaves originated from the same point. These were nerved and serrated like leaflets. Mostly these were widely uniserrated. Their colour was the same as in leaflets. New leaflets arose from the middle of nodal leaves (Plate 4.2 A. a, and A.b.). Two small, thin, leafy and greenish purple stipules with pointed tips appeared to arise at the base of each nodal leaf (4.2.A.a.). Average nodal leaf area, length and width are given in Table 4.2.

In *Rubus niveus* T. the leaves were pinnate with 5-7 on floricane and most commonly upto 11 leaflets found on primocane. The upper surface was green and glabrous and lower

surface was white and tomentose. The terminal leaflets were often large and lobed, lateral ones smaller and sessile. At every leaf axil there was an appearance of leaf and new shoot (Plate 4.2 A. c.).

The leaves of *Rubus paniculatus* S. were alternatively arranged, had simple petiolated and hairy leaves (Plate 4.2 A. d.).

The alternate arrangement of leaves and larger size of primocane leaves than floricane was similar in all the three species (Plate 4.2 A.a, A.b, A,c, and A.d).

4.2.2 Shape and size of leaflets

Leaves were compound and petiolated but simple leaves were found in *Rubus paniculatus* S. shape and size of leaflets varied considerably in each species and which is mentioned in Table 4.2.

In *Rubus macilentus* C. the leaves were 3-foliolated, Leaflets were always three in number and elliptic. Terminal one was large had long stalk while two lateral leaflets were small with short stalk (Plate 4.2 A.a.) and seldom they were sessile. The pinnate terminal leaves were observed, the lateral leaflets differed a little in size or were always equal. The primocane and floricane differ in their leaf sizes (4.2 B.a, B.b, B.c and B.d), average length and width and leaf area are mentioned in Table 4.2. In most cases, both the nodal leaves had almost same in size and shape. The nodal leaves were broader and arose at the leaf axils, (Plate 4.2 B.b and B.d). Bi-or trifurcation in one leaf while in other, all the three parts remained fused. (Plate 4.2 B.b and B.d).

In *Rubus niveus* T. had pinnate leaves with the terminal leaflet of floricane was larger than lateral leaflets which are almost similar in size and shape (Plate 4.2 B.f.), while in primocane the lower pair of leaflets were having large size when compared to terminal leaflet and the size of leaflets decreased from petiole to tip of the leaf (Plate 4.2 B.e.).

Rubus paniculatus S. lamina of leaf is broadly ovate, cordate and acuminate in shape, more or less serrated. Upper surface of leaf is green with coriaceous and lower surface densely white, tomentose and nerves were prominent beneath(Plate 4.2 B.g and B.h).

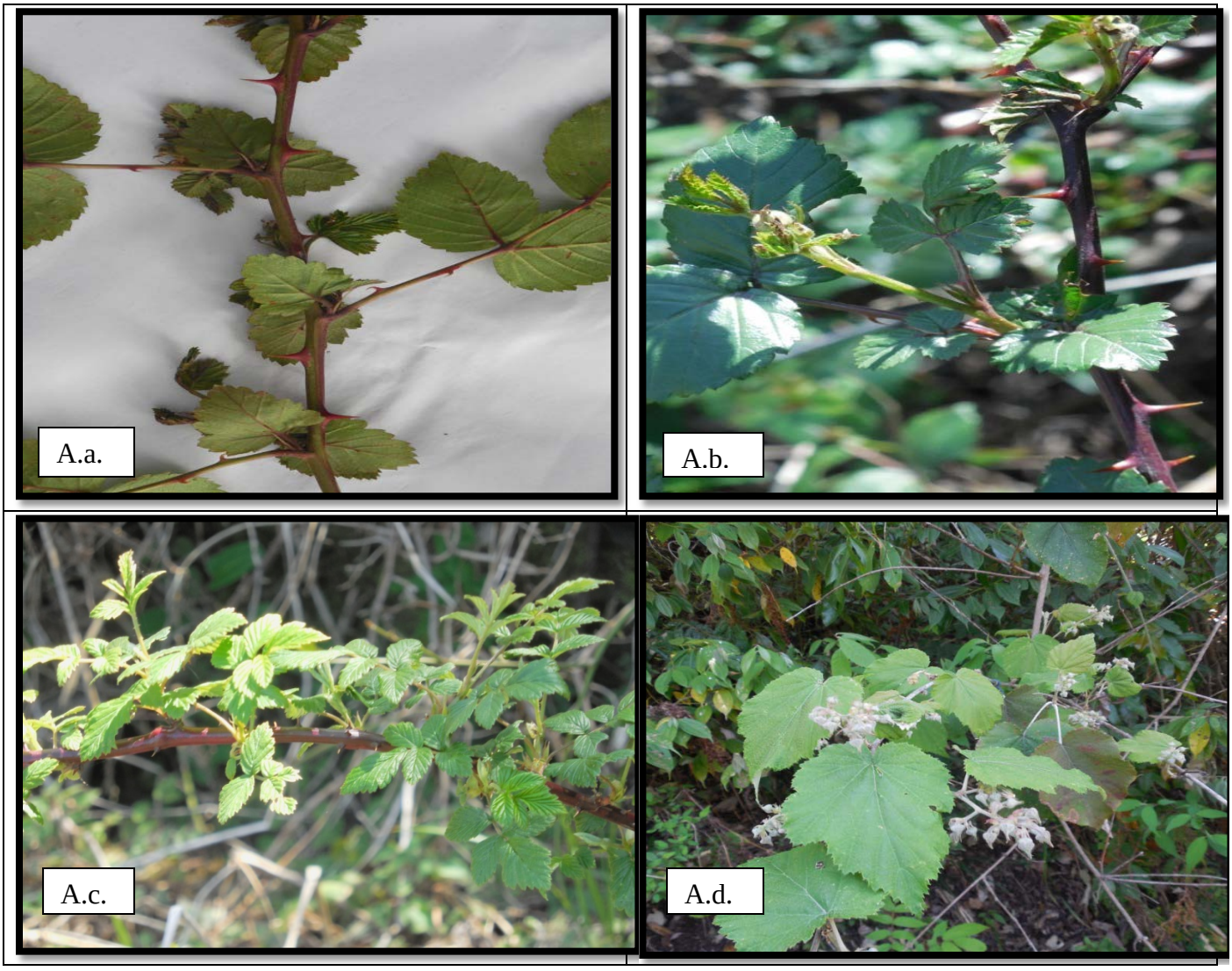


Plate 4.2 A. Leaf morphology (Phyllotaxy)

A.a and **A.b.** Phyllotaxy of *Rubus macilentus* C.

A.c Phyllotaxy of *Rubus niveus* T.

A.d Phyllotaxy of *Rubus paniculatus* S.

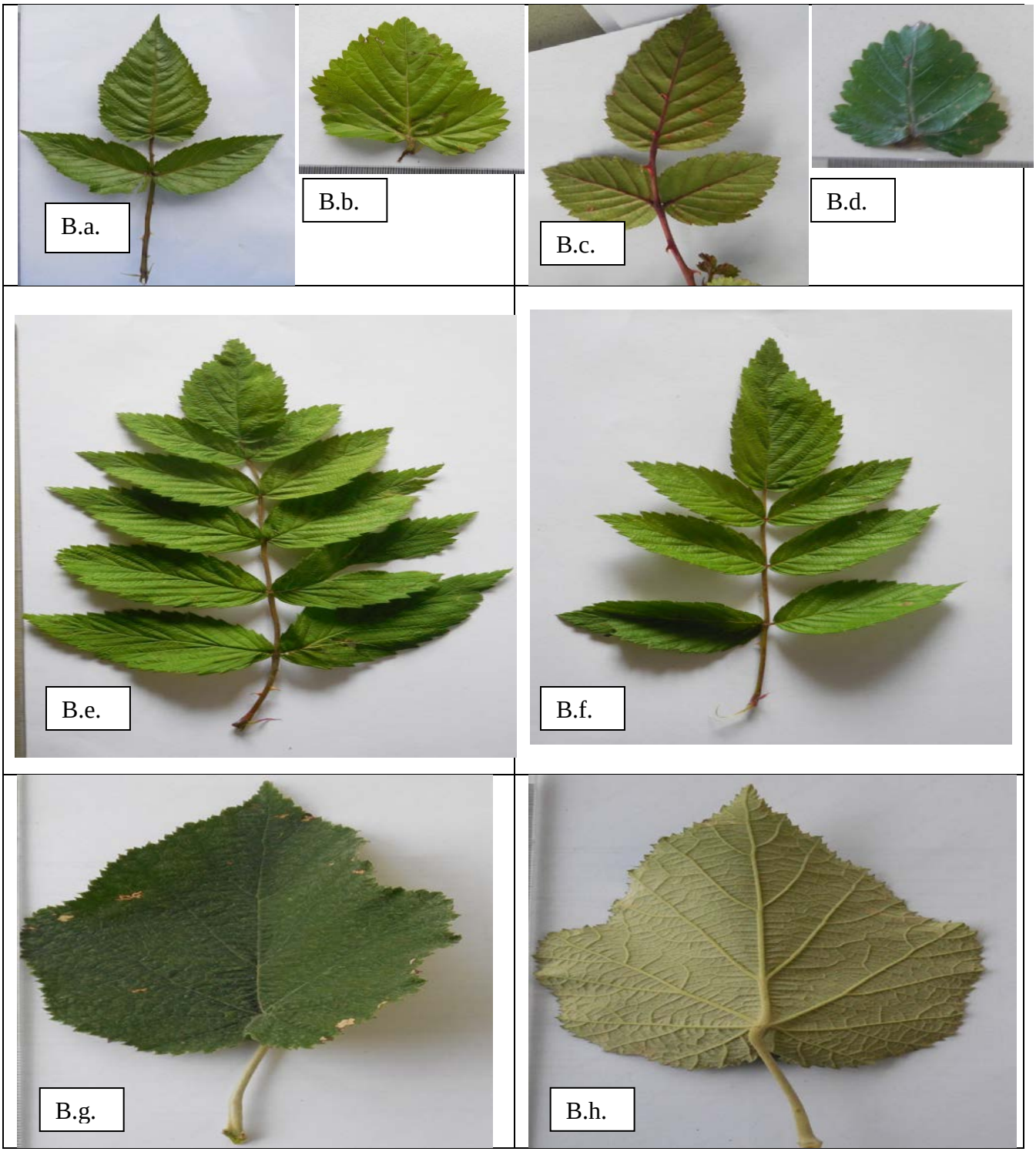


Plate 4.2 B. Leaf morphology (Leaf characters)

Rubus macilentus C. **B.a** Terminal leaf of primocane **B.b.** Nodal leaf of primocane

B.c. Terminal leaf of floricane **B.d.** Nodal leaf of floricane

Rubus niveus T. **B.e.** Primocane leaf **B.f.** Floricane leaf

Rubus paniculatus S. **B.g.** Upper surface of the leaf **B.h.** Lower surface of the leaf

4.2.3 Leaf area (cm^2)

The leaf area of primocane are found to be more than that of floricane in *Rubus macilentus* C. and *Rubus niveus* T. In case of *Rubus paniculatus* S. the newly emerged shoots and bearing shots had the same leaf size and shapes and numerical are given in Table 4.2.

Table 4.2 Leaf measurements of raspberry species under study

character	***Rubus macilentus* C.**		***Rubus niveus* T.**		***Rubus paniculatus* S.**
	Primocane	**Floricane**	**Primocane**	**Floricane**	
Terminal leaf					
Average length and width of terminal leaflet (cm)	**9.82X4.64**	7.18 X 4.06	6.95 X 4.30	8.15 X 4.63	-
Average length and width of lateral leaflet (cm)	6.72X3.92	4.55 X 3.13	**7.88 X 3.51**	6.075 X 2.96	-
Average length and width of terminal leaf/ whole leaf (cm)	8.27X4.28	5.42 X 3.44	7.39 X 3.94	6.76 X 3.52	**13.85X9.93**
Average length of petiole of terminal leaflet (cm)	**3.24**	3.15	1.6	1.55	-
Average length of petiole of terminal leaf / whole leaf (cm)	**7.84**	5.54	4.9	4.45	5.14
Leaf area (cm^2)	65.74	27.54	94.55	56.56	**97.74**
Nodal leaf					
Average length and width of nodal leaf (cm)	**3.31X3.16**	2.97 X 2.85	-	-	-
Leaf area (cm^2)	**4.24**	3.81	-	-	-

4.3 GROWTH BEHAVIOUR

4.3.1 General growth habit

The *Rubus macilentus* C. produced green coloured primocane in April month of 2016 (at the peak flowering period of previous season floricane), during first season the growth of primocane was very rapid till June third week and produced many secondary shoots. By the next year in June 2017 the primary and secondary shoots became reddish purple coloured and the shoot was called as floricane in which tertiary shoots were produced during January and the flowering occurred in same on both secondary and tertiary shoots, after flowering the death of floricane occurred. From April again new primocanes started appearing during the peak flowering of floricane for next year flowering.

The *Rubus niveus* T. also produced the primocane in the second week of April 2016 (at the initial periods of flowering of previous season floricane), the primocanes showed a rapid growth till the end of June and also produced the primary and secondary shoots which became deep reddish purple and produced the new shoots from secondary shoots during February moth of 2017 which is now called as floricane and the flowering occurred on primary, secondary as well as on tertiary shoots. The typical character of shoot was found to be natural mode of propagation through tip layering.

The *Rubus paniculatus* S. was perennial in growth behavior hence plant was a big shrub with many primary, secondary and tertiary branches. New tertiary shoots were produced from previous season's secondary shoots during the May, 2016 and the flowering occurred on tertiary shoots in May 2017, the new tertiary shoots for flowering in next year appeared with commencement of flowering of previous year's shoots. After flowering the drying of secondary shoots were seen.

4.3.2. Length of the shoots

In *Rubus macilentus* C., the bearing shoots viz., secondary shoots had grater shoot length (143.44 cm) than tertiary shoots (65.83 cm). it is observed in case of *Rubus niveus* T. the length of primary shoots (230.74 cm) was greater than that of secondary (72.55 cm) and tertiary shoots (25.73 cm) and also produced more number of inflorescence. While the bearing

shoots of *Rubus paniculatus* S. had lesser lengths (148.68 cm) as compared to newly produced next year bearing shoots (170.63 cm). From the table 4.3 it is reported that the shoots of *Rubus niveus* T. plants showed more growth than the other two species.

4.3.3 Diameter of the shoot

The diameter of the shoots of raspberry under study was kept on increasing throughout the season correlated with the increase in the length of the shoot. The length of the shoots after flower initiation almost remained constant. The table 4.3 shows the comparison between the increase in the diameter of the shoot of three species under study. The highest shoot diameter (0.93 cm) was recorded in *Rubus paniculatus* S. plants than other two species and produced more number of laterals per node which ranged from five to one, every lateral was not the bearing laterals among five only two to three were bearing flowers and fruits.

4.3.4 Emergence of the new leaves

The emergence of new leaves occurred during last week of January in case of *Rubus macilentus* C. while in R*ubus niveus* T. and *Rubus paniculatus* S. the new leaves emerged in first week of February. Arreangement of leaves is explained in phyllotaxy part of this chapter.

Table 4.3 Size of vegetative shoots of raspberry species

Species name	Size of shoots (l x w) at 10 days interval													
	Time of shoot emergence	**Average number of shoot observed**		**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**
***Rubus macilentus* C.**	April 2016	5	L(cm)	1.32	15.23	34.64	56.74	84.85	104.85	119.75	132.65	143.39	143.42	143.44	143.44
			W(cm)	0.05	0.12	0.19	0.22	0.27	0.31	0.39	0.45	0.52	0.54	0.56	0.56
	January 2017	5	L(cm)	1.46	4.63	12.32	20.84	32.73	45.74	57.73	63.74	65.78	65.8	65.81	65.83
			W(cm)	0.04	0.09	0.11	0.15	0.18	0.21	0.26	0.29	0.3	0.32	0.35	0.38
***Rubus niveus* T.**	April 2016	5	L(cm)	1.52	17.42	35.63	65.31	92.53	112.32	137.63	159.64	188.63	215.64	227.38	230.74
			W(cm)	0.05	0.14	0.19	0.24	0.29	0.36	0.42	0.58	0.68	0.71	0.74	0.76
	February 2017	5	L(cm)	0.93	7.64	16.74	25.64	37.85	49.74	59.75	67.84	72.48	72.5	72.52	72.55
			W(cm)	0.05	0.08	0.12	0.16	0.20	0.25	0.29	0.32	0.35	0.36	0.38	0.40
***Rubus paniculatus* S.**	May 2016	5	L(cm)	1.53	15.63	38.63	59.63	87.93	105.62	120.42	136.72	139.74	140.52	143.35	148.65
			W(cm)	0.07	0.15	0.20	0.27	0.36	0.42	0.50	0.62	0.70	0.81	0.88	0.93

4.4 FLORAL BIOLOGY

4.4.1 Bearing Behavior

4.4.1.1 Emergence of the inflorescence

Initiation of floral buds differed in the three wild species of raspberry under investigation. Floral buds started appearing at the first week of February in *Rubus macilentus* C. and in case of *Rubus niveus* T. the flower bud initiation occurred in second week of March, while it was seen in last week of March for *Rubus paniculatus* S.

Floral buds appeared on branches of 2nd year and other mature branches of floricane. Two types of inflorescence were noticed.

(i) Corymbose raceme which was both terminal and axillary (Plate 4.3 a, b, c and d). In axillary corymbose raceme, 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 one, two or three leaflets.

(ii) Panicle which were also both axillary and terminal (Plate 4.3 e and f). These were leafy but leaflets were very small in size. Axillary panicles also arose from the axils of main as well as lateral shoots.

Corymbose raceme was more frequent in nature than panicle. Inflorescence was lax in panicles, while it was compact in corymbose raceme in a cluster. 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 (Plate 4.3 c).

Table 4. 4 Characteristics of bearing shoots

SL. No.	Average Number of shoots observed	Average length of bearing shoot (cm)	Average number of inflorescence per bearing shoots	Average number of buds per inflorescence	Average number of flowers per inflorescence	Average number of flowers per bearing shoots
Rubus macilentus C.	5	143.39	55.6	3	3	166.8
Rubus niveus T.	5	230.74	105	28.14	27.83	2922.15
Rubus paniculatus S.	5	148.65	22	25.48	22.38	492.36

It was noticed that number of flowers in an inflorescence varied among three species of wild raspberry. *Rubus macilentus* C. had strictly 3 flowers per inflorescence and number of flowers varied from 3 to 35 in case of *Rubus niveus* T. both the species mostly had corymbose raceme type of inflorescence, while in *Rubus paniculatus* S. it varied from 1 to 30 flowers per inflorescence with panicle type of inflorescence and had a typical character that buds and flowers facing towards gravity or ground. The results with respect to emergence of flowers and inflorescence on bearing shoots are given in the table 4.4. Each floral axis was arranged in alternate manner on the flowering shoot. The internodal distance varied from species to species. Internodal distance of floral axis was more on lower portion and went on decreasing on the upper part. Near the tip, this distance was so short that flowers of 2-3 clusters appeared as in one cluster-(Plate 4.3 c).

4.4.2 Flower Bud Development

The observations on flower bud development were recorded from the time of buds started showing growth activity to till the balloon stage was reached. The *Rubus macilentus*

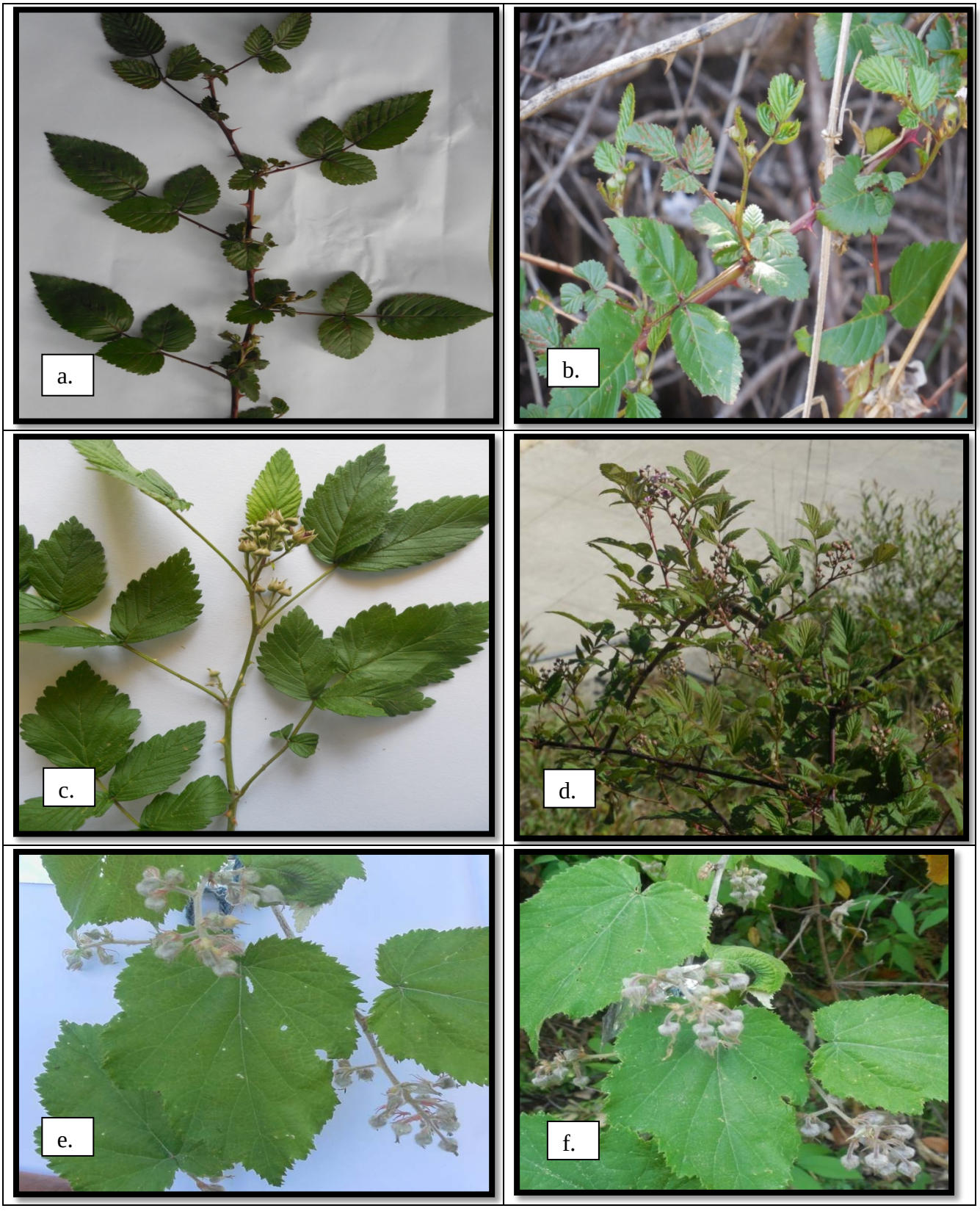


Plate 4.3 **a. b.** Bearing habit of *Rubus macilentus* C.

c. d. Bearing habit of *Rubus niveus* T.

e. f. Bearing habit of *Rubus paniculatus* S.

C. took maximum of 48 days from bud initiation to anthesis followed by *Rubus niveus* T. which took 40 days and minimum of 34 days were taken by *Rubus paniculatus* S. Maximum pre blooming bud size was notice in *Rubus macilentus* C. and *Rubus paniculatus* S. and minimum was recorded in *Rubus niveus* T.

4.4.2. 1 Stages of flower bud development

4.4.2. 1.1 *Rubus macilentus* C.

When buds arose, they were sessile and bracteates. On their maturity, they developed pedicels which were 1 to 1.5 cm long and hairy. Floral buds were arisen along with leaf-buds on lateral as well as on main shoots.

The whole period of bud development, from its appearance upto the full bloom stage, is shown in (Plate 4.4) and is divided into eight stages. The observations on the time required for development of each stage are given in table 4.5 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 0.1 cm. In this stage the pedicel was not prominent and bracts were not clear.

Stage II: In the II stage, 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. There was an appearance of pair of new lateral buds on pedicel of the old bud. In this stage 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 0.54 cm, diameter of the bud was 0.42 cm. Pedicel was prominent attached to the base of the leaf. The lateral buds were half of the size of the axiliary 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 0.66 cm and diameter of the bud was 0.53 cm.

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 0.71 cm and diameter of the bud was 0.62 cm,

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 0.85 cm and diameter of the bud was 0.72cm.

Stage VIII: In the eighth stage of development flower, average, length of the bud was 0.94 cm and diameter of the bud was 0.80 cm. 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.

Plate 4.4 Developmental stage of flower buds in *Rubus macilentus* C.

Table 4.5 Chronology of bud development stages from emergence to full bloom in *Rubus macilentus* C.

Number of buds observed	Days required for passing 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	10.6	9.4	6	4.8	2	1	45.4

4.4.2. 1.1 *Rubus niveus* T.

The whole period of bud development, from its appearance upto the full bloom stage, is shown in (Plate 4.5) and is divided into following six stages of development. . The observations on the time required for development of each stage are given in Table 4.6.

Stage I: The flower buds have just emerged and appeared very small, round, greenish in colour at this stage. The tiny buds were covered with pink tinged calyx lobes completely and tightly. A bract like structure was visible at the base of the bud. The average diameter and length of bud was 0.10 and 0.10 cm.

Stage II: Buds assumed oval shape with increased in size and two sepal tips appeared. Distinctive calyx lobes became visible. The final average length and diameter of the bud was 0.28 cm and 0.25cm. A pair of new lateral buds arrived on pedicel of the old bud and pedicel was prominent and distinguishable.

Stage III: There was further increase in diameter of buds than the length. The calyx lobes became slightly dark green distinguishable with light green edges and four calyx tips became visible. Average length and diameter of the bud was 0.36 cm and 0.34

cm respectively. In this stage pedicel is prominent, the flower bud is still oval in shape with the four prominent calyx tip.

Stage IV: The corolla were visible, the calyx started splitting from top to base. At this stage, sepal lobes became dark green with white distinctive edges and calyx tip became brownish purple. Average length of the bud was 0.47 cm and diameter of the bud was 0.40 cm. The lateral buds were half of the size of the axiliary bud.

Stage V: The pink petals were visible clearly but held tightly inside the calyx cup, the 5 calyx became loosen, Stigmas were visible at the centre of flower bud and stamens were enclosed inside the petals. Average, length of the bud was 0.55 cm and diameter of the bud was 0.53 cm.

Stage VI: The bud was partially opened at this stage. Stamens and stigmas came out of the petals and became clear. The petals became loose inside the calyx. Average length of the bud was 0.58 cm and diameter of the bud was 0.65 cm.

Table 4.6 Chronology of bud development stages from emergence to full bloom in *Rubus niveus* T.

Number of buds observed	**Days required for passing from one stage to next stage**					**Total days required**
	I to II	II to III	III to IV	IV to V	V to VI	
10	14	12	9	4	1	40
10	12	12	10	6	1	41
10	13	11	9	6	1	40
10	14	10	9	5	1	39
10	13	12	10	4	1	40
Average	13.2	11.4	9.4	5	1	40

Plate 4.5 Developmental stage of flower buds in *Rubus niveus* T.

4.4.2. 1.3 *Rubus paniculatus* S.

Floral bud development in the species *Rubus paniculatus* S. also divided in six distinct stages of bud development (Plate 4.6). The observations on the time required for development of each stage are given in Table 4.7.

Stage I: In first stage, the buds looks nearly round in shape and sepal lobes are non distinctive with straw yellow colour. The pedicel was not prominent. The average length and diameter of bud was 0.10 cm.

Stage II: the colour of sepals became dark green and the size of buds increased further. The shape of bud became oval and a calyx tip was visible but the calyx lobes were still indistinctive. The final average length and diameter of the bud was 0.21cm and 0.25cm.

Stage III: there was further increase in diameter of buds than the length. The calyx lobes became slightly dark green and prominently distinguishable with light reddish pink edges. Average length and diameter of the bud was 0.49 cm and 0.46cm respectively.

Stage IV: sepal lobes became dark green with reddish pink streaks at edges and calyx tip became reddish purple. Average length of the bud was 0.69 cm and diameter of the bud was 0.65 cm.

Stage V: All the calyx tips were visible and the size of buds increased further, the calyx lobes are arranged in such a way that the upper three prominent sepal lobes covered the lower two sepals. Stigma and stamens were enclosed inside the buds. Average length of the bud 0.83 cm and diameter of the bud was 0.77 cm.

Stage VI: The bud was assumed balloon shape. Stamens and stigmas were exerting pressure on the calyx lobes as a result calyx lobes became slightly lose hence opening of flower occurred from bottom to tip. The reddish pink colour restricted at edges and lower portion of the buds. The all 5 calyx tips were clearly visible. The petals

became loose inside the calyx. Average length of the bud 0.91cm and diameter of the bud was 0.84cm.

Table 4.7 Chronology of bud development stages from emergence to full bloom in *Rubus paniculatus* S.

Number of buds observed	Days required for passing from one stage to next stage					Total days required
	I to II	II to III	III to IV	IV to V	V to VI	
10	11	9	7	4	1	32
10	10	10	8	5	1	34
10	12	10	9	4	1	36
10	11	10	8	5	1	35
10	11	10	9	6	1	37
Average	11	9.8	8.2	4.8	1	34.8

4.4.2.2 Bud growth behavior of raspberry species

4.4.2.2 .1 *Rubus macilentus* C.

The data presented in figure. 4.1 was taken on alternative days from bud emergence to final size of buds just before opening, indicate that in the initial stages, the buds showed a slow growth rate with respect length and diameter, but after fourth stage of development the bud growth gradually increased and the maxing growth of buds was seen after sixth stage of bud development with maximum bud size of 0.96 x 0.80cm in length and width respectively.

4.4.2.2 .2 *Rubus niveus* T.

The data on growing bud was taken on alternative days to know the growth behavior of buds and presented in the figure. 4.2 indicated that the first three stages of bud development showed a slow growth rate with respect to length and width of buds. The later stages showed a rapid rate of increase in bud sizes with final length of 0.58cm and width of 0.65 cm.

Plate 4.6 Developmental stage of flower buds in *Rubus paniculatus* S.

Figure. 4.1 Flower bud growth from emergence to anthesis *Rubus macilentus* C.

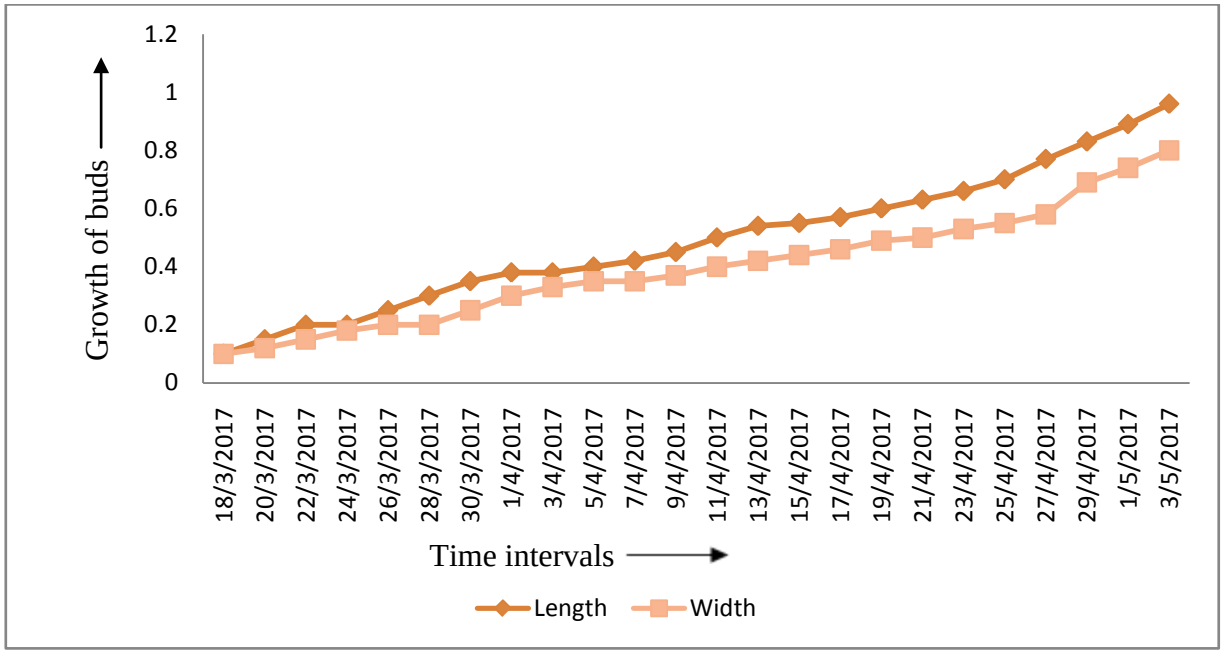
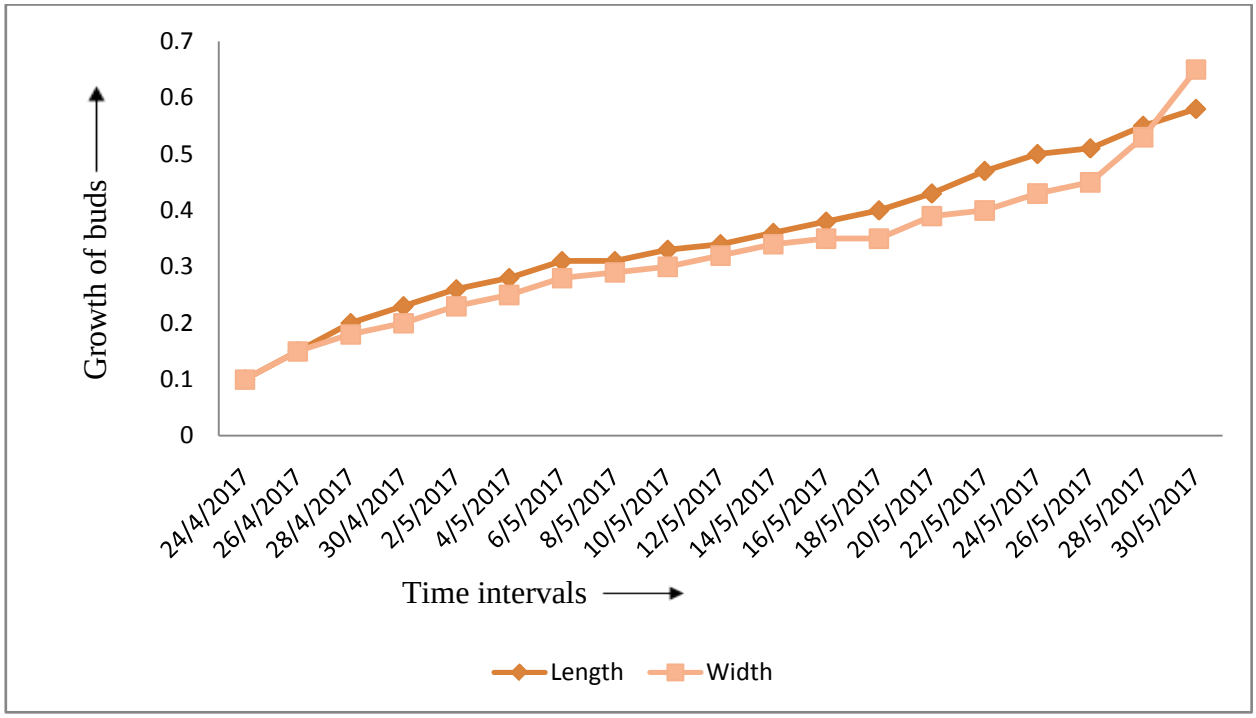


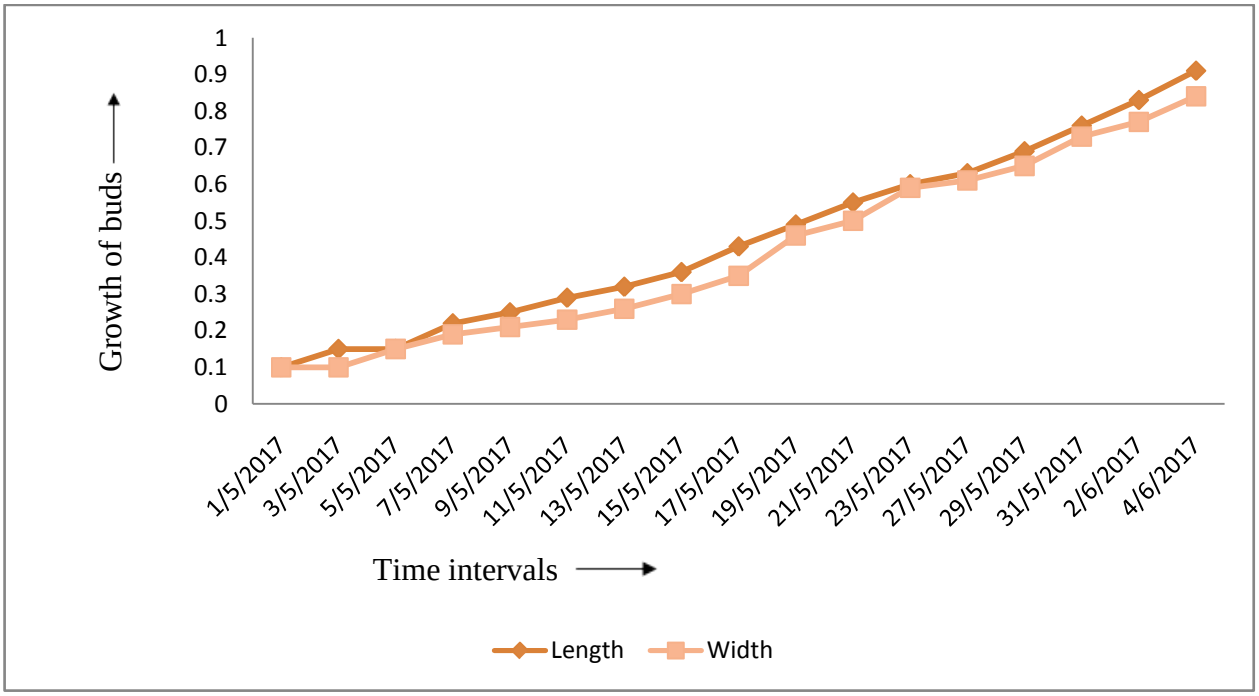
Figure. 4.2 Flower bud growth from emergence to anthesis *Rubus niveus* T.



4.4.2.2 .3 *Rubus paniculatus* S.

The data on buds from emergence to Anthesis was taken on alternative days and presented in the figure. 4.3 to know the number of days taken for Anthesis and bud sizes at different stages of bud growth. Initially bud growth was slow but in later stages it showed a rapid rate of bud development with 0.91 cm length and 0.84 cm width at balloon stage just before anthesis.

Figure. 4.3 Flower bud growth from emergence to anthesis *Rubus paniculatus* S.



4.4.3. Time and duration of flowering

Flowering season of *Rubus macilentus* C. (53 days) was quite longer than other two species. The flowering started from last week of March and continued upto second week of June with its peak in last week of April. While in *Rubus niveus* T. it started from last week of April and continued upto first week of June with its peak in last week of May. The total duration of flowering was recorded 45 days. Whereas in *Rubus paniculatus* S. flowering appeared first week of May and continued upto first week of June with its peak in last week of May. The duration of flowering with its peak periods is shown in Table 4.8

The results indicated that the *Rubus macilentus* C. showed early flowering and long duration (53 days) of flowering, followed by *Rubus niveus* T. for commencement and duration of flowering(45 days), while late and short duration of flowering (40 days) was observed in *Rubus paniculatus* S.

Table 4.8 Time and duration of flowering

SL. No.	Commencement of flowering	Full bloom (about 75% flowers opened)	Number of days required to attain full bloom	End of flowering	Duration of flowering
Rubus macilentus C,	18/3/2017	26/4/2017	30/4/2017	15/6/2017	53
Rubus niveus T.	24/4/2017	17/5/2017	21/5/2017	7/6/2017	45
Rubus paniculatus S.	1/5/2017	22/5/2017	24/5/2017	9/6/2017	40

4.4.4 Flower organization

Freshly opened flowers of raspberry plants were collected for visual observation and size measurement of their parts. The data thus, obtained are presented in Table 4.9.

Flowers of three species under study were regular, complete, perfect, actinomorphic, hermaphrodite or bisexual, hypogynous, petal colour varied from white to pink, bracteate and pedicillate; pedicel was hairy (Fig.64). It was found that abnormal flowers (Plate 4.9) appeared in addition to normal ones in two species of wild raspberry namely *Rubus macilentus* C. *and Rubus niveus* T. Larger flowers were noticed in *Rubus macilentus* C. (0.85 cm length and x2.35 cm width) followed by *Rubus paniculatus* S. (0.70 cm length and x 2.10 cm width) and smallest one was *Rubus niveus* T. (0.74 cm length x 1.10 cm width) the floral parts were explained in following heads.

4.4.4.1 *Rubus macilentus* C.

(i) Bracts: Bracts were formed before the appearance of buds, as the growth of buds and pedicle progressed the bracts remained small, which is a stipule like structure on pedicle.

(ii) Calyx: Five, gamoseplous, free at the terminal end; persistent, lobed, lobes ovate and tomentose with acute apex (Plate 4.7 a). Sepals were green but they also developed light reddish-purple colour at early stages of bud development. Small white, silky hair had covered the calyx as the bud stages progressed the hairiness declined. Average sepal length was1.05 cm and width 0.30cm and longer than petals (Plate 4.7 b).

Sepal abnormality was noticed in the *Rubus macilentus* C. flowers. The flowers with 4 sepals were considered abnormal. Abnormal sepal or sepals thus increased the distance between adjacent sepals (Plate 4.9 a, b, c and d). They were shorter and less broader in size than the normal ones, the abnormal sepals were unequal in sizes and changed shapes (Plate 4.9 a, b and c). Flowers with 4 sepals were smaller in size in comparison to normal flowers.

(iii) Corolla: Five, polypetalous, white in colour sometimes pink tinge can be seen at the edges of the petals, actinomorphic, lobed, long-stalked and obovate having wavy margins. They were broad on the upper portion and tapered downwardly. Average petal length was 1.02 cm and width was 0.63cm. Sometimes they were broader than longer. 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.

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 (Plate 4.9 a, b and c). 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 (Plate 4.9 a, b and c). Abnormal petals also shortened the distance between adjacent

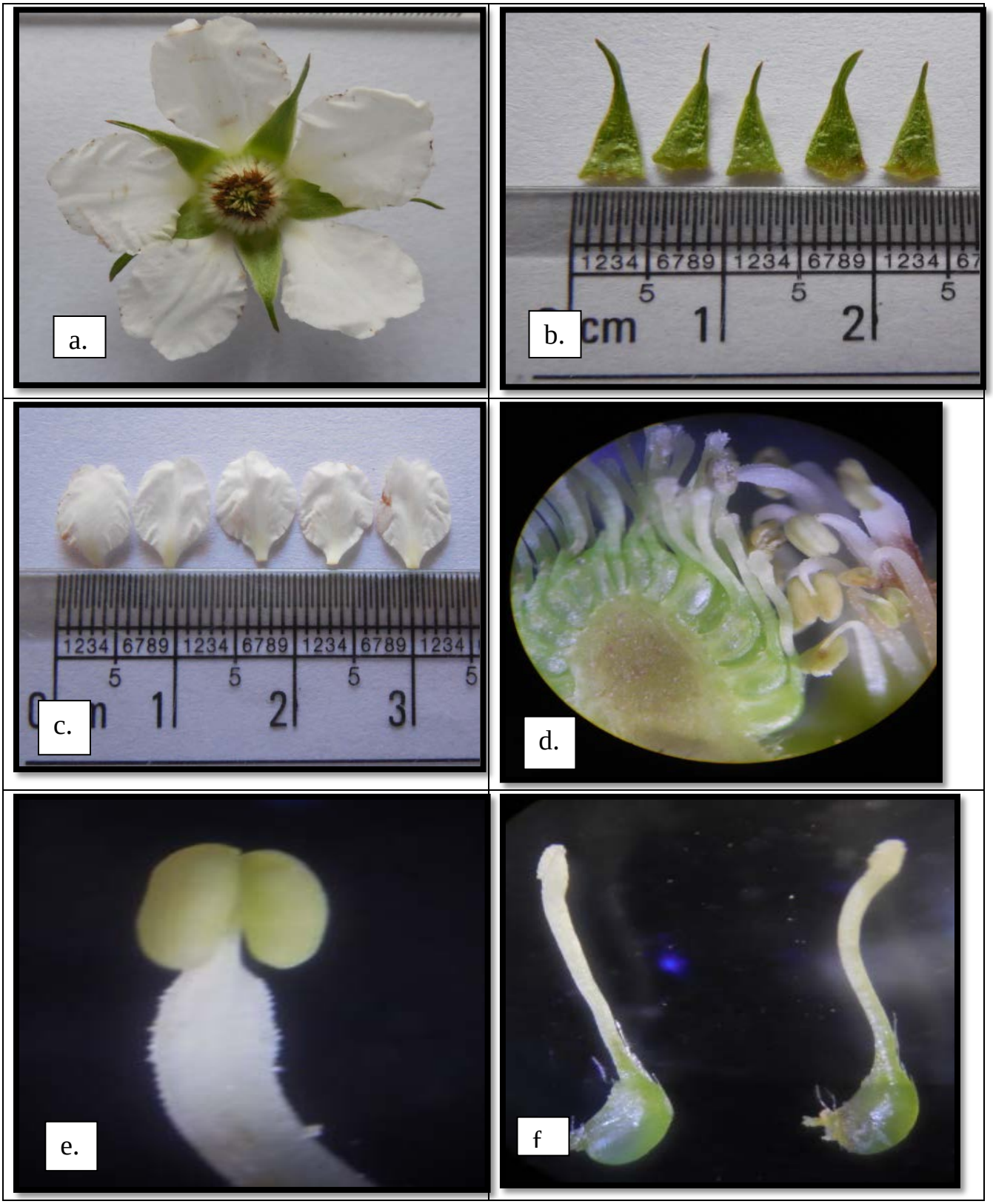


Plate 4.7 Flower organization of *Rubus macilentus* C.

a. Flower **b.** Calyx **c.** Corolla **d.** Arrangement of stamens and pistils

e. Stamen **f.** Pistils

petals and disturbed their alternate arrangement with sepals. It was further observed that the flower with 4 petals were associated with 4 sepals (Plate 4.9 a) having alternate arrangement, while 6 and 7 petals were associated with 5 sepals (Plate 4.9 b and c).

(iv) Androecium: Stamens were numerous, polyandrous, free and arranged in four whorls i.e. one above the other (Plate 4.7 d). Inner most whorl with shortest stamens and outer most with longest stamens (Plate 4.9 e). Anthers were creamish or light yellow in colour, bithecous, dorsifixed and average length of stamens was 0.32cm. Androecium surrounded the gynoecium in a circle. Stamens were observed to be in three different positions (Plate 4.7 b) with respect to stigmas: (i) lying above the stigmas (ii) lying below the stigmas and (iii) lying at the same level with stigmas.

(v) Gynoecium: Ovary was polycarpellary, apocarpous, superior and crowded on a convex receptacle (Plate 4.7 d). Stigmas and style were creamish white with green coloured ovary (Plate 4.7 f). Aveage length of pistil was 0.22 cm.

4.4.4.2 *Rubus niveus* T.

(i) Bracts: When buds appeared, they were bracteate which were green, lanceolate and sometimes developed greenish-purple colour on the outer side. Bracts were larger than buds in their initial stages enclosing the cluster of buds. Each bud had its own bract.

(ii) Calyx: Five, gamoseplous, free at the terminal end; persistent, lobed, lobes ovate and tomentose with acute apex (Plate 4.8 a). The calyx lobes were green to light purplish in colour, the nerves were not seen prominently. Average length and width of the calyx was 0.57 cm and 0.25 cm (Plate 4.8 b).

While normal number of sepal is 5, flowers with 4, 6 and 7 sepals were considered abnormal. Abnormal sepal or sepals were arisen either from the inner side (Plate 4.9 d, e and f) or in between the normal sepals, thus shortening the distance between adjacent sepals. They were shorter and less broader in size than the normal ones, and sometimes transparent and their middle portion was thicker and darker. When abnormal sepal was present, mostly rest of the sepals were of

unequal sizes and changed shapes. Flowers with 4 sepals were smaller in size in comparison to others (Plate 4.9 d).

(iii) Corolla: Five, polypetalous, pink to white coloured, sticky, membranous and transparent when young; actinomorphic, lobed, long-stalked and obovate having wavy margins. They were broad on the upper portion and tapered downwardly (Plate 4.8 c). Petal length was on an average 0.38 cm and width was 0.36 cm. Sometimes they were broader than longer. They were caducous and fell down 1-2 days after dehiscence. They were shorter than sepals having alternate arrangement with them.

Normal number of petals is 5, flowers with 4 and 6 petals were placed under abnormal category of flowers (Plate 4.9 d e and f). Abnormal petals were arisen between the normal petals. They were of the size and shape of the normal petal. Abnormal petals also shortened the distance between adjacent petals and disturbed their alternate arrangement with sepals. It was further observed that in a flower, 4 and 6 petals were mostly associated with 4 and 6 sepals respectively (Plate 4.9 d and e), having alternate arrangement, even sometimes 6 petals associated with 7 sepals (Plate 4.9 f).

(iv) Androecium: stamens were numerous, polyandrous and arranged in single whorl (Plate 4.8 a). Anthers were creamish or light yellow in colour, bithecous with pinkish tinge at the longitudinal grooves of anther (Plate 4.8 e), dorsifixed. Sometimes the stamens with pinkish filaments with white tinge at the tip of the filament were also noticed. Androecium surrounded the gynoecium in a circle (Plate 4.8 a). Stamens were lying above, below and at the same level with stigmas (Plate 4.8 d). Average length of androecium was 0.25 cm.

(v) Gynoecium: Ovary was polycarpellary, apocarpous, superior and crowded on a convex receptacle (Plate 4.8 d). Style was pink coloured thread like and silky with green glossy ovary (Plate 4.8 f). Stigmas were sparsely distributed and creamish white in colour. On an average length of gynoecium was 0.35 cm.

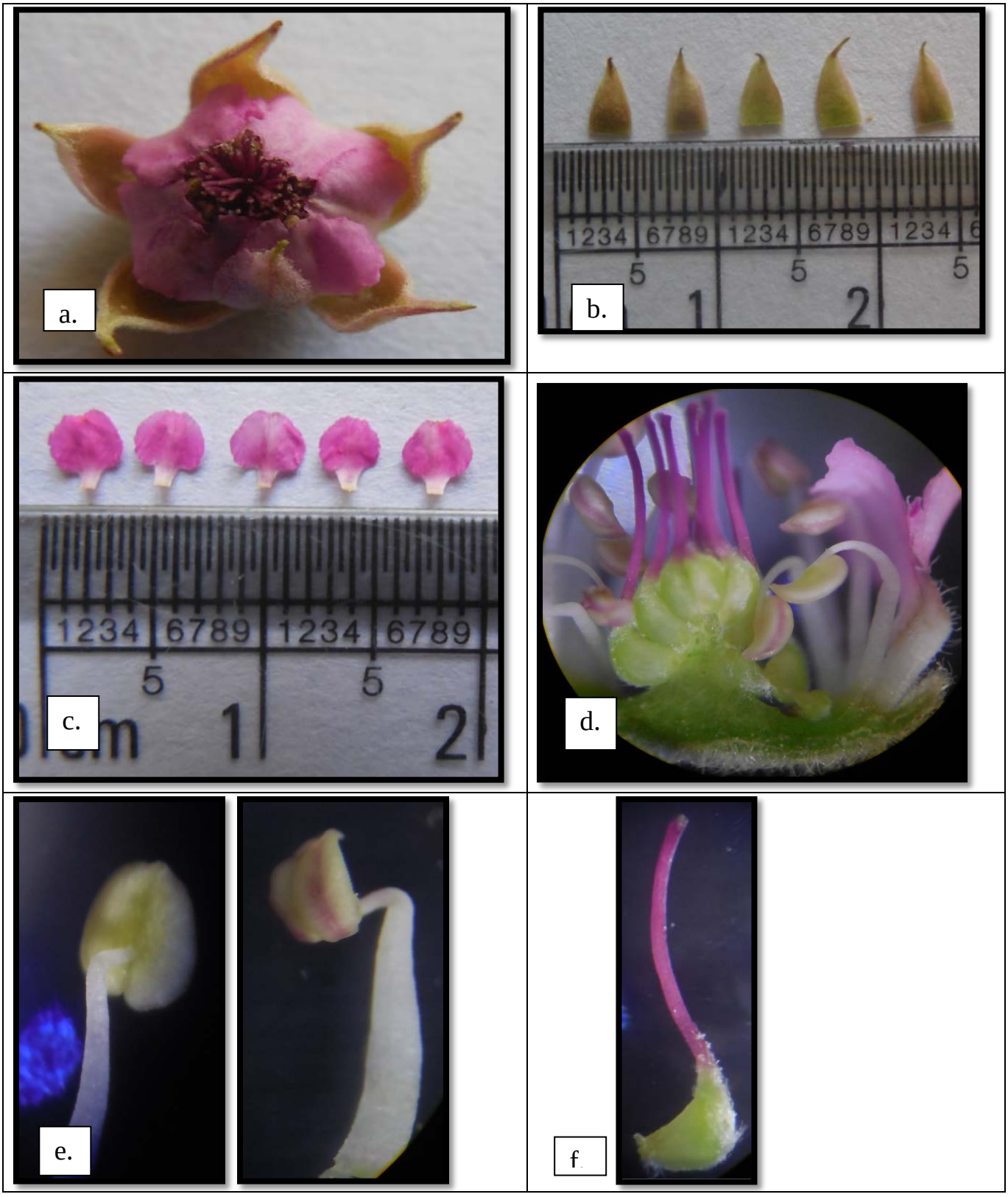


Plate 4.8 Flower organization of *Rubus niveus* T.

a. Flower **b.** Calyx **c.** Corolla **d.** Arrangement of stamens and pistils

e. Stamens **f.** Pistil

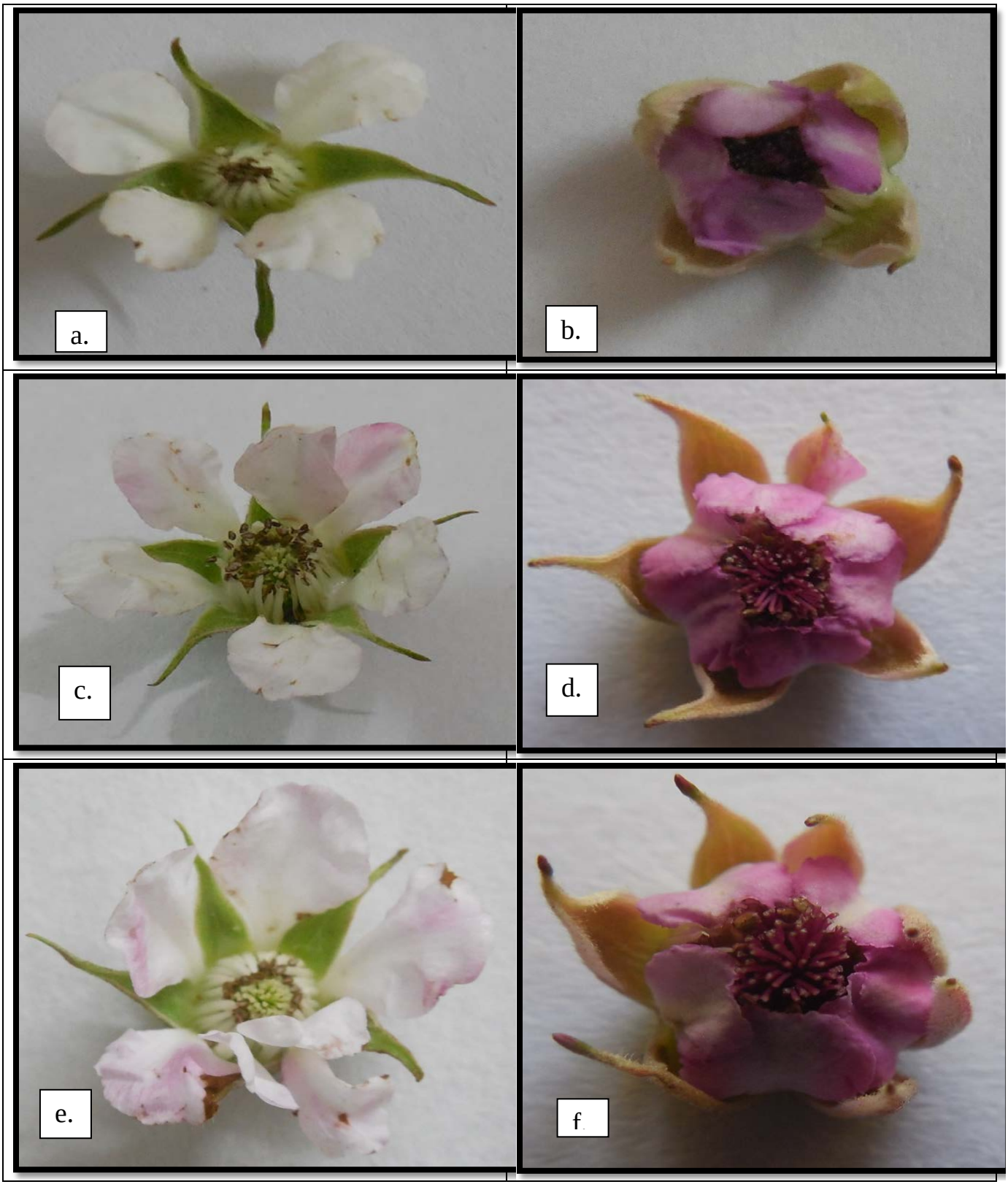


Plate 4.9 Floral abnormalities

***Rubus macilentus* C.** **a.** Flower with 4 sepals and 4 petals **b.** Flower with 5 sepals and 6 petals **c.** Flower with 5 sepals and 7 petals.

***Rubus niveus* T.** **d.** Flower with 4 sepals and 4 petals **e.** Flower with 6 sepals and 6 petals. **f.** Flower with 7 sepals and 5 petals

4.4.4.3 *Rubus paniculatus* S.

(i) Bracts: Buds had bracts which were straw yellow in colour more hairy.

(i) Calyx: Gamosepalous (united at the base) more or less wooly and non glandular or stalked glands and the lobes bent back, valvate and lanceolate, Nerved; central nerve was very prominent, straight and other small nerves were also straight. Sepals were green but they also developed greenish purple colour on inner and outer sides (Plate 4.10 b). Minute, white, silky hair were present on both sides but frequency was more on outer side. Margins were slightly bent inwards. Sepal length was 0.92 cm and width was 0.43 cm.

(iii) Corolla: Five, white in colour, actinomorphic, lobed, long-stalked and obovate. They were broad on the upper portion and tapered downwardly (Plate 4.10 c). On an average the length of petal was 0.42 cm and width was 0.25 cm. The petals were alternatively arranged with the sepals and petals were smaller than the sepals.

(iv) Androecium: Polyandrous, free and arranged in four whorls i.e. one above the other (Plate 4.10 a). Inner most whorl with shortest stamens and outer most with longest stamens. Anthers were larger in size as compared to other two species, had creamish or light yellow in colour, bithecous and 0.54 cm long (Plate 4.10 e). Androecium surrounded the gynoecium freely in a circle (Plate 4.10 a).

(v) Gynoecium: Ovary was polycarpellary, apocarpous, superior and crowded on a convex receptacle (Plate 4.10 d). Stigma was reddish from margin and inside it was white with sunken appearance; style was white with light green coloured ovary. The styles were curvy before the anthesis (Plate 4.10 f); on the day of Anthesis the styles became straight and highly receptive. Average length of gynoecium was 0.52 cm.

Table 4.9 Dimension of different flower parts

Species name	Number of flowers	Size of flowers		Size of pedicle	Corolla		Calyx		Androecium		Gynoecium	
		Length (cm)	Width (cm)	Length (cm)	Number	Size (l×w) cm	Number	Size (l×w) cm	Number	Size(L) cm	Number	Size(L) cm
Rubus macilentus C.	10	0.85	2.35	1.53	5	1.02×0.63	5	1.058×0.306	126.2	0.32	112.6	0.22
Rubus niveus T.	10	0.74	1.1	1.26	5	0.38×0.36	5	0.57×0.25	51.9	0.25	103.9	0.35
Rubus paniculatus S.	10	0.70	2.1	1.35	5	0.42×0.25	5	0.92×0.43	107.25	0.54	28	0.52

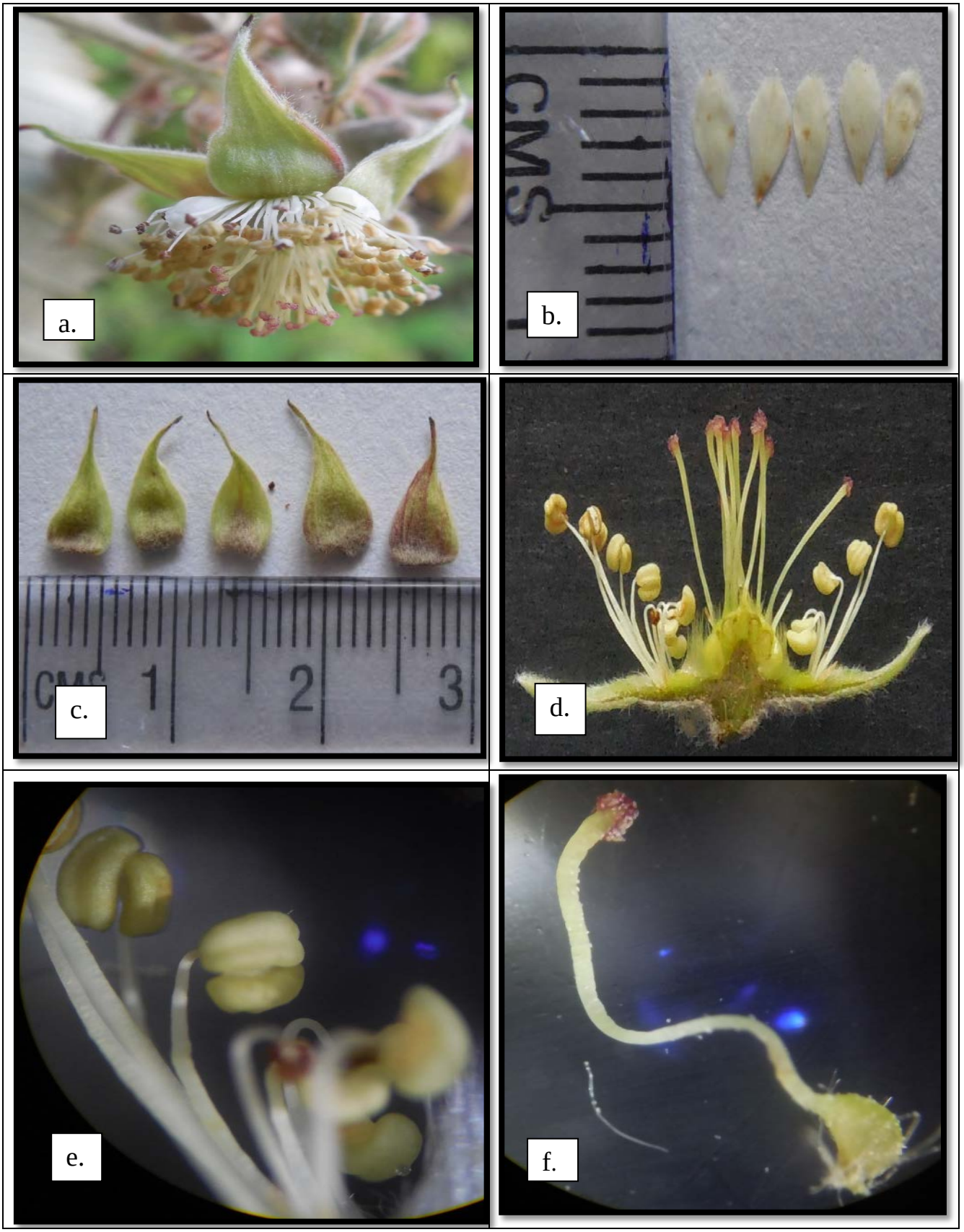


Plate 4.10 Flower organization of *Rubus paniculatus* S.

a. Flower **b.** Corolla **c.** Calyx **d.** Arrangement of stamens and pistils

e. Stamen **f.** Pistils

4.4.5Anthesis

Anthesis was the first opening of flowers. It plays an important role in pollination, fertilization and fruit set in allogamous flowers. Raspberry fruit set depends on amount of cross pollination takes place between the flowers hence bee activity can increase the per cent of fruit set.

4.4.5.1Time of Anthesis

4.4.5.1.1 *Rubus macilentus* C.

The data presented in Table 4.10 indicate that the main period of anthesis varied from 6.00 am to 4.00 pm. The maximum average anthesis (41.43%) was recorded between morning 10 am to 12 noon, followed by 8 am. to 10 am. with 23.71% flower opened 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.

4.4.5.1.2 *Rubus niveus* T.

From the data of table 4.11, it was inferred that the anthesis in *Rubus niveus* T. started between 6 am to 8 am and extended up to 4 pm with the peak period (40.96%) reached between 10 am to 12 noon, followed by the period between 12 noon to 2pm with 26.39%. The antesis of flower buds was found to be lowest (6.82%) between 6 am to 8 am.

4.4.5.1.3 *Rubus paniculatus* S.

As per the observation recorded (Table 4.12) in ***Rubus paniculatus* S.** showed maximum anthesis (33.96%) between 10 am. to 12 noon, followed by 24.94% anthesis occurred from 12 noon to 2 pm. The anthesis period extended from 6 am to 6 pm.The minimum anthesis (0.20%) was observed between 4 pm to 6pm.

Table 4.10: Average time of anthesis of *Rubus macilentus* C.

Date of observation	Total number of flower	Percentage of flowers opened at different time interval						Humidity	Temperature
		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	6.57	23.71	41.43	20.29	8.00	0.06	57.14	14.85

Table 4.11 Average time of anthesis of *Rubus niveus* T.

Date of observation	Total number of flower	Percentage of flowers opened at different time interval						Humidity	Temperature
		6 am - 8am	8 am - 10am	10 am - 12noon	12noon -2pm	2pm- 4pm	4pm- 6pm	Max Min	Max Min
13/5/2017	50	5.64	15.23	38.58	30.23	10.55	0.00	50	18
14/5/2017	50	5.13	16.43	42.73	26.26	9.83	0.00	50	19
15/5/2017	50	7.34	16.47	42.12	24.74	10.12	0.00	40	20
16/5/2017	50	7.95	17.86	40.27	25.86	8.72	0.00	60	19
17/5/2017	50	7.73	15.63	41.76	25.28	9.95	0.00	60	19
18/5/2017	50	7.43	17.32	40.73	24.74	10.75	0.00	50	20
19/5/2017	50	6.53	15.71	40.53	27.64	9.94	0.00	60	19
Average	50	6.82	16.37	40.96	26.39	9.98	0.00	52.85	19.14

Table 4.12 Average time of anthesis of *Rubus paniculatus* S.

Date of observation	Total number of flower	Percentage of flowers opened at different time interval						Humidity Max Min	Temperature Max Min
		6 am - 8am	8 am - 10am	10 am - 12 noon	12 noon-2pm	2pm-4pm	4pm-6pm		
18/5/2017	50	8.49	16.94	31.20	23.16	17.39	0.40	40	19
19/5/2017	50	6.92	18.97	35.57	21.06	12.91	0.40	50	18
20/5/2017	50	9.57	19.14	33.53	25.35	11.91	0.00	40	19
21/5/2017	50	7.91	17.93	35.93	27.74	10.26	0.00	50	18
22/5/2017	50	9.69	17.28	34.71	23.08	11.79	0.62	50	18
23/5/2017	50	11.35	17.16	33.08	26.75	11.73	0.00	40	19
24/5/2017	50	7.39	15.29	33.73	27.43	13.71	0.00	60	18
Average	50	8.76	17.53	33.96	24.94	12.81	0.20	47.14	18.42

4.4.5.2 Mode of Anthesis

4.4.5.2 .1 *Rubus macilentus* C. and *Rubus niveus* T.

Mode of Anthesis of *Rubus macilentus* C. and *Rubus niveus* T. was found to be same, five different stages (Plate 4.11 and 4.12) of anthesis were noticed and are explained below:

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 open. 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.

A typical character of *Rubus niveus* T. is that it takes one and half days for complete opening of flower. At the end of first day (one day before anthesis) of opening of flower it remain in third stage of anthesis and completes fourth and fifth stages on next day (on the day of anthesis) before noon.

4.4.5.2 .2 *Rubus paniculatus* S.

The mode of anthesis was classified in to five different stages (Plate 4.13) which are elaborated below:

Stage I: On the day of anthesis, the buds became balloon shaped and a small split in the bottom of the bud was noticed, probably due to the pressure developed by the stamens and pistils, since they remain curved or pressed inside the buds until the complete Anthesis occur. The sepals were arranged in such a way that outermost first sepal overlaps the inner second and third sepals which further overlap the fourth and fifth sepal. The anthesis occurs from outermost first sepal to inner most fifth sepal.

Stage II: the complete slitting of the calyx can be noticed and the outer most sepal splits open and became free by exposing the stamens and petals were not prominently visible.

3Stage III: there was loosening of second and third sepals thus 25% of the flower was opened by exposing stamens and pistils but they remained curvy and one or small white petals were visible.

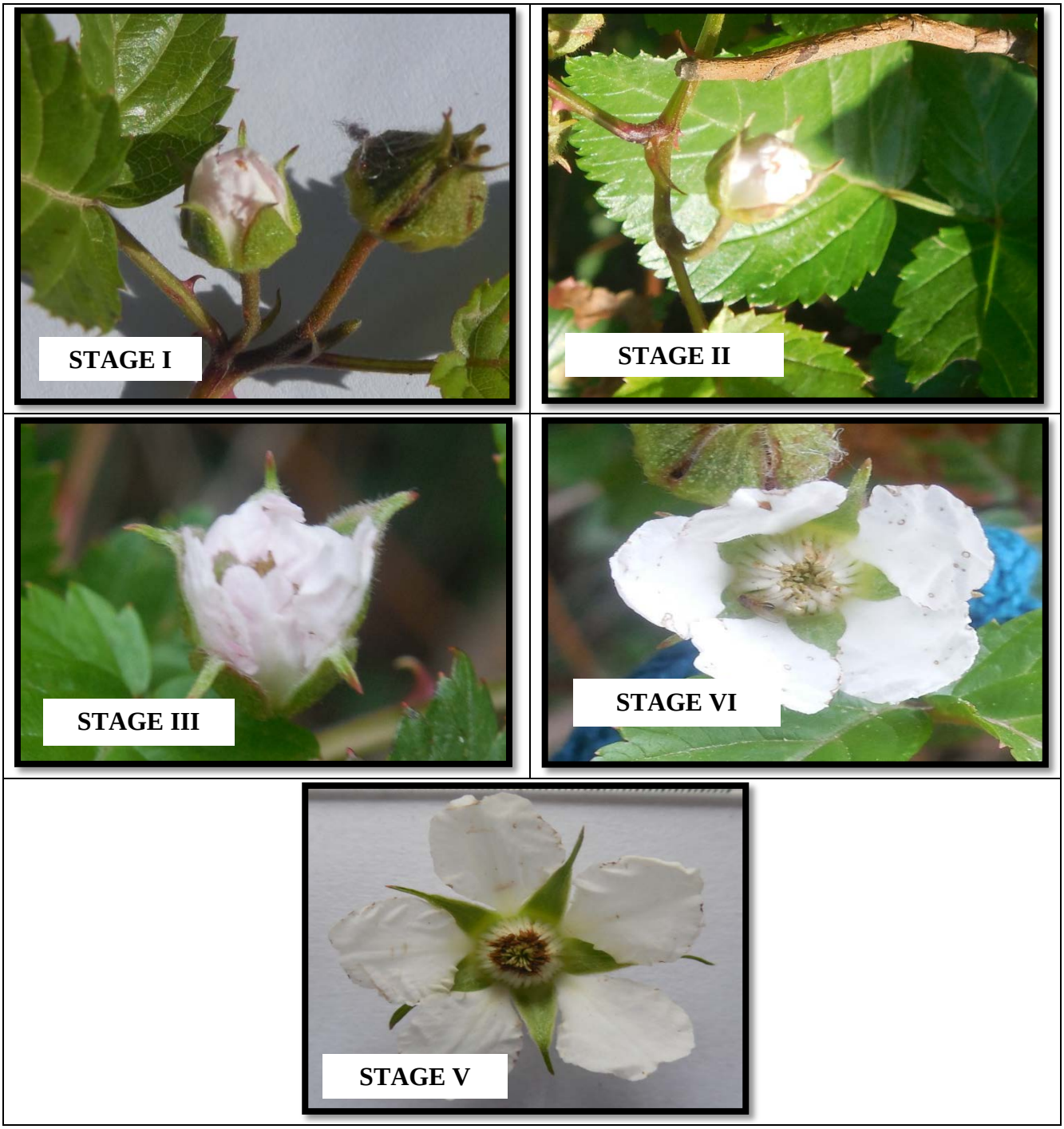


Plate 4.11 Stages of anthesis of *Rubus macilentus* C.

Plate 4.12 Stages of anthesis of *Rubus niveus* T.

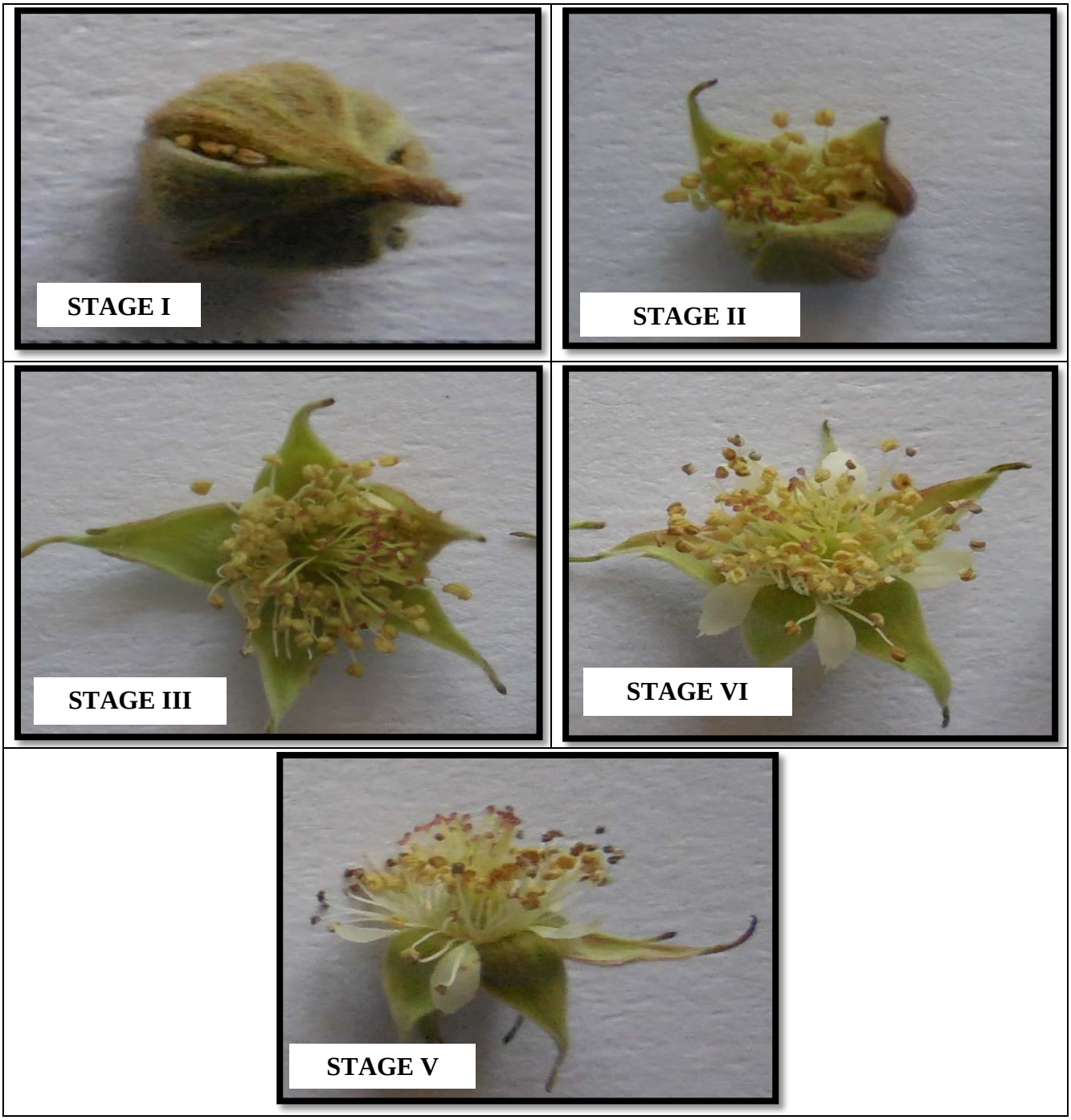


Plate 4.13 Stages of anthesis of *Rubus paniculatus* S.

Stage IV: All the sepals and petals were opened, but the petals remain slightly free and stretched out wards. The stamens and pistils became slightly straight.

Stage V: The complete opening of flower was seen by complete stretching of stemans, pistils, petals and sepals slightly bent or curved towards pedicel.

4.4.6 Anther dehiscence

Anther dehiscence means, the first bursting of anthers to release of pollen grains from the pollen sac. The anther dehiscence takes place after the completion of microsporogenesis. Raspberry plants were allogamous but autogamy was also noticed to some extent.

4.4.6.1 Time of anthers dehiscence

4.4.6.1.1 *Rubus macilentus* C.

From the data as presented in Table 4.13 and Plate 4.14 is seen that 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% of the anther dehiscence was noticed on the day of anthesis, remaining 29.36% was occurred on next day of anthesis.

4.4.6.1. 2 *Rubus niveus* T.

Sometimes, the dehiscence of anthers was noticed even before anthesis with some anthers left undehisced for the following day. It took place 1-2 days before the opening of flowers (protandry) in some flowers. It was observed in most of the cases that dehiscence took place one day after anthesis and started from 6 am and continued upto 4pm (Table 4.14). Maximum (40.85%) dehiscence took place between 10 am to 12noon and it continued for 3-4 hours. Minimum (6.56%) dehiscence was noticed between 6am to 8am data given in Table 4.14.

4.4.6.1. 3 *Rubus paniculatus* S.

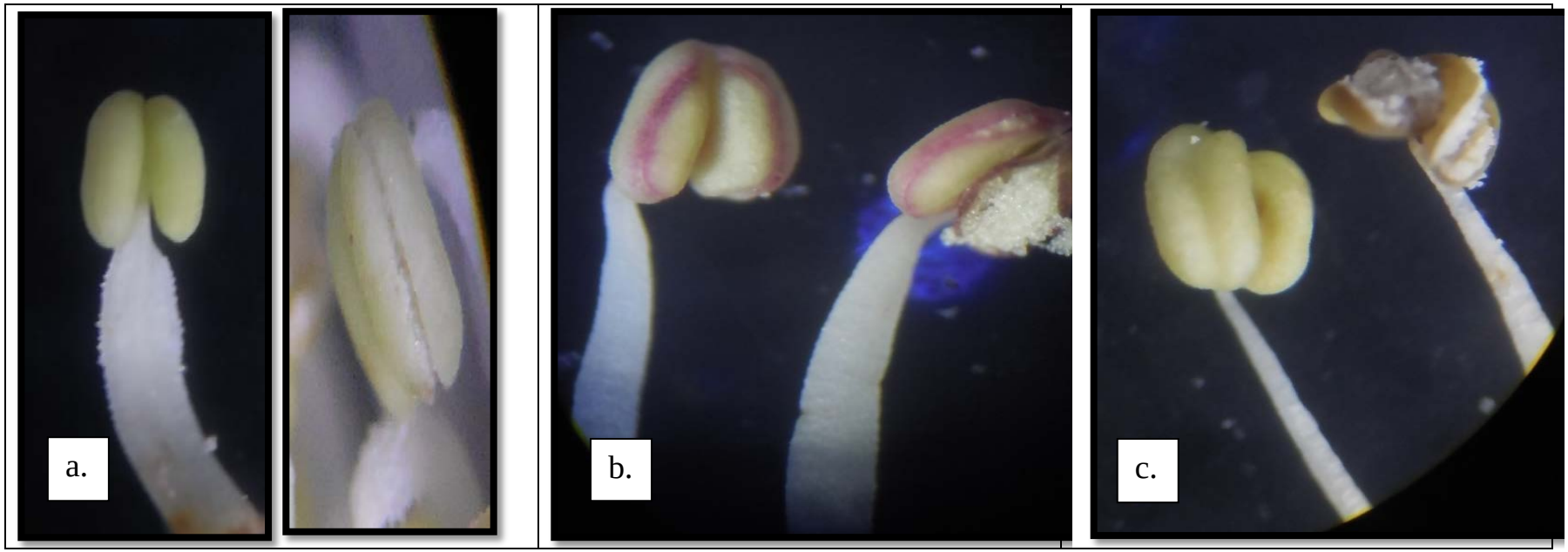
The dehiscence was seen to take place only after the flowers had opened completely. From the data (Table 4.15) it is seen that dehiscence was maximum (28.57%) between 10am to 12 noon, followed by 26.18% of dehiscence was noticed between 12 noon to 2 pm Minimum anther dehiscence was reported between 4pm to 6pm (Table 4.15).

Table 4.13 Time of anthers dehiscence in *Rubus macilentus* C.

Date of observation	Total number of anthers observed	Percentage of anthers dehisced at different time interval						Humidity	Temperature
		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	5.98	12.19	20.29	17.11	10.71	4.36	49.63	14.57

Table 4.14 Time of anthers dehiscence in *Rubus niveus* T.

Date of observation	Total number of anthers observed	Percentage of anthers dehisced at different time interval						Humidity	Temperature
		6 am - 8am	8 am - 10am	10 am - 12noon	12noon - 2pm	2pm- 4pm	4pm- 6pm		
20/5/2017	53	5.66	15.09	37.73	24.53	16.98	0.00	50	18
21/5/2017	51	7.84	17.65	43.14	23.53	13.73	0.00	40	19
22/5/2017	47	6.38	14.89	42.55	25.53	10.64	0.00	50	18
23/5/2017	58	6.90	15.52	39.66	22.41	15.52	0.00	60	18
24/5/2017	51	5.88	13.73	41.18	27.45	11.76	0.00	40	19
25/5/2017	57	7.02	15.79	42.11	22.81	12.28	0.00	50	19
26/5/2017	48	6.25	16.67	39.58	25.00	12.50	0.00	50	19
Average	52.1429	6.56	15.62	40.85	24.47	13.34	0.00	48.57	18.57



***Rubus macilentus* C.** ***Rubus niveus* T.** ***Rubus paniculatus* S.**

Plate 4.14 Mode of anther dehiscence

Table 4.15 Time of anther dehiscence in *Rubus paniculatus* S.

Date of observation	Total number of anthers observed	Percentage of anthers dehisced at different time interval						Humidity	Temperature
		6 am - 8am	8 am - 10am	10am - 12noon	12noon-2pm	2pm-4pm	4pm-6pm		
19/5/2017	101	2.97	8.91	27.72	24.75	8.91	0.39	50	19
20/5/2017	106	3.77	6.60	30.19	28.30	6.60	0.19	60	18
21/5/2017	102	3.92	7.84	28.43	26.47	7.84	0.00	50	19
22/5/2017	115	2.61	8.70	30.43	27.83	6.95	0.00	50	18
23/5/2017	104	3.85	8.65	29.81	27.88	8.65	0.38	50	18
24/5/2017	109	2.75	7.34	27.52	25.69	6.42	0.00	40	19
25/5/2017	112	3.57	6.25	25.89	22.32	8.03	0.00	50	19
Average	107	3.35	7.76	28.57	26.18	7.63	0.14	50	18.57

4.4.6.2 Mode of anther dehiscence

With the opening of flower, petals were spread out first accompanied by stamens. At times, stamens were bent over stigmas. The anthers dehisced in a longitudinal fashion was seen in all three species under study. At the time of opening, they were distinctly creamish yellow in colour, sticky and formed a longitudinal slit on the lobes (Plate 4.14 a, b and c). As the time advanced, they turned brown in colour. The anther dehiscence occurs from outer whorl to inner whorls. Pollens were yellow, dry and powder formed.

4.5 POLLEN STUDIES

4.5.1 Number of pollens per anther

On an average number of pollens per anther and flower in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus* pani*culatus* S. found to be around 900 and 96,525, 1100 and 57,090 and 1260 and 1,59,012 respectively. The highest quantities of pollens are produced by *Rubus*

paniculatus S. followed by *Rubus macilentus* C. and then *Rubus niveus* T. the values are given in Table 4.16.

Table 4.16 Number of pollens per anther and per flower.

Observation	***Rubus macilentus* C.**	***Rubus niveus* T.**	***Rubus paniculatus* S.**
Number of pollens per anther	900±120	1100±98.73	1260±114.93
Number of pollens per flower	96,525	57,090	1,59,012

4.5.2 Morphology of pollen grains in different conditions

Mature anthers were collected from freshly opened flowers which dehisced in sunshine and were examined under microscope. Individual pollen grains were pale yellow in color whereas in mass the pollen grains looked golden yellow in color and dry pollen grains were The shape of pollen grains in glycol and dry conditions were oblong, ellipitic and tricolpate, while in acetocarmine and water pollen grains looked round in shape. The values are given in Table 4.17.

The pollen grains of *Rubus macilentus* C. exhibited the maximum average size (length x width) of 31.29 x 16.12 µ in dry conditions, followed by glycerine (30.74 x 17.68 µ). The minimum size of pollen grains (25.23 µ x 23.51 µ) was recorded under the acetocarmine conditions, followed by water (28.61 x 28.28 µ).

While The maximum average pollen grains size(length x width) of *Rubus niveus* T. was recorded in glycerine, (32.11 µ x 17.02 µ), followed by dry conditions (30.73 µ x 17.28 µ) and the minimum size was reported in acetocarmine (26.01 µ x 25.81 µ), followed by water (30.42 µ x 29.98 µ).

The maximum pollen grains size (length x width) of 35.87 x 17.73 µ was noticed in dry conditions, followed by glycerine (35.44 µ x 16.52 µ). The minimum size of pollen grains

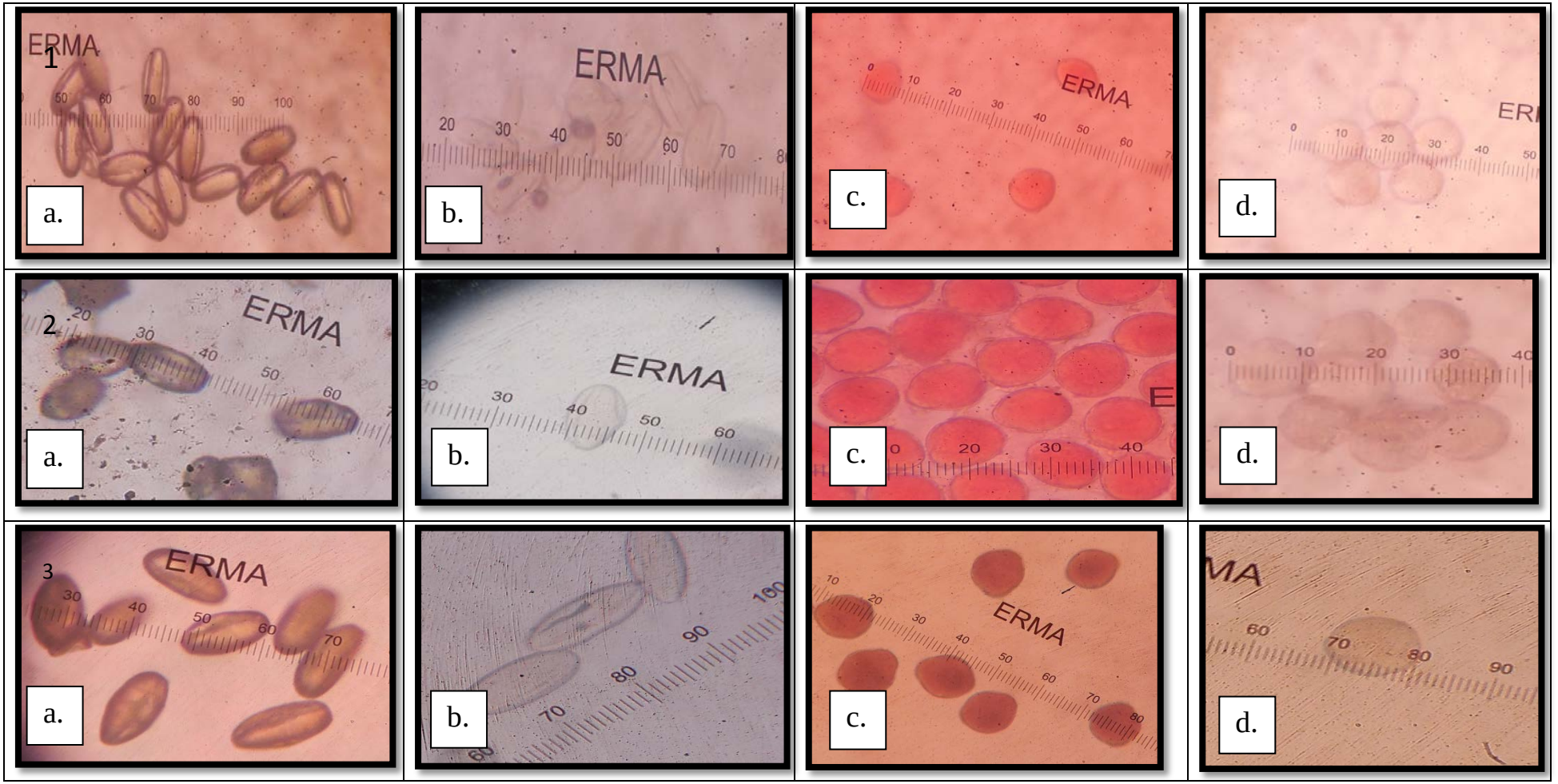


Plate 4.15 Morphology of pollens under different conditions 1. *Rubus macilentus* C. 2. *Rubus niveus* T. 3. *Rubus paniculatus* S. (a. Under dry condition **b.** Glycerine **c.** Acetocarmine **d**.Water)

(27.89 µ x 26.92 µ) was recorded under the acetocarmine conditions, followed by water (29.69 µ x 28.54 µ) in case of *Rubus paniculatus* S.

Table 4.17 Morphology of pollen grains in different conditions

Treatment	***Rubus macilentus* C. pollen ± SE(m)**		***Rubus niveus* T. pollen ± SE(m)**		***Rubus paniculatus* S. pollen ± SE(m)**	
	Length (µ)	**Width (µ)**	**Length (µ)**	**Width (µ)**	**Length (µ)**	**Width (µ)**
T1 - Control (Water)	28.61±0.16	**28.28±0.11**	30.42±0.41	**28.54±0.38**	29.69±0.48	**29.98±0.52**
T2- Glycerine	**30.74±0.19**	17.68±0.24	**32.11±0.32**	17.02±0.35	**35.44±0.27**	16.52±0.39
T3 - Acetocarmine	25.23±0.15	**23.51±0.12**	26.01±0.36	**25.81±0.38**	27.89±0.49	**26.92±0.47**
T4 – Dry condition	**31.29±0.34**	16.12±0.11	**30.73±0.49**	17.28±0.53	**35.87±0.43**	17.73±0.39
SE(d)	0.31	0.22	0.56	0.59	0.60	0.63
C.D.	0.65	0.46	1.19	1.23	1.27	1.33
C.V.	1.87	1.78	3.31	4.58	3.25	4.82

µ - micron (unit of pollen tube length)

4.5.3 Pollen viability and longevity

It was evident from the Table 4.18 that the viability of pollen grains of *Rubus macilentus* C. and *Rubus niveus* T. under study was high. The viability of fresh pollen grain was recorded 100%, while that of *Rubus paniculatus* S. was 73.33% and low viability as compared to other two species under study. The germinability of pollen grains of three wild species under study, stored under room conditions have been presented in Table 4.18.

The results of *Rubus macilentus* C. indicate that after seven days of storage, the viability of the pollen grains totally declained. The sharp decline was seen between third and fifth day of observation.

The raspberry wild species *Rubus niveus* T. showed maximum pollen longevity as compared to other two species under study. From the Table 4.18, we concluded that the pollen viability became nil on ninth day of storage under room condition.

Rubus paniculatus S. showed low viability (73.33%) as compared to other two species under study. The results in table 4.18 indicate that the pollen longevity was lost after seventh day of observation.

Table 4.18 Viability and longevity of pollens

Species	Longevity of pollens at different days intervals					
	1st day	3rd day	5th day	7th day	9th day	11th day
Rubus macilentus C.	100%	94.00%	56.00%	32.66%	0.00%	-
Rubus niveus T.	100%	96.66%	76.66%	38.66%	9.33%	0.00%
Rubus paniculatus S.	73.33%	70.00%	45.33%	16.66%	0.00%	-

4.5.4 Pollen germination

The results indicated that among three wild species of raspberry under study, *Rubus macilentus* C. showed best results for pollen germination and pollen tube length when compared to other two spices, the *Rubus macilentus* C. showed early germination (6 hours) while *Rubus niveus* T. and *Rubus paniculatus* S. showed delayed germination (24 hours), since the observations of pollen germination for *Rubus macilentus* C. were taken at 6, 18, 24 and 48 hours whereas for *Rubus niveus* T. and *Rubus paniculatus* S. the observations were made at 24, 48 and 72 hours after planting of pollen grains in artificial media of sucrose, boric acid and gibberellic acid at different concentration combinations and water served as control.

4.5.4.1 *Rubus macilentus* C.

The pollen germination in *Rubus macilentus* C. starts from 6 hours after planting and continued upto 48 hours (Table 4.19). The maximum pollen germination (90.54%) and pollen tube length (1,164.19 µ) was recorded 48 hours after planting, followed by 24 hours after planting with the pollen germination of 88.84% and pollen tube length of 1,084.61 µ, while the minimum pollen germination and pollen tube length (12.63% and 42.17µ) was recorded 6 hours after planting of pollen grains.

Among all the treatments T_{13} (10% Sucrose + 2ppm Gibberellic acid) and T_{12} (5% Sucrose + 1ppm Gibberellic acid) ranks first with maximum pollen germination (90.54%) and pollen tube length (1,164.19µ), followed by T_4 (0.3% Boric acid + 15% Sucrose) and T_{13} (10% Sucrose + 2ppm Gibberellic acid) with pollen germination (87.63%) and pollen tube length (1,051.85µ), while the minimum value (pollen germination 68.11% and pollen tube length 92.27µ) was recorded after 6 hours of pollen planting with T_{13} (10% Sucrose + 2ppm Gibberellic acid) and T_{12} (5% Sucrose + 1ppm Gibberellic acid).

The data on pollen germination and pollen tube length after 6 hours of planting in raspberry (*Rubus macilentus* C.) is presented in Table 4.19 and 4.20. the data revealed that all treatments for pollen germination were significant and for pollen tube length all treatments are significant except T_6 (0.5% Boric acid + 25% Sucrose) when compared to control (water).The maximum pollen germination percentage (68.11%) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid). The minimum pollen germination percentage (12.63%) was recorded with T_1 (Water). The maximum pollen tube length (92.277 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which were statistically at par with treatment T_{13} and T_{14} recording 90.41 µ and 85.15 µ respectively. Minimum pollen tube length (42.17µ) was recorded T_1 (Water).

After 18hours of pollen planting the results (Table 4.19 and 4.20) recorded indicated that all the treatments were significant for pollen germination, the treatment T_2 was not significant for pollen tube length as compared to water. Maximum pollen germination percentage (85.71%) was recorded with T_4 (0.3% Boric acid + 15% Sucrose) which was statistically at par with treatment T_{12} recording (85.24). The minimum pollen germination

percentage (45.83%) was recorded with T_1 (Water). The maximum pollen tube length (244.74 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which were statistically at par with treatment T_{13} (234.89 µ). Minimum pollen tube length (68.04 µ) was recorded T_1 (Water) which was statistically at par with treatment T_2 (78.86 µ)

The Table 4.19 and 4.20 indicated that after 24 hours of germination the treatments T_2, T_6, T_8 and T_{11} were non-significant for pollen germination and remaining treatments were significant, all the treatments for pollen tube length were significant when compared to control (water). Pollen germination percentage (88.84%) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid) was recorded to be maximum and the minimum pollen germination percentage (42.73%) was recorded with T_7 (0.1% Boric acid + 1ppm Gibberellic acid). The maximum pollen tube length (1,084.61 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid). Minimum pollen tube length (98.52 µ) was recorded T_1 (Water).

The results obtained after 48 hours of planting of pollen grains for germination (Table 4.19 and 4.20) indicated that there was no significant difference among the treatments T2, T5, T6, T7, T8, T9.T10 and T11, the treatments T3, T4, T12, T13, T14, T15 and T16 showed significant differences when compared to control. The maximum pollen germination percentage (90.54%) (Plate 4.16) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid). The minimum pollen germination percentage (40.49%) was recorded with T_6 (0.5% Boric acid + 25% Sucrose) which was statistically at par with treatment T_7 (40.64 µ). The maximum pollen tube length (1,164.19 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which were statistically at par with treatment T_{13} and T_{14} recording 90.41 µ and 85.16 µ respectively. Minimum pollen tube length (77.46 µ) was recorded T_1 (Water).

4.5.4.2 *Rubus niveus* T.

The observations (Table 4.21 and 4.22) on pollen germination of *Rubus niveus* T. were recorded after 24, 48 and 72 hours after planting and the maximum pollen germination (62.96%) and pollen tube length (214.98 µ) was recorded after 72 hours after planting with the treatments T_{15}, followed by 48 hours after planting of pollens resulted in maximum pollen germination of 54.16% and pollen tube length of 201.25 µ with treatment T_{15} was recorded. Among the data recorded on pollen germination at 24 hours after planting the maximum

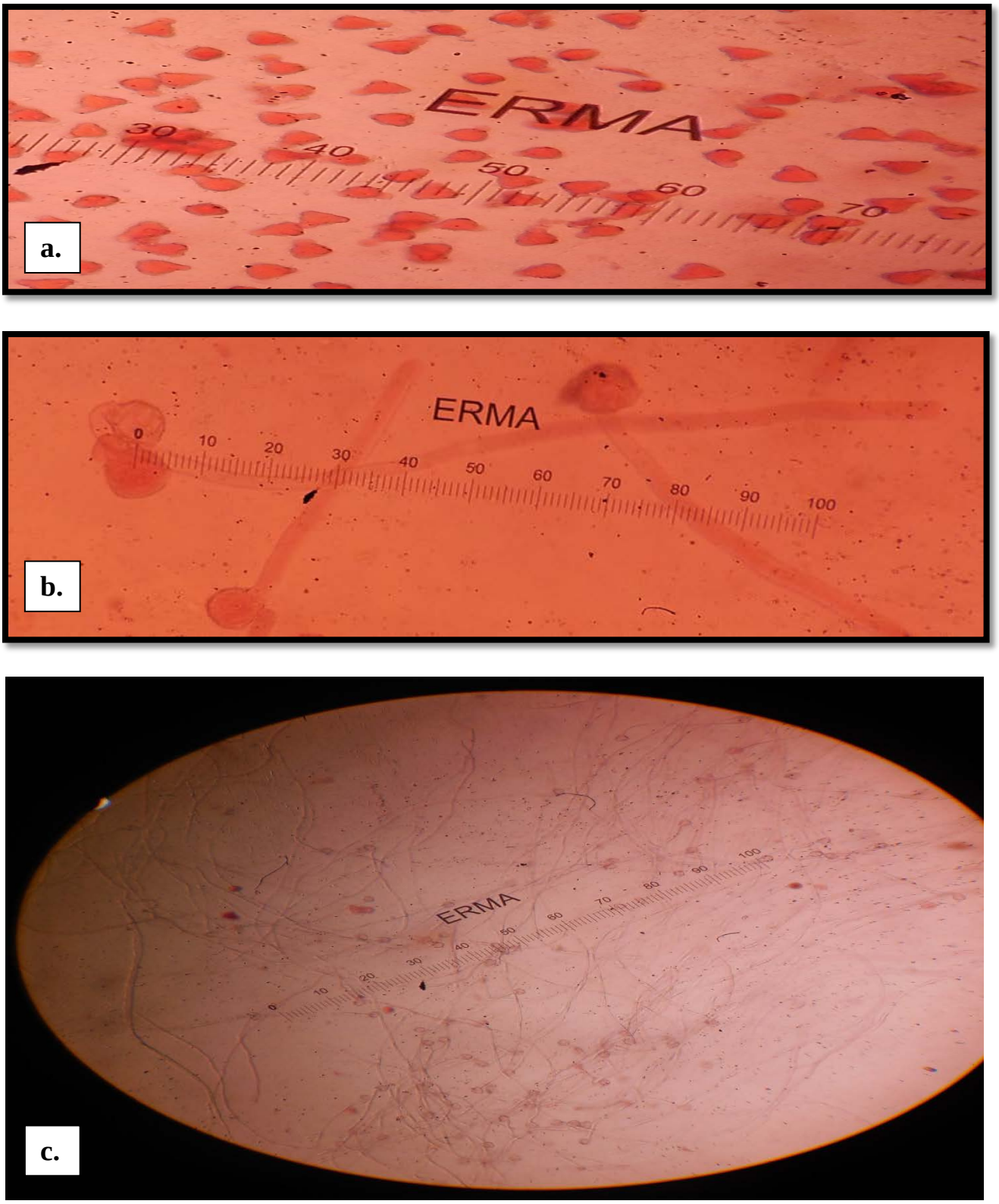


Plate 4.16 Pollen germination : **a**. Initiation of pollen grain germination **b.** Germinated pollen grain with pollen tube **c.** 90% pollen germination

pollen germination (20.06%) and pollen tube length (102.16µ) was observed with the treatment T_{15}. The data on pollen germination and pollen tube length in raspberry (*Rubus niveus* T.) is presented in Table 4.21 and 4.22. It was observed that all the treatments at different intervals of time (24, 48 and 72 hours) were significant for pollen germination and pollen tube length when compared to control (water).

The data (Table 4.21 and 4.22) revealed that the maximum pollen germination percentage (20.06%) was recorded with T_{15} (20% Sucrose + 4ppm Gibberellic acid) after 24 hours of planting, which was statistically at par with treatment T_6 (19.97%). The minimum pollen germination percentage (16.31%) was recorded with T_2 and no germination was seen in T_1 control (Water). The maximum pollen tube length (102.16µ) was recorded with T_{15} (20% Sucrose + 4ppm Gibberellic acid) which was statistically at par with treatment $T_{16,}$ T_{10} and T_{14} recording 98.19 µ, 90.203 µ and 93.59 µ respectively. Minimum pollen tube length (37.17µ) was recorded T_2.

The observations taken on pollen germination after 48 hours data on pollen germination and pollen tube length in raspberry (*Rubus niveus* T.) is presented in Table 4.21 and 4.22 The data revealed that the maximum pollen germination percentage (54.16 %) was recorded with T_{15} (20% Sucrose + 4ppm Gibberellic acid). The minimum pollen germination percentage (27.76%) was recorded with T_2 and no germination was seen in T_1 control (Water). The maximum pollen tube length (201.25 µ) was recorded with T_{15} (20% Sucrose + 4ppm Gibberellic acid) which were statistically at par with treatment T_{16} (200.54 µ). Minimum pollen tube length (16.63 µ) was recorded T_1 (Water).

The results presented in the Table 4.21 and 4.22 indicated that after 72 hours of pollen incubation the maximum pollen germination percentage (62.96 %) was recorded T_{15} (20% Sucrose + 4ppm Gibberellic acid). The minimum pollen germination percentage (6.94 %) was recorded with T_1 (Water). The maximum pollen tube length (214.97µ) was recorded with T_{15} (20% Sucrose + 4ppm Gibberellic acid) which was statistically at par with treatment T_{16} and T_{14} (209.273 µ) and (205.77 µ) respectively. Minimum pollen tube length (25.22 µ) was recorded in T_1 (Water).

4.5.4.3 *Rubus paniculatus* S.

The observations on pollen germination of *Rubus paniculatus* S. were recorded after 24, 48 and 72 hours after planting. The maximum pollen germination (52.34%) and pollen tube length (135.22 µ) was recorded after 72 hours after planting with the treatment T_{16}, followed by 48 hours after planting of pollens resulted in maximum pollen germination of 37.82% pollen tube length of 99.18 µ with treatment T_{16} was recorded. Among the data recorded on pollen germination at 24 hours after planting the maximum pollen germination (9.66%) and pollen tube length (88.05µ) was observed with the treatment T_{16}. The data on pollen germination and pollen tube length in raspberry (*Rubus niveus* T.) is presented in Table 4.23 and 4.24. It was observed that all the treatments at different intervals of time (24, 48 and 72 hours) were significant for pollen germination and pollen tube length when compared to control (water).

The data on pollen germination and pollen tube length in raspberry (*Rubus paniculatus* S.) is presented in Table 4.23 and 4.24. The data revealed that after 24 hours of pollen planting, the maximum pollen germination percentage (9.66%) was recorded with T_{16} 25% (Sucrose + 5ppm Gibberellic acid). The minimum pollen germination percentage (5.35 %) recorded with T_2 and no germination was seen in T_1 control (Water). The maximum pollen tube length (88.05 µ) was recorded with T_{16} (25% Sucrose + 5ppm Gibberellic acid) which was statistically at par with treatment $T_{6,}$ $T_{9,}$ $T_{10,}$ T_{11} and T_{15} recording 81.57 µ, 75.43 µ, 80.35 µ, 77.31 µ and 76.22 µ respectively. Minimum pollen tube length (0 µ) was recorded T_1- Water (Control).

The data on pollen germination and pollen tube length in raspberry (*Rubus paniculatus* S.) taken after 48 hours of pollen planting is presented in Table 4.23 and 4.24. The data revealed that the maximum pollen germination percentage (37.82 %) was recorded with T_{16} 25% (Sucrose + 5ppm Gibberellic acid). The minimum pollen germination percentage (3.05%) was recorded with T_1 (Water). The maximum pollen tube length (99.18 µ) was recorded with T_{16} (25% Sucrose + 5ppm Gibberellic acid) which was statistically at par with treatment $T_{11,}$ $T_{14,}$ $T_{15,}$ T_6 and T_{10} recording 89.37 µ, 89.26 µ, 90.05 µ, 88.21 µ and 88.61 µ respectively. Minimum pollen tube length (0 µ) was recorded T_1 (Water).

The data on pollen germination and pollen tube length in raspberry (*Rubus paniculatus* S.) was observed after 72 hours of pollen incubation and the results are presented in Table 4.23 and 4.24. The data revealed that the maximum pollen germination percentage (52.34 %) was recorded with T_{16} 25% (Sucrose + 5ppm Gibberellic acid). The minimum pollen germination percentage (9.68 %) was recorded with T_1 (Water). The maximum pollen tube length (135.22µ) was recorded with T_{16} (25% Sucrose + 5ppm Gibberellic acid) which was statistically at par with treatment T_{14} (124.38 µ). Minimum pollen tube length (14.18µ) was recorded T_1 (Water).

Table 4.19 Pollen germination percentage of *Rubus macilentus* C. after different time of incubation.

Treatments	Pollen germination (%)±SE(m) (6hours)	Pollen germination (%)±SE(m) (18hours)	Pollen germination (%)±SE(m) (24hours)	Pollen germination (%)±SE(m) (48hours)
T1- Water(Control)	12.63±0.27	45.83±0.27	64.41±0.21	73.76±0.18
T2- 0.1% Boric acid + 5% Sucrose	35.43±0.33	68.29±0.24	46.57±0.23	46.45±0.22
T3 - 0.2% Boric acid + 10% Sucrose	46.69±0.40	81.81±0.25	85.68±0.19	86.68±0.18
T4- 0.3% Boric acid + 15% Sucrose	41.53±0.27	85.71±0.26	86.2±0.17	87.63±0.21
T5- 0.4% Boric acid + 20% Sucrose	37.82±0.26	83.87±0.15	78.75±0.11	73.78±0.18
T6 - 0.5% Boric acid + 25% Sucrose	37.06±0.10	77.43±0.26	62.75±0.15	40.49±0.16
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	24.53±0.29	76.12±0.18	42.73±0.16	40.64±0.22
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	41.19±0.21	61.17±0.13	62.48±0.24	64.86±0.14
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	58.54±0.31	66.92±0.53	68.11±0.09	69.97±0.13
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	52.91±0.14	61.85±0.12	56.33±0.11	53.48±0.23
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	34.73±0.32	55.12±0.40	49.35±0.12	49.13±0.12
T12 - 5% Sucrose + 1ppm Gibberellic acid	56.82±0.29	85.24±0.17	85.72±0.13	87.12±0.12
T13 - 10% Sucrose + 2ppm Gibberellic acid	68.11±0.11	80.71±0.26	88.84±0.11	90.54±0.15
T14 - 15% Sucrose + 3ppm Gibberellic acid	48.71±0.16	71.33±0.12	76.57±0.24	79.67±0.23
T 15 - 20% Sucrose + 4ppm Gibberellic acid	43.75±0.27	67.87±0.14	72.88±0.17	75.79±0.21
T16 - 25% Sucrose + 5ppm Gibberellic acid	40.14±0.29	64.87±0.14	72.89±0.14	73.77±0.14
SE(d)	0.38	0.36	0.24	0.26
$CD_{0.05}$	0.77	0.73	0.49	0.52
C.V.	1.09	0.62	0.42	0.46

Table 4.20 Pollen tube length of *Rubus macilentus* C. after different time of incubation.

Treatments	Pollen tube length (µ)±SE(m) (6hours)	Pollen tube length (µ)±SE(m) (18hours)	Pollen tube length (µ)±SE(m) (24hours)	Pollen tube length (µ)±SE(m) (48hours)
T1- Water(Control)	42.17 ± 3.33	68.04 ± 3.03	98.52 ± 0.65	77.463 ± 12.68
T2- 0.1% Boric acid + 5% Sucrose	51.53±2.66	78.857 ± 3.04	405.587 ± 4.14	380.12 ± 8.65
T3 - 0.2% Boric acid + 10% Sucrose	60.66 ± 1.63	150.123 ± 7.53	419.72 ± 2.32	404.5 ± 6.27
T4- 0.3% Boric acid + 15% Sucrose	55.61 ± 2.16	126.19 ± 5.60	516.263 ± 1.36	532.667 ± 6.76
T5- 0.4% Boric acid + 20% Sucrose	51.47 ± 3.23	117.493 ± 6.44	487.843 ± 2.64	531.013 ± 16.25
T6 - 0.5% Boric acid + 25% Sucrose	46.427 ± 1.69	114.52 ± 6.54	437.493 ± 2.93	410.497 ± 6.69
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	57.207± 2.35	94.527 ± 6.66	615.503 ± 2.62	785.49 ± 7.43
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	64.147 ± 2.28	101.14 ± 10.10	691.49 ± 3.32	806.097 ± 1.93
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	70.257 ± 3.33	157.49 ± 4.69	816.253 ± 3.71	926.023 ± 9.65
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	76.443 ± 1.69	137.203 ± 6.38	729.867 ± 4.63	861.057 ± 11.14
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	70.28 ± 2.77	164.73 ± 5.29	785.487 ± 5.81	921.83 ± 10.14
T12 - 5% Sucrose + 1ppm Gibberellic acid	92.277 ± 3.32	244.74 ± 3.89	1,084.61 ± 7.68	1,164.19 ± 14.77
T13 - 10% Sucrose + 2ppm Gibberellic acid	90.413 ± 2.70	234.893 ± 8.86	994.97 ± 4.06	1,051.85 ± 10.67
T14 - 15% Sucrose + 3ppm Gibberellic acid	85.157 ± 3.28	218.07 ± 4.65	913.74 ± 1.76	928.43 ± 6.49
T 15 - 20% Sucrose + 4ppm Gibberellic acid	81.457 ± 3.23	215.307 ± 6.43	707.76 ± 3.43	825.223 ± 6.41
T16 - 25% Sucrose + 5ppm Gibberellic acid	75.963 ± 2.93	206.43 ± 5.01	591.363 ± 3.40	795.803 ± 5.99
SE(d)	3.86	8.73	5.35	13.51
$CD_{0.05}$	7.89	17.87	10.96	27.65
C.V.	7.06	7.04	1.01	2.32

µ - micron (unit of pollen tube length)

Table 4.21 Pollen germination percentage of *Rubus niveus* T. after different time of incubation.

Treatments	Pollen germination (%)±SE(m) (24hours)	Pollen germination (%)±SE(m) (48hours)	Pollen germination (%)±SE(m) (72hours)
T1- Water(Control)	0.00±0.00	0.00±0.00	6.94±0.05
T2- 0.1% Boric acid + 5% Sucrose	16.31±0.09	27.76±0.07	35.46±0.07
T3 - 0.2% Boric acid + 10% Sucrose	16.95±0.04	33.93±0.06	39.84±0.05
T4- 0.3% Boric acid + 15% Sucrose	17.23±0.10	38.83±0.05	42.74±0.07
T5- 0.4% Boric acid + 20% Sucrose	17.96±0.08	44.83±0.05	53.93±0.05
T6 - 0.5% Boric acid + 25% Sucrose	19.97±0.07	48.73±0.07	62.75±0.05
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	16.72±0.10	30.94±0.06	36.86±0.07
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	17.25±0.05	35.75±0.07	40.87±0.05
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	18.03±0.06	39.22±0.09	46.94±0.05
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	18.94±0.07	46.77±0.06	54.85±0.06
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	17.80±0.09	43.04±0.06	50.03±0.06
T12 - 5% Sucrose + 1ppm Gibberellic acid	17.53±0.08	34.15±0.07	37.93±0.05
T13 - 10% Sucrose + 2ppm Gibberellic acid	17.87±0.04	40.14±0.07	43.76±0.05
T14 - 15% Sucrose + 3ppm Gibberellic acid	18.62±0.09	44.94±0.05	49.04±0.06
T 15 - 20% Sucrose + 4ppm Gibberellic acid	20.06±0.06	54.16±0.06	62.96±0.05
T16 - 25% Sucrose + 5ppm Gibberellic acid	18.98±0.09	47.04±0.07	44.06±0.08
SE(d)	0.11	0.09	0.08
$CD_{0.05}$	0.22	0.18	0.17
C.V.	0.79	0.28	0.23

Table 4.22 Pollen tube length of *Rubus niveus* T. after different time of incubation

Treatments	Pollen tube length (µ)±SE(m) (24hours)	Pollen tube length (µ)±SE(m) (48hours)	Pollen tube length (µ)±SE(m) (72hours)
T1- Water(Control)	0.00±0.00	16.63±4.59	25.22±3.86
T2- 0.1% Boric acid + 5% Sucrose	37.17± 4.32	60.31±1.75	69.48±5.09
T3 - 0.2% Boric acid + 10% Sucrose	51.52 ± 2.57	71.45±3.74	80.92±3.61
T4- 0.3% Boric acid + 15% Sucrose	59.83 ± 4.50	78.58±4.67	92.09±7.38
T5- 0.4% Boric acid + 20% Sucrose	72.42 ± 3.61	81.08±4.33	100.52±4.68
T6 - 0.5% Boric acid + 25% Sucrose	61.20 ± 3.31	70.97±4.38	63.68±4.09
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	77.29 ± 3.26	90.16±4.28	118.05±5.90
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	84.31 ± 5.26	93.77±5.06	110.98±8.01
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	82.43 ± 2.30	101.86±5.68	130.59±6.33
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	90.20 ± 4.26	108.78±2.96	142.89±5.59
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	59.88 ± 4.97	116.21±3.09	153.21±8.33
T12 - 5% Sucrose + 1ppm Gibberellic acid	82.77 ± 3.04	153.84±5.04	171.4±8.60
T13 - 10% Sucrose + 2ppm Gibberellic acid	87.85 ± 5.44	162.20±5.86	189.47±5.78
T14 - 15% Sucrose + 3ppm Gibberellic acid	93.59 ± 5.07	186.30±6.71	205.77±9.12
T 15 - 20% Sucrose + 4ppm Gibberellic acid	102.16 ± 7.33	201.25±2.36	214.98±11.94
T16 - 25% Sucrose + 5ppm Gibberellic acid	98.19 ± 7.32	200.54±6.64	209.27±9.38
SE(d)	6.40	6.59	10.05
$CD_{0.05}$	13.1	13.48	20.56
C.V.	10.99	7.19	9.47

µ - micron (unit of pollen tube length)

Table 4.23 Pollen germination percentage of *Rubus paniculatus* S. after different time of incubation

Treatments	Pollen germination (%)±SE(m) (24hours)	Pollen germination (%)±SE(m) (48hours)	Pollen germination (%)±SE(m) (72hours)
T1- Water(Control)	0.00±0.00	3.05± 0.08	9.68±0.16
T2- 0.1% Boric acid + 5% Sucrose	5.35±0.11	22.63±0.11	31.73±0.08
T3 - 0.2% Boric acid + 10% Sucrose	5.62±0.16	23.75±0.12	38.81±0.09
T4- 0.3% Boric acid + 15% Sucrose	7.80±0.14	28.14±0.13	40.56±0.05
T5- 0.4% Boric acid + 20% Sucrose	9.52±0.17	31.57±0.13	45.8±0.06
T6 - 0.5% Boric acid + 25% Sucrose	8.25±0.13	31.46±0.11	30.67±0.09
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	6.59±0.15	22.61±0.09	32.33±0.08
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	7.80±0.09	24.57±0.14	35.85±0.06
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	8.42±0.10	25.69±0.13	38.75±0.07
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	8.89±0.08	27.56±0.10	41.84±0.05
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	8.06±0.06	28.29±0.13	42.78±0.08
T12 - 5% Sucrose + 1ppm Gibberellic acid	6.88±0.08	27.75±0.11	39.95±0.04
T13 - 10% Sucrose + 2ppm Gibberellic acid	6.94±0.06	30.68±0.07	43.78±0.06
T14 - 15% Sucrose + 3ppm Gibberellic acid	7.67±0.09	34.83±0.08	45.58±0.07
T 15 - 20% Sucrose + 4ppm Gibberellic acid	9.07±0.08	36.71±0.07	49.09±0.08
T16 - 25% Sucrose + 5ppm Gibberellic acid	9.66±0.07	37.82±0.09	52.34±0.07
SE(d)	0.15	0.15	0.11
$CD_{0.05}$	0.31	0.32	0.23
C.V.	2.54	0.69	0.36

Table 4. 24 Pollen tube length of *Rubus paniculatus* S. after different time of incubation

Treatments	Pollen tube length (µ)±SE(m) (24hours)	Pollen tube length (µ)±SE(m) (48hours)	Pollen tube length (µ)±SE(m) (72 hours)
T1- Water(Control)	0.00±0.00	0.00±0.00	14.18± 0.77
T2- 0.1% Boric acid + 5% Sucrose	35.51±1.7	40.48±1.57	37.51±4.60
T3 - 0.2% Boric acid + 10% Sucrose	52.70±3.06	60.65±6.63	57.09±5.37
T4- 0.3% Boric acid + 15% Sucrose	62.43±3.64	61.93±6.52	57.07±6.33
T5- 0.4% Boric acid + 20% Sucrose	69.88±4.97	73.73±5.04	77.47±3.68
T6 - 0.5% Boric acid + 25% Sucrose	81.57±2.65	88.22±5.31	83.72±5.04
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	62.13±8.40	63.51±4.70	69.72±7
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	66.71±5.90	71.32±5.75	80.04±7.34
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	75.43±5.71	78.07±3.38	82.95±3.39
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	80.35±2.24	88.61±3.05	93.36±4.74
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	77.32±4.76	89.37±4.68	94.15±7.29
T12 - 5% Sucrose + 1ppm Gibberellic acid	63.54±6.65	74.09±4.31	92.63±9.6
T13 - 10% Sucrose + 2ppm Gibberellic acid	69.72±7.00	80.58±6.56	102.19±6.27
T14 - 15% Sucrose + 3ppm Gibberellic acid	74.32±5.65	89.26±6.33	124.38±6.63
T 15 - 20% Sucrose + 4ppm Gibberellic acid	76.22±4.30	90.05±1.88	109.79±4.97
T16 - 25% Sucrose + 5ppm Gibberellic acid	88.05±4.34	99.18±4.27	135.22±5.37
SE(d)	6.94	6.75	8.28
$CD_{0.05}$	14.21	13.81	16.95
C.V.	13.13	11.51	12.37

µ - micron (unit of pollen tube length)

4.6 STIGMA RECEPTIVITY

4.6.1 Visual observation

In order to study stigma receptivity of raspberry wild species under study by visual observation, the stigmas of different age group viz., three day, two day and one day before anthesis, on the day of anthesis and one day, two day and three days after anthesis were recorded with the help of a hand lens. Stigma which were plump and shiny with stigmatic secretion were considered to be receptive while those having dull brownish appearance and lacking in secretions were considered to be non receptive.

4.6.1.1 Stigma receptivity of *Rubus macilentus* C. by visual method

1 - Three days before anthesis the stigma was white, light cream in colour, dull with short length.

2 - Two days before anthesis, the stigma was green, dark cream in color, less shiny and style length was short.

3 - One day before anthesis the stigma was dark cream in color, appearance of shine, style was healthy.

4 - On the day of anthesis, the stigmatic surface completely turned to creamish colour. It showed the highest amount of sugary secretion and fresh appearance. The pollination takes place at this stage leads to the highest amount of fruit set.

5 - One day after anthesis, ovary size increased, brown colored stigma, stigmatic surface bilobed.

6 - Two day after anthesis, basal portion swollen, style constriction at the middle portion and shininess disappeared.

7 - Three day after anthesis, swelling of ovary was observed, style became brown complete disappearance of shininess.

4.6.1.2 Stigma receptivity of *Rubus niveus* T. by visual method

1 - Three days before anthesis stigma was white, short, prominent and there was no presence of any sugary secretion on the surface.

2 - Two days before anthesis, the stigmatic surface was little bigger than the previous day and the surface was more whitish, prominent and bigger than before. There was slight appearance of any sugary secretion on the surface of the stigma.

3 - One day before anthesis the stigmatic surface was at its maximum length and start showing little amount of sugary secretion. The stigmatic surface starts to turn light creamish colour.

4 - On the day of anthesis, the stigmatic surface completely turned to creamish colour. It showed the highest amount of sugary secretion and fresh appearance. The pollination takes place at this stage leads to the highest amount of fruit set.

5 - One day after anthesis, flower showed the little shrunken stigmatic surface. The colour of the stigma was still creamish in colour and there was a depleted amount of sugary secretion on the surface of the stigma.

6 - Two day after anthesis, stigma shows the more shrunken stigmatic surface. The colour of the stigma was still creamish in colour and there was a depleted amount of sugary secretion on the surface of the stigma.

7 - Three day after anthesis, stigma shows the more shrunken stigmatic surface. The colour of the stigma is started to turn from creamish to brown colour and there is a depleted amount of sugary secretion on the surface of the stigma and it starts to dry.

4.6.1.3 Stigma receptivity of *Rubus paniculatus* S. by visual method

1 - Three days before anthesis stigma was white with pinkish margins, short, prominent and there was no presence of any sugary secretion on the surface, the style was more curved and fused at the centre of flower.

2 - Two days before anthesis, the stigmatic surface was little bigger than the previous day and the surface was more whitish, prominent than before. There was slight appearance of some sugary secretion on the surface of the stigma and the styles were less curvy than before but still fused.

3 - One day before anthesis the stigmatic surface was at its maximum length and start showing little amount of sugary secretion. The stigmatic surface starts to turn light creamish colour and styles became slightly free.

4 - On the day of anthesis, the stigmatic surface completely turned to creamish colour. It showed the highest amount of sugary secretion and fresh appearance. The pollination takes place at this stage leads to the highest amount of fruit set, the styles became completely straight and was showing maximum receptivity.

5 - One day after anthesis, flower showed the little shrunken stigmatic surface. The colour of the stigma was still creamish in colour and there was a depleted amount of sugary secretion on the surface of the stigma, again the styles became slightly curvy.

6 - Two day after anthesis, stigma shows the more shrunken stigmatic surface. The colour of the stigma was still creamish in colour and there was a depleted amount of sugary secretion on the surface of the stigma and styles were curvier than before.

7 - Three day after anthesis, stigma shows the more shrunken stigmatic surface. The colour of the stigma is started to turn from creamish to brown colour and there is a depleted amount of sugary secretion on the surface of the stigma and it starts to dry and curved styles.

4.6.2 Fruit set method

The stigma receptivity assessed through fruit set method by selecting the different flower buds of different age groups were emasculated and pollinated with fresh pollen. From the results of Table 4.25 it was concluded that the highest amount of stigma receptivity was recorded on the day of anthesis. The flower is highly cross pollinated in nature. Among the three species of raspberry under study, *Rubus macilentus* C. and *Rubus niveus* T. showed good fruit set and in *Rubus paniculatus* S. fruit set was not observed.

4.6.2.1 *Rubus macilentus* C.

As per the observation shows in the Table 4.25 highest amount of fruit set percentage (73.33%) was obtained on the day of anthesis. Followed by one day after and one day before anthesis anthesis which shows 60.00% and 56.00% of fruit set respectively

4.6.2.2 *Rubus niveus* T.

From the results recorded with respect to stigma receptivity by fruit set method in *Rubus niveus* T. indicated that highest amount of fruit set percentage (86.66%) was obtained on the day of anthesis which is recorded in the Table 4.25 Followed by one day after and one day before anthesis which showed 66.66% and 76.66% of fruit set respectively.

4.6.2.3 *Rubus paniculatus* S.

No fruit set was recorded.

Table 4.25 Stigma receptivity by fruit set method

	Percentage of fruit set		
Age of stigma	*Rubus macilentus* C.	*Rubus niveus* T.	*Rubus paniculatus* S.
Three days before anthesis	0.00%	0.00%	0.00
Two days before anthesis	23.33%	30.00%	0.00
One day before anthesis	56.66%	66.66%	0.00
Day of anthesis	73.33%	86.66%	0.00
One day after anthesis	60.00%	76.66%	0.00
Two days after anthesis	50.00%	56.66%	0.00
Three days after anthesis	30.00%	36.66%	0.00

4.7 POLLINATION STUDIES

In raspberry flowering the pollination mainly mediated through honey bees and ants hence, cross pollination was naturally observed.

4.7.1 Autogamy / Self pollination

When the flowers were subjected to self pollination by bagging them with butter bag to save them from the contamination, the flower showed good amount of fruit set, since the raspberry flower was hermaphrodite. The maximum fruit set on self pollination was reported in *Rubus niveus* T. (81.93%), followed by *Rubus macilentus* C. (71.63%), while no fruit set was noticed in *Rubus paniculatus* S.(Table 4.26).

4.7.2 Geitonogamy

Maximum geitonogamy of 83.20% was noticed in *Rubus niveus* T. followed by 75.93% was observed in *Rubus macilentus* C. and geitonogamy was not successful in *Rubus paniculatus* S. (Table 4.26).

4.7.3 Cross pollination

The raspberry species are cross pollinated and some extent of self pollination is also seen. From the data presented in tables 4.27, it is clear that the maximum fruit set was recorded on crossing between *Rubus niveus* T. X *Rubus macilentus* C. (80.00%), followed by *Rubus niveus* T. X *Rubus paniculatus* S. (72.06%) and minimum was noticed in *Rubus macilentus* C. X *Rubus niveus* T. (68.29%), followed by *Rubus macilentus* C. X *Rubus paniculatus* S. (56.75%). No fruit set was seen when *Rubus paniculatus* S. was used as female parent with in *Rubus macilentus* C. and *Rubus niveus* T.

4.7.4 Natural pollination

The plants were allowed to pollinate openly with natural pollination mediators like honey bees or ants. It was observed (Table 4.26) that maximum of 88.93% of fruit set in *Rubus paniculatus* S., where the other methods of pollination carried out under study were failed and 88.64% of fruit set was reported in *Rubus niveus* T. under natural pollination while

84.40% of fruit set was seen in *Rubus macilentus* C. which reveals that the pollinators play a key role in fruit set increments which shows better results over any other methods of pollination. After third day of natural pollination of *Rubus paniculatus* S. clumping of stigmas were seen upon which the pollens were germinating.

4.7.5 Self incompatibility

Self incompatibility of 0.92 was recorded in *Rubus niveus* T. followed by 0.85 was obtained in *Rubus macilentus* C. and could not able to determine the incompatibility in *Rubus paniculatus* S. due to failure of pollination methods. The results indicated that the species *Rubus macilentus* C. and *Rubus niveus* T. are compatible (Table 4.26).

4.7.6 Apomixes

The apomixes was not found among three species of raspberry.

Table 4. 26: Mode of pollination

Pollination method	Percentage of fruit set		
	***Rubus macilentus* C.**	***Rubus niveus* T.**	***Rubus paniculatus* S.**
Autogamy	71.63	81.93	0.00
Geitonogamy	75.93	83.2	0.00
Natural or open pollination	84.40	88.64	88.93
Self incompatibility	0.85	0.92	0.00
Apomixes	0.00	0.00	0.00

Table 4.27 Inter specific crosses

Interspecific crosses	Percentage of fruit set
Rubus macilentus C. X *Rubus niveus* T.	68.29
Rubus macilentus C. X *Rubus paniculatus* S.	56.75
Rubus niveus T. X *Rubus macilentus* C.	**80.00**
Rubus niveus T. X *Rubus paniculatus* S.	72.06
Rubus paniculatus S. X *Rubus macilentus* C.	0.00
Rubus paniculatus S. X *Rubus niveus* T.	0.00

4.8 FRUIT SET

4.8.1 Time of fruit set

Observation on the time of fruit set revealed that the species *Rubus macilentus* C. set fruits between second week of April to last week of June 2017, whereas in *Rubus niveus* T. the fruit set started from first week of May to last week of June and *Rubus paniculatus* S. showed the fruit set from third week of May to last week of June. The fruit set in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. took 30 days, 26 days and 23 days respectively from commencement of flowering to first fruit set.

4.8.2 Percentage of fruit set

Under the present study, average initial fruit set percentage was of *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. was 55.73%, 73.42% and 68.75% of initial fruit set over an average of 166.8, 2,922.15 and 492.36 numbers of flowers per branch (Table 4.28).

Table 4.28 Fruit set percentage

Species	Average number of flower per branch	Percentage of initial fruit set	Fruit retention
Rubus macilentus C.	166.8	55.73%	99.52%
Rubus niveus T.	2,922.15	73.42%	97.63%
Rubus paniculatus S.	492.36	68.75%	88.71%

Chapter-5

DISCUSSION

CHAPTER 5

DISCUSSIONS

The present investigation on the Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya was carried out with respect to morphological characters for identification and documentation, flowering habit and duration, pollen characteristics and fruit set under different modes of pollination. The results of the present studies are discussed below:

5.1 CANE MORPHOLOGY

Rubus paniculatus S. showed maximum cane height, diameter of stem, number of laterals per plant and diameter of laterals compared to other two species under study. The plant with maximum height also had maximum girth of stem, number of laterals and diameter of laterals which was in accordance to Kapil (1988) who reported that, if plant height increases, girth of stem and other branches will also increases. Jenning and Dale (1982) also reported that cane diameter was positively correlated with cane height in red raspberries. Variability in plant height may affect the other characters like branching, stem girth, leaflet size and nodal leaf size and shape. Cole (1957) has suggested that branching habit is partly genetically and partly environmentally controlled.

The species *Rubus niveus* T. and *Rubus macilentus* C. had purple coloured floricanes, the variation in pigment intensity among the different species may be due to the conversion of more anthocyanin pigments. Purple colour on petioles and sometimes on leaf veins is also due to the accumulation of anthocyanin. It was also reported that plants growing at higher altitude and shady areas in forest developed purpler colour of the stem than that of open areas and in lower altitudes.

Prickles found on stem, branch and leaf were superficial in origin and irregularly distributed. Kapil (1988) reported that the walls of bristles present on stem and branches are thickened with a deposit of silica or calcium carbonate Prickles, bristles and hair help in defence mechanism from external factors.

5.2 LEAF MORPHOLOGY

The raspberry species under study had compound leaf except *Rubus paniculatus* S. which had simple leaf and were alternatively arranged on bearing shoots. *Rubus macilentus* C. had trifoliate leaves with two basal or nodal leaves arose from each node on primocane and floricane and had terminal leaflet was larger with long stalk while two lateral leaflets were small with short stalk or sessile, prickles were present on the leaf mid ribs and petioles. Typically, leaves were pinnate in shape. Lateral leaflets differed a little in size or were always equal and had ovate – lanceolate, acuminate, which was in accordance with Kapil (1988) who reported nodal leaves were broader and arose at the leaf axils in case of *Rubus ellipticus* S.

Lateral leaflets are sometimes missing as reported by Collett (1921) but the present observation differs from the above as two lateral leaflets were always found along with terminal one in all the populations of *Rubus macilentus* C. However, sometimes a single leaf was present terminally from the axil of which floral axis was arisen. Kachroo and Daftri (1968) reported that *Rubus fruticosus* L. bears leaves and flowers during late spring to early autumn and is practically leafless during winter whereas *Rubus ellipticus* S. was evergreen and bear leaves in all the seasons and flowers in winters to late spring. The present observation is in confirmation with that of Bailey and Bailey (1959) that the floricanes which bear flowers and fruits in the second year May have different foliage.

Rubus niveus T. the leaves were pinnate in shape, while that of *Rubus paniculatus* S. was heart shaped simple leaf with upper surface dark green with sunken veination, on lower surface white tomentose. Collett (1921) reported glabrous leaves *Rubus ellipticus* S. in the Flora Simlensis, but hairy and prickly leaves were observed during the present study from all the three species, although very less and minute hairs were present on the upper surface of leaves. Bailey (1958) also reported glabrous upper surface of leaves in *Rubus ellipticus* S.

Bi-and trifurcation in nodal leaves may be attributed to the marginal meristematic activity which may be of short duration and contributes a little to the lateral extension of the nodal leaf.

5.3 GROWTH BEHAVIOUR

During first year, the growth of the plants was very rapid for four months later it reduced, by the next year the plants produced few new laterals and their growth was rapid till the reproductive parts appearance in all the three species, which was in line with Vasilakakis (1978) who reported that the shoots of fall-bearing cultivars continue to elongate until the terminal inflorescence appears and vegetative growth ceases. Inflorescences continue to develop basipetally to certain node of fall-bearing cultivars, normally do not form a terminal inflorescence in the first year of growth. In the spring of the second year, axillary buds give mixed shoots similar to those of fall-bearing cultivars and yield the full crop.

5.4 FLORAL BIOLOGY

5.4.1 Bearing habit

The flower buds appeared on second year shoots, i.e. floricanes of *Rubus macilentus* C. and *Rubus niveus* T., while in *Rubus paniculatus* S. flower buds appeared on one year old shoots. The entire flowering period vary from March to June for above all the three species. According to Bailey (1932), flower primordia are initiated in the axillary buds usually in the autumn, develop in the spring and finally primary laterals grow out, bear their crop after which the second year branch or floricane dies down to ground level. Brainerd and Peitersen (1920) working with *Rubus* species showed that the vegetative parts of the plant can be modified much by the environment than the reproductive parts, thus confirming experimentally the common belief of taxonomists that the latter organs are generally more reliable as diagnostic criteria to separate different species within the genus. In the present observations also, vegetative parts as well as reproductive parts showed more plasticity among the species.

5.4.1.1 Emergence of inflorescence

Flowering in raspberry species under study proceeded from top to lower portions, thereby lengthening the duration of flowering period as well as making best use of the pollinators. Production of large number of inflorescences and flowers at the ends of branches provided greater attraction for insects, the majority of which were flying insects. The present

observation of corymbose raceme inflorescence was observed in *Rubus macilentus* C. and *Rubus niveus* T. is in agreement with that of Parmar and Kaushal (1982). It is also in line with that of Collett (1921) who reported axillary and terminal panicles in *Rubus ellipticus* S. However, the panicles were observed in *Rubus paniculatus* S. in the present observations. Flower size and shape showed variability which appeared between species and within a population, same plant and even within the same branch. This is suggestive of intermorphotypic and intramorphotypic morphological variations.

5.4.2 Flower bud development

Eight stages of flower bud development were observed in *Rubus macilentus* C., while in *Rubus niveus T.* and *Rubus paniculatus* S. six stages were noticed. The total time required for flower bud to reach the anthesis for *Rubus macilentus* C., while in *Rubus niveus T.* and *Rubus paniculatus* S. was 53 days, 45 days and 40 days respectively. The growth of flower buds in all the raspberry wild species under present investigation showed slow growth for initial days. The differences in flower bud development period may be due to the genetic makeup of the individual species, which appears to be a principle factors like in controlling flower bud development. During the development of flower bud in *Rubus macilentus* C. the buds were green with more silky hair on outer surface, while in *Rubus niveus* T. The buds were pinkish coloured and *Rubus paniculatus* S. had straw coloured buds with dense silky hair.

Tamura *et al.* (1987) reported that the flower bud development period as determined on the basis of length/diameter ratio appeared to be a good indicator for observing flower bud development. There were seven bud development stages in apple. The diameter of flower primordial was a suitable indicator of the stage of flower bud development. Asada (1987) reported that the morphological development of flower bud in early spring with various apple cultivars that L/D ratio (length/diameter) was similar for all the cultivars rising from 0.25 at dormancy to 0.65 at flowering.

The developmental phase of the flower into eight stages shows its close similarity with those observed by Teaotia and Chauhan (1963) in ber bud development. Singh et al. (1970), Desai and Patil (1978) and Ranawade *et al.* (1983) observed 7 stages of ber bud development.

It is clear from our data that growth of bud was very slow during early stages but it was accelerated after fourth stage in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. but the maximum bud size was achieved after eighth stage of *Rubus macilentus* C., and sixth stage of *Rubus niveus* T. and *Rubus paniculatus* S.

5.4.3 Time and duration of flowering

In raspberry wild species, the flowering of *Rubus macilentus* C., started from last week of March upto second week of June and total period was recorded as 53 days which was quite longer. In case of *Rubus niveus* T. the flowering commenced on last week of April and continued upto first week of June, with total of 45 days. *Rubus paniculatus* S. showed flowering from first week of May to first week of June and total period was recorded as 40 days. The peak period of flowering of *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. was 48 days, 30 days and 22 days from first flower opening. As per the observation, the commencement of flowering in three raspberry wild species under hilly conditions of Uttarakhand takes place between the second week of March to second week of June. This variation among the raspberry species under study may be due to that, the time of flowering in raspberries is related to temperature, plant age, vigour and termination of vegetative growth (Waldo 1934, Vasilakakis *et al*. 1980).

Kumar (1996) studied the flowering behavior of apple and found that all the six cultivars studied attained peak flowering within 4 to13 days after starting of flowering. Flowering duration varied from 15 to 18 days in first year of study, where as it varied from 12 to19 days in second year of study. All the cultivars that lowered within 3 to 8 days exhibited sufficient overlapping of full bloom period. Daniels (1922) 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) 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.

Our observation is based on the assumption that many biennials require a low temperature for flowering (Leopold and Kriedermann 1975). Temperature provides salient climatic cue for plants which is a ready signal of seasonal changes. In the present case, flowering responses to temperature can be considered as an ecological coordination of flowering times. The photoperiodic effect is also important of all environmental factors upon the initiation of flowering which shows great variation between different plants. It involves the exposure of plants to different lengths of light and darkness.

In the present study, floral buds showed rapid growth towards the end of the flowering, it may be due to photoperiodic induction occurs in leaves and the flowering stimulus moves out from the leaves to the meristems where flowers are to be initiated by a flowering hormone or florigen (Swamy and Krishnarnurty 1980). Often the speed of flowering (interval between induction and the flower appearance) and the number of flowers formed increase as the number of consecutive inductive photoperiods increases. However, the photoperiodic conditions essential for flower induction may not be optimal for flower development in December and low synthesis of florigen may be one of the reasons.

5.4.4 Flower morphology

The flower characteristics are more stable characters than the morphological characters which are more prone to variation. Family Rosaceae is characterized by primitive features like numerous and indefinite number of stamens and pistils and the advanced condition of perigyny and epigyny. Above all it is well known for the diversity of fruit-types.

Flowers of three species under present study were regular, complete, perfect, actinomorphic, hermaphrodite or bisexual, hypogynous, petal colour varied from white to pink, bracteate and pedicillate; pedicel was hairy. The number of petals and sepals were five. The average numbers of pistils for *Rubus macilentus* C., *Rubus niveus* T.and *Rubus paniculatus* S. were on an average 112.6, 103.9 and 28 and stamens were 126.2, 51.9 and 107.25 respectively. Ashman *et al.* (2012) 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) observed that raspberry flowers were white to pink in color, small about 0.5 to 1.5 cm and were initiated in the second year of planting. The gynoecium consisted of 60 to 80 ovaries, each of which develops into a drupelet. There were 60 to 90 stamens. 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

Length of sepals, petals, pistils and stamens of *Rubus macilentus* C. *Rubus niveus* T. and *Rubus paniculatus* S. was observed as 1.05 cm, 1.02 cm, 0.22 cm and 0.32cm, 0.57 cm, 0.38 cm, 0.35 cm and 0.25 cm and 0.92 cm, 0.42 cm, 0.52 cm and 0.54 cm respectively, which is in accordance with the result of Kumar (1988) who found that the sepals length was between 0.52 cm to 0.82 cm in different cultivars of apple. The maximum length of pistils was 1.36 cm and minimum was 0.63 cm. Bhartiya *et al.* (1983) reported that the length of stamens ranged from 0.63 to 0.96 cm and the pistils from 1.00 to 1.12 cm.

In present study, the abnormal flowers appeared in addition to normal ones in two species of wild raspberry namely *Rubus macilentus* C. and *Rubus niveus* T. which was in accordance to Kapil (1988) reported flowering in *Rubus ellipticus* S. was accompanied by abnormal flowers along with normal flowers and the abnormality in petals was more frequent in comparison to sepals. Parmar and Kaushal (1982) mentioned an off-season bloom in December with most of the flowers abnormal and it is reported that the increase in number of petals and/or sepals is a tendency towards evolution.

In the flowers of *Rubus macilentus* C. *and Rubus niveus* T, it was observed that the stamens formed a compact whorl completely enclosing the stigma. Such condition would appear favourable for the transfer of pollen from anthers to stigma within a single flower but in case of *Rubus paniculatus* S. the stamens were curving outwards from the centre of the flower. In the present studies, three types of stamen arrangement were seen in the natural populations of all the three species under study. Nybom (1986) reported two types of stamen arrangement in senescing flowers of *Rubus* cultivars. He suggests that in the self-pollinating species, stamens curve over the pistils so that the anthers are in contact with the stigmas but in

cross-pollinating species, the stamens are at the same level with stigmas and curve outwards away from them.

5.4.5 Anthesis

The observations on the anthesis were recorded at two hours interval from 6.00 am to 6.00 pm in all the cultivars under study. The low rate of anthesis was recorded in morning hours. The maximum anthesis of all the three raspberry species under study was observed between 10.00 am to 12.00 noon with highest mean percentage of anthesis in *Rubus macilentus* C. and *Rubus niveus* T. and *Rubus paniculatus* S. was 41.43%, 40.96% and 33.96% respectively recorded. The percentage of anthesis increased gradually from 8.00 am to 2.00 pm after which it declined gradually up to 6.00 pm. According to the Kumar *et al.* (1996) the percentage anthesis increased gradually from 8:00 am to 2:00 pm., after which it decreased in all the apple cultivars. While Sharma (1970) reported that the anthesis occurred between 6.00 am to 6.00 pm with gradual increase up to 12.00 noon and then decreased slowly which ended by 6.00 pm in apple cultivars.

The early flowering was observed in *Rubus macilentus* C. followed by *Rubus niveus* T. and *Rubus paniculatus* S. Zeevaart (1964) reported that nucleic acid synthesis is also involved in flowering induction at the apical bud. The exact mechanism involved in the opening of flowers is not clear. However, according to Swamy and Krishnamurthy (1980), petals also synthesize a substantial amount of auxins along with anthers during the limited period of flower opening. Auxin and growth regulators are also believed to cause the opening and closing of flowers in relation to day and night or vice versa and affect flower closure after pollination.

5.4.6 Anther dehiscence

The rate of anther dehiscence was recorded to be higher between 10.00 am to 12.00 noon. Further dehiscence started after opening of flower in all the plants studied. The peak period of dehiscence was recorded between 10.00 am to 12.00 noon in all the three species under study with the maximum of 18.38%, 40.85% and 28.57% in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. The dehiscence almost completed between 4.00 pm

to 6.00 pm. It was observed that 70.64% and 73.63% of anthers dehisced on the day of Anthesis in *Rubus macilentus* C. and *Rubus paniculatus* S. while in *Rubus niveus* T. almost all the anthers dehisced on the day of Anthesis. Similar dehiscence pattern has been reported by Bekey and Lawrence (1985) who found that the anther dehiscence began in a few anthers of a flower almost immediately after the split of the calyx. Sharma (1970) reported that the dehiscence in apple varieties begin at 6.00 am with a progressive increase till 12.00 noon, ending by 6.00 pm. The dehiscence in general started almost at 8.00 am and ended around 4.00 pm.

5.5 POLLEN STUDY

5.5.1 Number of pollens per anther and per flower

In present study, the maximum number of pollens per anther was reported in *Rubus paniculatus* S. followed by *Rubus niveus* T. then in *Rubus macilentus* C. and the number of pollens per flower was maximum with *Rubus paniculatus* S. followed by *Rubus macilentus* C. and *Rubus niveus* T. this is because *Rubus paniculatus* S. had larger anther number and size than other two species, while in *Rubus macilentus* C. even though the number of pollens per anthers were less but number of stamens were more per flower than *Rubus niveus* T. the present results on line with Tianming *et al.* (2000), who reported that the pollen grain mass of pear Xiangli (*Pyrus bretschneideri* R.) was about 4000 in each anther.

5.5.2 Pollen morphology

Variation is the fundamental basis for natural selection, leading to the origin of a new species with new complements of characters. Pollen being the carrier of plant heredity, its variations reflects the change undergone and transmitted to the next generation by the plant. Nair (1974) reported that pollen organization is more or less stable within the same species and when there is a deviation from the general type, it brings about the biological change in the species. It is imperative that the pollen also undergoes change in its morphological characters and that change provide an important tool to analyse and interpret the taxonomic relationship and evolutionary trends of the plants concerned. In recent years, pollen morphology is being put to greater use in phylogeny and evolution. Pollens and spores are

often formed in tetrads before they loosen and become separate entities. The tetrad stage is of importance as far as the origins of certain morphological characters of exine are concerned.

Study of pollen morphology indicated elliptic and tricolpate shape of fresh pollen grains. The *Rubus paniculatus* S. exhibited maximum pollen size (35.87µ length X 17.73µ width) in dry condition, followed by *Rubus macilentus* C. (31.29µ length X 16.12 µ width) and minimum size (30.73 µ length X 17.28 µ width) was recorded in *Rubus niveus* T. Under glycerine the maximum size(35.44 µ length X 16.52 µ width) was recorded in *Rubus paniculatus* S. followed by *Rubus niveus* T.(32.11 µ length X 17.02 µ width) and minimum (30.74 µ length X 17.68 µ width) was recorded in *Rubus macilentus* C. In water maximum size (30.42 µ length X 28.54 µ width) was recorded in *Rubus niveus* T. followed by *Rubus paniculatus* S. (29.69 µ length X 29.98 µ width) and minimum (28.61 µ length X 28.28 µ width) was recorded in *Rubus macilentus* C. and under acetocarmine condition the *Rubus paniculatus* S. showed maximum (27.89 µ length X 26.92 µ width) followed by *Rubus niveus* T. (26.01 µ length X 25.81 µ width) and the minimum pollen size (25.23 µ length X 23.51 µ width) was recorded in *Rubus macilentus* C. The shape of pollen grains in glycerine and dry conditions were oblong and in acetocarmine and water pollen grains looked round in shape. The pollen viability was recorded 100% through acetocarmine test.

Similar observations were recorded on freshly dehisced anthers by Bist and Sharma (1986) who found that the fresh pollen grains of apple were elliptic and tricolpate and in acetocarmine solution the pollen grains assumed the triangular shape. The average length of fresh pollen ranged from 33.96 to 47.95µ in different cultivar, whereas the breadth ranged from 23.97 to 29.97 µ. The average length and breadth of pollen grain in aniline oil ranged from 38.29 to 47.95 µ and 21.31 to 26.64 µ respectively in some apple cultivars. Ali (1962), Chauhan (1966) and Rajput (1967) observed that the shape of the pollen grains in dry state was elliptical. While the shape of the pollen grains was observed to be roundish when mounted in 2% acetocarmine.

Reitsma (1966) reported that the pollen grains of Rosaceae are tricolpate and striate. Tricolpate and stephanocolporate grains occur as normal variation. He has organized 13 types of pollen grains within this family. Froydix (1981) reported subprolate shape in *Rubus*

chamaemorus and spinuliferous exine. The importance of shape differences in taxonomy is not very significant because it is not a stable character at all.

5.5.3 Pollen viability

In the present investigation, pollen grain viability of *Rubus macilentus* C. and *Rubus niveus* T. was 100%, while that of *Rubus paniculatus* S. had 73.33%. Gercekcioglu *et al.* (2000) studied the pollen grains of eight different pome and stone fruit cultivars (plum, peach, sweet cherry and apple cultivars) and observed pollen viability in the range of 71.53 to 81.78 per cent. Otterbacher *et al.* (1983) showed that high temperature resulted in rapid loss of viability of raspberry pollen. The germinability of pollen grains stored under room conditions indicated that after three days of storage, the pollen grains were quite normal and showed 46.66% germination. But after seven days of storage, the germinabilty was decreased rapidly and after nine days of storage, the germination percentage totally declined. Similar observation recorded by the Asma (2008) where the pollen viability and germination ratios were determined for eight apricot cultivars. The results indicated that viable, semi viable and dead pollen rates differed among cultivars, where Roksana had the least amount of viable pollen (41.50%).

5.5.4 Pollen germination

Amongst all the treatment combinations used for pollen germination of raspberry under study indicated that the *Rubus macilentus* C. had maximum pollen germination (90.54%) and pollen tube length (1,164.19 µ) after 48 hours after planting with 10% Sucrose + 2ppm Gibberellic acid and 5% Sucrose + 1ppm Gibberellic acid respectively, followed by *Rubus niveus* T. for pollen germination (62.96%) and tube length (214.98 µ) was achieved with 20% Sucrose + 4ppm Gibberellic acid and the minimum pollen germination (52.34%) and tube length (135.22 µ) was reported in *Rubus paniculatus* S. with 25% Sucrose + 5ppm Gibberellic acid after 72 hours of planting. Similar observations were taken by Wertheim (1996) who reported that 13 to 89 per cent pollen germination took place in different apple cultivars in a solution containing 15 per cent sucrose + 15 ppm boric acid + 150 ppm calcium nitrate.

Kumar (1996) reported that the sucrose solution of 9 to 10 per cent concentration was the most effective for pollen germination of apple cultivars. Abdel (1999) reported 73.30 to 86.10 per cent pollen germination in 15 per cent sucrose solution in three apple cultivars. The best pollen tube length was observed with 25% sucrose + 0.4% boric acid solution which ranged from 26.66 µ to 284.37µ. According to Rawat *et al*. (2003) the pollen grains of peach were large, equitriangular, tricolporate with thick exine. The highest pollen germination (83.78%) and pollen tube length (1259.01µ) were recorded in 10% sucrose solution.

Larsson (1969) reported that pollens of 2x *Rubus* germinated best on 15% sucrose medium, whereas 20-25% sucrose was best for 4x material. A 20% sucrose-agar medium was satisfactory for germination study of the pollen from tetraploid blackberries but gave poor results on a diploid clone (Perry and Moore 1985). They further reported that blackberry pollens loses viability quickly and is short-lived when stored at room temperature.

5.6 STIGMA RECEPTIVITY

In order to study stigma receptivity by visual observation, stigmas of different age group viz., three day, two day and one day before anthesis, on the day of anthesis and one day, two day and three day after anthesis were recorded with the help of a hand lens. On the day of anthesis the stigma receptivity was high as compared to the one and two days prior and after anthesis in all raspberry species under study. The stigma receptivity was investigated by fruit set method in all the three species. 23.33%, 56.66% 73.33%, 60.00%, 50.00% and 30.00% was recorded in *Rubus macilentus* C., while in *Rubus niveus* T. the fruit set of 30.00%, 66.66%, 86.66%, 76.66% 56.66% and 36.66% was observed on pollinating the stigmas of two day, one day before anthesis, on the day of anthesis, one day, two day and three days after anthesis respectively. No fruit set was seen in *Rubus paniculatus* S. due to pollination failure. Bekey (1985) reported that the peak receptivity period of eight cultivars of red raspberry lasted from 1 to 4 days. Most cultivars set as well with one day of hand pollination at the peak of the receptivity period as with three days of consecutive pollination.

5.7 POLLINATION STUDIES

The maximum fruit set on cross pollination, geitonogamy and autogamy was reported in *Rubus niveus* T., followed by *Rubus macilentus* C., while no fruit set was noticed in *Rubus paniculatus* S. Daubeny (1971) recorded that flowers pollinated only with their own pollen or that from adjacent flowers set nearly 57 to 77 % when open pollinated. Shanks (1969) observed that without any additional pollination a berry developed frequently in raspberry, although it was relatively small and had a tuft of unpollinated pistils at its center. It was seen that in *Rubus macilentus* C. and *Rubus niveus* T., some flowers showed dehiscence before anthesis and position of stamens above the stigmas which may lead to self-pollination. These flowers showed reduced fruit size. Chaudhari (1966) reported that raspberries in India have zero to five percent self-pollination, though these are naturally cross-pollinated plants. Perry and Moore (1985) reported smaller fruits and generally decreased seed number per fruit due to self pollination in some cultivars of blackberries.

The maximum fruit set was recorded on crossing between *Rubus niveus* T. X *Rubus macilentus* C., followed by *Rubus niveus* T. X *Rubus paniculatus* S. and minimum was noticed in *Rubus macilentus* C. X *Rubus niveus* T., followed by *Rubus macilentus* C. X *Rubus paniculatus* S. No fruit set was seen when *Rubus paniculatus* S. was used as female parent with in *Rubus macilentus* C. and *Rubus niveus* T. the present results are inline with the finding of Bors (2005) who did the crosses of *Fragaria moschata* × *Fragaria nubicola, Fragaria moschata* × *Fragaria viridis,* and their reciprocal combinations were done to create tetraploids for eventual introgression into octoploid cultivars of *Fragaria* × *ananassa,* which indicate the cross compatibility of the *Fragaria* species.

In present study self incompatibility of 0.92 was recorded in *Rubus niveus* T. followed by 0.85 was obtained in *Rubus macilentus* C. and could not able to determine the incompatibility in *Rubus paniculatus* S. due to failure of pollination methods. The results indicated that the species *Rubus macilentus* C. and *Rubus niveus* T. are compatible. Under natural pollination maximum fruit set in *Rubus paniculatus* S., followed by *Rubus niveus* T. and minimum was reported in *Rubus macilentus* C. Rajput and Singh (1967) reported that the cross pollination resulted in the highest percentage of fruit set and self pollination resulted in

the lowest fruit set in strawberry cultivars. The pollination methods failure in *Rubus paniculatus* S. may be due to self incompatibility as well as cross incompatibility with the species under study. The fruit set was seen on natural pollination it might be due to partenocarpy or pollens might be coming from fourth compatible species apart from the species under study. The actual mechanism is yet to be study.

The apomixes was not found among three species of raspberry under study, but apomixes has been reported in *Rubus* species. It was caused when the stamens were shorter than pistils, shrunken and non-dehiscent. As a result, there was failure of stamen development or the transformation of male sex-organs into female ones (Gattschalk and Kaul 1974).

5.8 FRUIT SET

In *Rubus macilentus* C. fruit set occurs between second week of April to last week of June 2017, whereas in *Rubus niveus* T. the fruit set started from first week of May to last week of June and *Rubus paniculatus* S. showed the fruit set from third week of May to last week of June. The fruit set in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. took 30 days, 26 days and 23 days respectively from commencement of flowering to first fruit set this variation is because they started ripening with the rise in temperature at the end of April month. The flowers which opened in the first week of April, became unripe fruits after 30days and started ripening in the month of May when the temperature further increased.

Fruit-ripening occurs only at higher temperature because heat is required for the maturation of fruit. Thus, it is clear that temperature significantly affected the number of days to fruit ripening maximum fruit-ripening was seen in May and June under Bharsar condition, when maximum temperature was 28.9° C. In *Fragaria*, fruit ripening was earlier as growing temperature was higher (Gruppe and Khanzari 1975) and in *Malus* there was a significant negative correlation between the temperature from June to September and picking date (Luton and Hamer 1983). In *Prunus cerasus*, on the other hand, time to fruit-ripening was hardly affected by temperature (Tukey 1952).

Chapter-6

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

The present investigations were conducted on on various aspects of morphology, floral biology and pollination studies in natural populations of wild species of raspberry namely, *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. in College of Horticulture, V.C.S.G UUHF, Bharsar, Pauri Garhwal, U.K. which are most important pre-requisites for any improvement and hybridization programme. These aspects are yet not received much attention in raspberry wild species under temperate conditions in the hills. The hills and valley areas of Uttarakhand state offer suitable climate for growing raspberry species and thus, there seems to be a big scope of these fruits. The salient finding of the study are being summarized in the following paragraphs:

6.1 CANE MORPHOLOGY

Raspberry species are bi-annual and perennial, found growing wildly in all types of forests as undergrowth, along the roadsides and near water. It grows in a wide range of altitude from 600 to 2200 m above mean see level. Three wild species of raspberry showed lot of variability in their habitat and morphological characters. The maximum cane height, diameter of stem, number of laterals per plant and diameter of laterals was found in *Rubus paniculatus* S. followed by *Rubus niveus* T. except for number of laterals and minimum was noticed in *Rubus macilentus* C. but had more number of laterals than *Rubus nivues* T. *Rubus macilentus* C. had five angled primocane and four angled tertiary shoots in floricanes, the green coloured primocane on maturity turned to purple colour and the same colour changes were notice in *Rubus nivues* T.

6.2 LEAF MORPHOLOGY

The raspberry wild species under study showed variability with respect to Phyllotaxy. Two nodal leaves, which always appeared at each node in *Rubus macilentus* C. and had strictly trifoliated leaves, bi-or trifurcated nodal leaves were observed frequently. While *Rubus niveus* T. had 5 to 7 pinnate leaflets in floricane but in primocane 11 leaflets were present. *Rubus paniculatus* S. had simple with heart shaped leaves. Such types of abnormal leaflets were probably originated due to the damage caused by external factors to the growing leaf tips. These may also be either due to increase or decrease in

cell number, change of nutritional status, genetic mechanisms etc. Various other phenotypic characters like leaflet length, leaflet width, prickles on stem, leaflets, venation, number and type of dentations etc. have been discussed in detail in the text. They showed marked variations in plant height, stem girth, branching, shape and size of leaflets, dentations, inflorescence etc. These are considered to be due to the result of various ecological, environmental, topographical and physiological factors.

6.3 GROWTH BEHAVIOUR

The *Rubus macilentus* C. produced primocane in April month of 2016, during first season the growth of primocane was very rapid till June third week and produced many secondary shoots. By the next year in January 2017, the tertiary shoots were produced and the flowering occurred in same on both secondary and tertiary shoots, after flowering the death of floricane occurred. The *Rubus niveus* T. also produced the primocane in the second week of April 2016. The primary and secondary shoots which became deep reddish purple and produced the new shoots from secondary shoots during February moth of 2017 which is now called as floricane and the flowering occurred on primary, secondary as well as on tertiary shoots. The typical character of shoot was found to be natural mode of propagation through tip layering. The *Rubus paniculatus* S. was perennial in growth behavior hence plant was a big shrub with many primary, secondary and tertiary branches. New tertiary shoots were produced from previous season's secondary shoots during the May, 2016 and the flowering occurred on tertiary shoots in May 2017, the new tertiary shoots for flowering in next year appeared with commencement of flowering of previous year's shoots. After flowering the drying of secondary shoots were seen.

6.4 FLORAL BIOLOGY

6.4.1 Bearing Behavior: Two types of inflorescence were observed (i) Corymbose raceme and (ii) panicle (both axillary and terminal). The former was found in *Rubus macilentus* C. and *Rubus niveus* T. while later was found in *Rubus paniculatus* S. with flowers and buds facing towards gravity. Average maximum length of bearing shoot, average number of inflorescence per bearing shoots, average number of buds per inflorescence, average number of flowers per inflorescence and average number of flowers per bearing shoots was observed in *Rubus niveus* T. followed by *Rubus paniculatus* S. and minimum was recorded in *Rubus macilentus* C.

6.4.2 **Flower Bud Development:** Eight stages of flower bud development were observed in *Rubus macilentus* C. and the average total time required for flower bud to reach the anthesis was 45.4 days, while in *Rubus niveus* T. and *Rubus paniculatus* S. six stages of flower bud development were noticed with 40 and 34.8 days of average total time required for flower bud to reach the anthesis. The growth of flower buds in all the three wild species of raspberry under present investigation showed slow growth for initial days, later stages showed a rapid flower bud growth. The differences in flower bud development period may be due to the genetic makeup of the individuals, which appears to be a principle factors like in controlling flower bud development.

6.4.3 **Time and duration of flowering:** In *Rubus macilentus* C., the flowering started from last week of March and continued upto second week of June with its peak in last week of April while in *Rubus niveus* it started from last week of April and continued upto first week of June with its peak in last week of May. The total duration of flowering was recorded as 45 days. Whereas in *Rubus paniculatus* S., flowering appeared first week of May and continued upto first week of June with its peak in last week of May and total duration of flowering recorded was 40 days. The *Rubus macilentus* C. showed early and long duration (53 days) of flowering, followed by *Rubus niveus* T. for commencement and duration of flowering (45 days), while late and short duration of flowering (40 days) was observed in *Rubus paniculatus* S.

6.4.4 **Flower organization:** The flowers of three species under study, were regular, hermaphrodite, pedicillate, actinomorphic, bracteolate, complete, perfect, and hypogynous. Petal colour varied from white to pink and pedicel was hairy.

Flowers of *Rubus macilentus* C. was white with an average size (hight x diameter) of 0.85 x 2.35 cm and consisted of following four whorls: five Sepals (gamasepalous and united at the base), Corolla- five, Androecium - perigynous, indefinite (126.2), the stamens were generally arranged in four whorls, inner two whorls were small and outer two whorls were longer which were arranged freely. Anthers were small, bilobed dithecous, introse and basifixed; Gynoecium were on an average 112.6 in number. In *Rubus niveus* T. the flower had five sepals and petals. The petal colour ranged from pink to white, the size of the flower was less (0.74

X1.10cm) than other two species, the style was pink with green ovary on an average 103.9 in number and stamens were arranged freely in single whorl and were of equal lengths. *Rubus paniculatus* S. had flower with white petals and the petals were shorter than the sepals, androecium and gynoecium. The average size of the flower (hight x diameter) was 0.70 X 2.10 with on an average 28 gynoeciums and 107.25 androeciums. The pistils and stamens were curvy before anthesis after anthesis they became straight.

It was found that in addition to normal flowers, abnormal flowers were also appeared in *Rubus macilentus* C. and *Rubus niveus* T. The flower with 4 petals were associated with 4 sepals and had alternate arrangement, while 6 and 7 petals were associated with 5 sepals of *Rubus macilentus* C. and in *Rubus niveus* T. 4 and 6 petals were mostly associated with 4 and 6 sepals respectively had an alternate arrangement, even sometimes 6 petals were associated with 7 sepals.

6.4.5 **Anthesis:** The period of anthesis varied from 6 am to 6 pm with the peak period of anthesis with 41.43%, 40.96% and 33.96% had reached between 10 am to 12 noon for *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. Respectively. With the rise in temperature and decrease in relative humidity, the time and duration of anthesis were hastened. The mode of anthesis was divided into five stages and the splitting of buds observed from top to base of the buds in *Rubus macilentus* C. and *Rubus niveus* T. while in *Rubus paniculatus* S. the buds were cracked from base to top due to pressure exerted by the androecium and gynoecium.

6.4.6 **Anther dehiscence:** In most of the flowers the dehiscence started just after complete anthesis in all the species under study. In *Rubus macilentus* C. sometimes the anther dehiscence occurred before anthesis. The longitudinal mode of anther dehiscence, the anther lobes marginally bursting generally from top to base was observed in all the raspberry species under study. The major dehiscence period of the day was recorded between 10 am to 4 pm. The highest number of anthers i.e. 20.29%, 40.85% and 28.57% of dehisced between 10 am to 12 noon. Dehiscence complete on the day of anthesis in case of *Rubus niveus* T. while the dehiscence of 70.64% and 73.63% was recorded in *Rubus macilentus* C. and *Rubus paniculatus* S. respectively on the day of anthesis.

6.5 POLLEN STUDIES

6.5.1 Number of pollens per anther: On an average number of pollens per anther in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. was found to be around 900, 1100 and 1260 and number of pollens per flower was 96,525, 57,0901 and 1,59,012 respectively. The highest quantities of pollens are produced by *Rubus paniculatus* S. followed by *Rubus macilentus* C. and then *Rubus niveus* T.

6.5.2 **Morphology of pollen grains in different conditions:** Difference in the shape and size of the pollen grains was observed under different condition. The maximum length of 35.87µ and 35.44µ was recorded in *Rubus paniculatus* S. under dry and glycerine condition with oblong shape, while the maximum pollen width of 29.98µ and 26.92µ was recorded in water and acetocarmine respectively. While in *Rubus niveus* T. maximum pollen length of 32.11µ and 30.73µ was recorded in glycerine and dry conditions respectively and the maximum width of 28.54µ and 25.81µ was recorded in water and acetocarmine respectively. The maximum pollen length of 31.29µ and 30.74µ was recorded under glycerine and dry condition respectively in case of *Rubus macilentus* C. whereas the maximum pollen width of 28.28µ and 23.51µ was recorded under water and acetocarmine respectively. When the pollen grains were hydrated or placed in acetocarmine they took the spherical shape.

6.5.3 **Pollen viability and longevity:** In the present investigation, pollen grain viability of both *Rubus macilentus* C. and *Rubus niveus* T. flowers was 100% on the day of anthesis under natural condition. While in *Rubus paniculatus* S. the pollen viability was 73.33%. The pollen grains of *Rubus macilentus* C. and *Rubus paniculatus* S. shows viability for 7 days from anthesis. Whereas, the maximum pollen longevity of 9 days was recorded in *Rubus niveus* T. but in *Rubus paniculatus* S. the flowers showed a rapid decrease in the viability compared to other two species under natural condition.

6.5.4 **Pollen germination:** The raspberry species *Rubus macilentus* C. showed early (6 hours) and maximum pollen germination percentage (90.54%) with maximum pollen tube length (1,164.19 µ) recorded after 48 hours of pollen planting in 10% Sucrose + 2ppm Gibberellic acid and 5% Sucrose + 1ppm Gibberellic acid media respectively. *Rubus niveus* T. and *Rubus paniculatus* S. showed delayed germination

(24 hours). The maximum pollen germination percentage (62.96%) and pollen tube length (214.98µ) was recorded with 20% Sucrose + 4ppm Gibberellic acid and 20% Sucrose + 4ppm Gibberellic acid respectively, after 72 hours of pollen incubation in *Rubus niveus* T. *Rubus paniculatus* S. recorded maximum pollen germination (52.34%) and pollen tube length (135.22µ) with 25% Sucrose + 5ppm Gibberellic acid, after 72 hours of pollen planting.

6.6 STIGMA RECEPTIVITY

The studies on stigma receptivity by visual observation of all the raspberry species under study showed that fresh, shiny, sticky substance was present on stigmatic surface on the day of anthesis which gradually decreases and disappears after seven days of anthesis, in case of *Rubus paniculatus* S. the pistils and stamens remained curvy before the anthesis, on the day of anthesis they became completely straight, one day after anthesis again they became slightly curved. The stigma receptivity was investigated by fruit set method in all the three species, in *Rubus macilentus* C. stigma was found more receptive on the day of anthesis and one day after anthesis with 73.33% and 60.00% fruit set respectively, while in *Rubus niveus* T. with 86.66% and 76.66% fruit set on the day and one day after anthesis respectively. In *Rubus paniculatus* S., fruit set was not recorded due to pollination failure and stigma was more receptive on the day of anthesis.

6.6 POLLINATION STUDIES

The maximum fruit set on self pollination was reported in *Rubus niveus* T. (81.93%), followed by *Rubus macilentus* C. (71.63%), while no fruit set was noticed in *Rubus paniculatus* S. Maximum geitonogamy of 83.20% was noticed in *Rubus niveus* T. followed by 75.93% was observed in *Rubus macilentus* C. and geitonogamy was not successful in *Rubus paniculatus* S. maximum fruit set was recorded on crossing between *Rubus niveus* T. X *Rubus macilentus* C. (80.00%), followed by *Rubus niveus* T. X *Rubus paniculatus* S. (72.06%) and minimum was noticed in *Rubus macilentus* C. X *Rubus niveus* T. (68.29%), followed by *Rubus macilentus* C. X *Rubus paniculatus* S. (56.75%). No fruit set was recorded when *Rubus paniculatus* S. was used as female parent with in *Rubus macilentus* C. and *Rubus niveus* T.

Under open /natural pollination maximum fruit set of 88.93% recorded in *Rubus paniculatus* S. where other methods of pollination were failed to set fruit, while in *Rubus niveus* T. and *Rubus macilentus* C., 88.64% and 84.40% fruit set respectively, was recorded under natural mode of pollination, which reveals that the pollinators play a key role in fruit set increments which shows better results over any other methods of pollination. After third day of natural pollination of *Rubus paniculatus* S. clumping of stigmas were seen upon which the pollens were germinated. the species *Rubus macilentus* C. and *Rubus niveus* T. are compatible and could not able to determine the compatibility in *Rubus paniculatus* S. due to failure of pollination methods.

6.7 FRUIT SET

Rubus macilentus C. set fruits between second week of April to last week of June 2017, whereas in *Rubus niveus* T. the fruit set started from first week of May to last week of June 2017 and *Rubus paniculatus* S. showed the fruit set from third week of May to last week of June 2017. The fruit set in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. took 30 days, 26 days and 23 days respectively from commencement of flowering to first fruit set but as the temperature increases the ripening process accelerated.

Under the present study, average initial fruit set percentage of *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. was 55.73%, 73.42% and 68.75% of initial fruit set over an average of 166.8, 2,922.15 and 492.36 numbers of flowers per branch, but the maximum fruit retention (99.52%) was observed in *Rubus macilentus* C., *Rubus niveus* T. (97.63%) and *Rubus paniculatus* S. (88.71%).

6.8 CONCLUSION

Under hilly conditions of Uttarakhand, *Rubus macilentus* C. flower pass through eight stages, while *Rubus niveus* T. and *Rubus paniculatus* S. passed through six stages of flower bud development which showed slow growth during early stages and a rapid growth towards later stages. Time and duration of flowering is varies from 18th March to 15th June for *Rubus macilentus* C. and 24th April to 7th June for *Rubus niveus* T. while for *Rubus paniculatus* S. 1st May to 9th June. Peak anthesis of flower bud in all the three raspberry species under study varies from 10am – 12noon and peak anther dehiscence of

flowers varies from 10 am – 12 noon. The pollen viability of *Rubus macilentus* C. and *Rubus niveus* T. flowers was 100% while that of *Rubus paniculatus* S. was 73.33%. Pollen longevity of *Rubus macilentus* C. and *Rubus paniculatus* S. was upto 7 days whereas, pollens of *Rubus niveus* T. were viable upto 9 days under normal room temperature. When compared to others two species, the highest pollen germination (90.54%) and pollen tube length (1,164.19µ) was observed in *Rubus macilentus* C. with 10% Sucrose + 2ppm Gibberellic acid and 5% Sucrose + 1ppm Gibberellic acid respectively, after 48 hours of pollen planting in the media. But maximum pollen tube growth (244.74µ to 1,084.61µ) occurred between 18 hours to 24 hours.Stigma receptivity was highest on the day of anthesis in all the raspberry species under study. Raspberry species under study produced maximum fruits on natural pollination, while in *Rubus niveus* T. and *Rubus paniculatus* S. showed good fruit set on cross pollination and geitonogamy than any other method of pollination. The crosses between *Rubus niveus* T. X *Rubus macilentus* C. showed maximum fruit set than other interspecific pollination.

From the above observation it is conclude that, under hilly conditions of Uttarakhand, March to June is the best time to carryout breeding programmes since flowers of all the three species are available with maximum receptivity of stigma on the day of anthesis, The present study gave the estimate of time and duration of various flowering stages. though the fruit set on natural mode of pollination yielded more fruits, the crossing and geitonogamy also produced good amount of fruit set. The above information can be utilized in future breeding programmes that involve extensive crossing works. This research works serve as a vital step in further crop improvement programmes. The quality parameters may be studied further in future.

Chapter-7

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CHAPTER 7

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APPENDICES

APPENDIX- I

Appendix I: ANOVA Table for lenth of *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	3	66.341	22.114	10.236	0.00000
Error	20	13.207	2.160		
Total	23	109.548			

Appendix II: ANOVA Table for diameter of *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	3	282.125	94.042	7.651	0.00000
Error	20	15.833	12.292		
Total	23	527.958			

Appendix III: ANOVA Table for lenth of *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	3	122.094	40.698	42.116	0.00000
Error	20	19.327	0.966		
Total	23	141.421			

Appendix IV: ANOVA Table for diameter of *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	3	625.055	208.352	201.966	0.00000
Error	20	20.632	1.032		
Total	23	645.688			

Appendix V: ANOVA Table for lenth of *Rubus paniculatus* S.

SV	df	SS	MSS	F cal	SV
Treatment	3	293.093	97.698	88.756	0.00000
Error	20	22.015	1.101		
Total	23	315.107			

Appendix VI: ANOVA Table for diameter of *Rubus paniculatus* S.

SV	df	SS	MSS	F cal	SV
Treatment	3	802.108	267.369	221.118	0.00000
Error	20	24.183	1.209		
Total	23	826.292			

Appendix VII: ANOVA Table for 6hour of pollen germination *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	8007.112	533.807	2503.049	0.00000
Error	32	6.824	0.213		
Total	47	38,873.280			

Appendix VIII: ANOVA Table for 18hour of pollen germination *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	6,002.982	400.199	2,107.128	0.00000
Error	32	6.078	0.190		
Total	47	6,009.060			

Appendix IX: ANOVA Table for 24 hour of pollen germination *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	9,817.828	654.522	7,710.210	0.00000
Error	32	2.716	0.085		
Total	47	9,820.545			

Appendix X: ANOVA Table for 48hour of pollen germination *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	13,360.636	890.709	9,049.671	0.00000
Error	32	3.150	0.098		
Total	47	13,363.786			

Appendix XI: ANOVA Table for 24 hour of pollen germination *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	240.551	16.037	470.125	0.00000
Error	32	1.092	0.034		
Total	47	241.643			

Appendix XII: ANOVA Table for 48 hour pollen germination *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	2,878.218	191.881	5,399.459	0.00000
Error	32	1.137	0.036		
Total	47	2,879.355			

Appendix XIII: ANOVA Table for 72 hour pollen germination *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	4,360.455	290.697	14,955.875	0.00000
Error	32	0.622	0.019		
Total	47	4,361.077			

Appendix XIV: ANOVA Table for 24 hour pollen germination *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	965.451	64.363	3,649.168	0.00000
Error	32	0.564	0.018		
Total	47	966.016			

Appendix XV: ANOVA Table for 48 hour pollen germination *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	6,891.013	459.401	39,112.526	0.00000
Error	32	0.376	0.012		
Total	47	6,891.388			

Appendix XVI: ANOVA Table for 72 hour pollen germination *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	7,676.994	511.800	50,050.960	0.00000
Error	32	0.327	0.010		
Total	47	7,677.321			

Appendix XVII: ANOVA Table for 6hour pollen tube length *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	11,132.68	742.179	33.208	0.00000
Error	32	715.181	22.349		
Total	47	11,847.86			

Appendix XVIII: ANOVA Table for 18 hour pollen tube length *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	146317.9	9,754.53	85.255	0.00000
Error	32	3,661.30	114.416		
Total	47	149979.2			

Appendix XIX: ANOVA Table for 24 hour pollen tube length *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	2835223	189014.9	4392.263	0.00000
Error	32	1377.075	43.034		
Total	47	2836600			

Appendix XX: ANOVA Table for 48hour pollen tube length *Rubus macilentus* C.

SV	df	SS	MSS	F cal	SV
Treatment	15	3,749,281.829	249,952.122	8,402,577.922	0.00000
Error	32	0.952	0.030		
Total	47	3,749,282.781			

Appendix XXI: ANOVA Table for 24 hour pollen tube length *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	30804.06	2053.604	33.403	0.00000
Error	32	1967.374	61.48		
Total	47	32771.44			

Appendix XXII: ANOVA Table for 48 hour pollen tube length *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	131113.6	8740.905	134.294	0.00000
Error	32	2082.804	65.088		
Total	47	133196.4			

Appendix XXIII: ANOVA Table for 72 hour pollen tube length *Rubus niveus* T.

SV	df	SS	MSS	F cal	SV
Treatment	15	148401.6	9893.439	65.295	0.00000
Error	32	4848.617	151.519		
Total	47	153250.2			

Appendix XXIV: ANOVA Table for 24 hour pollen tube length *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	20,477.48	1,365.17	18.882	0.00000
Error	32	2,313.65	72.302		
Total	47	22,791.13			

Appendix XXV: ANOVA Table for 48 hour pollen tube length *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	26,401.67	1,760.11	25.773	0.00000
Error	32	2,185.33	68.292		
Total	47	28,586.99			

Appendix XXVI: ANOVA Table for 72 hour pollen tube length *Rubus paniculatus* D.

SV	df	SS	MSS	F cal	SV
Treatment	15	41,924.61	2,794.97	26.909	0.00000
Error	32	3,323.76	103.867		
Total	47	45,248.37			

ABSTRACT

ABSTRACT

Name of the student : Hemavati Hiregoudar **Id. Number** : 15209
Year of admission : 2015 **Degree** : M.Sc. Horticulture (Fruit Science)
Major Field : Fruit Science **Department** : Fruit Science
Minor Field : Genetics and Plant Breeding
Title of Thesis : **"Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya"**

The present investigations entitled "Studies on Morphology, Floral Biology and Pollination Behaviour of Different Wild Species of Raspberry (*Rubus* species) of Garhwal Himalaya" was carried out in College of Horticulture, VCSG, UUHF, Bharsar, from April 2016 – June 2017. The different parameters of morphology, floral biology and pollination were recorded on the basis of time period. Raspberry species under study showed wide variability with respect to cane morphology and leaf morphology as well as phyllotaxy, which can be efficiently used in identification and documentation of *Rubus* species. The result showed that the raspberry species produces primocane and floricane with different morphological characters. The floral biology study revealed that the total span of flower bud development from bud emergence to the anthesis has been described into eight different stages in *Rubus macilentus* C. and six stages in *Rubus niveus* T. and *Rubus paniculatus* S. the time required for flower bud to reach the anthesis was 45.4, 40 and 34.8 days for *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. respectively. The peak time of anthesis was 10am to 12noon for all the three species with maximum of 41.43% in *Rubus macilentus* C. and 40.96% in *Rubus niveus* T., while in *Rubus paniculatus* S. 33.96% of anthesis was recorded. The major dehiscence period of the day was recorded between 10 am to 4 pm for *Rubus niveus* T. while in *Rubus macilentus* C. and *Rubus paniculatus* S. it was ended between 10 am to 6 pm. The highest number of anthers dehisced between 10 am to 12 noon for all the three species. The maximum of 20.29%, 40.85% and 28.57% dehiscence was recorded in *Rubus macilentus* C., *Rubus niveus* T. and *Rubus paniculatus* S. respectively. The highest quantities of pollens were produced by *Rubus paniculatus* S. (1,59,012 pollens/flower) followed by *Rubus macilentus* C. (96,525 pollens/flower) and then *Rubus niveus* T. (57,090 pollens/flower). Among the different media tested, for the raspberry species under study, the size (length × width) of pollen grains was maximum (35.87 X 17.73µ) in dry condition for *Rubus paniculatus* S. when compared to other two species. *Rubus macilentus* C. recorded maximum pollen germination (90.54%) and pollen tube length (1,164.19 µ) after 48 hours of planting with 10% Sucrose + 2ppm Gibberellic acid and 5% Sucrose + 1ppm Gibberellic acid respectively and *Rubus niveus* T. recorded maximum pollen germination (62.96%) and tube length (214.98µ) after 72 hours of pollen incubation with 20% Sucrose + 4ppm Gibberellic acid, while in *Rubus paniculatus* S., after 72 hours of pollen planting in 25% Sucrose + 5ppm Gibberellic acid recorded highest pollen germination (52.34%) and pollen tube length (135.22µ). Best receptivity of stigma on the day of anthesis has been witnessed by 73.33% and 86,66% of fruit set in *Rubus macilentus* C. and *Rubus niveus* T. while in *Rubus paniculatus* S. by visual method it was confirmed. The cross pollination of *Rubus niveus* T. X *Rubus macilentus* C. recorded maximum (80.00%) fruit set, while *Rubus paniculatus* S. reported highest (88.93%) fruit set on open pollination. The investigation is concluded that the best time and duration of raspberry for production and breeding programme is from middle of April to last week of June under hilly condition of Uttarakhand.

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सांराश

नाम–हेमावती हिरेगौडर
प्रवेश का वर्ष – 2015
मुख्य विषय– फल विज्ञान
लघु विषय– आनुंवाशिकी एवं पादप प्रजनन

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विभाग– फल विज्ञान

शोध का शीर्षक– गढ़वाल हिमालय की विभिन्न हिंशालू वन्य प्रजातियों (रूबस प्रजातिया) की आकारिकी, पुष्प जीव विज्ञान एवं परागण व्यवहार का अध्ययन।

वर्तमान शीर्षक "गढ़वाल हिमालय की विभिन्न हिशालूं वन्य प्रजातियों (रूबस प्रजातिया) की आकारिकी, पुष्प जीव विज्ञान एवं परागण व्यवहार का अध्ययन" औद्यानिकी महाविद्यालय वि0च0सिं0ग0 उत्तराखण्ड औद्यानिकी एवं वानिकी विश्वविद्यालय भरसार में अप्रैल 2016 से जून 2016 तक किया गया। आकारिकी, पुष्प जीव विज्ञान और परागण के विभिन्न मापदण्डों को समय के आधार पर दर्ज किया गया था। अध्ययन के तहत हिंशालू प्रजातियों ने लता और पत्ती के आकारिकी के साथ–साथ पर्णविन्यास के सम्बन्ध में व्यापक परिवर्तनशीलता दिखाई है जो रूबस प्रजातियों की पहचान और प्रलेखन में कुशलता से उपयोग किया जा सकता है। शोध नतीजा यह दर्शाता है कि हिंशालू प्रजातिया विभिन्न आकारिकी वर्णो के साथ प्राइमोकेन और फ्लोरिकेन का उत्पादन करती हैं, पुष्प विज्ञान के अध्ययन से पता चला कि पुष्पों की कली के विकास में, कली अंकुरण की उपस्थिति से लेकर पुष्प खिलने तक की अवधि, रूबस मेसीलेन्टस मे 8 तथा पेनिकुलेटस व निवियस में 6 चरणों में विभाजित किया गया। स्फुटन तक पहुचने के लिए पुष्प कली को क्रमशः 45.4, 40.00,और 34.8 दिन रूबस मेसीलेन्टस, रूबस निवियस, व रूबस पेनीकुलेटस, के समय की आवश्यकता हुई। सभी तीन प्रजातियों में स्फुटन का शिखर समय सुबह 10.00 बजे से दोपहर 12.00 बजे तक था, रूबस मेसिलेन्टस मे अधिकतम 41.43 प्रतिशत और रूबस पेनिकुलेटस में 33.96 प्रतिशत स्फूटन दर्ज किया गया था। प्रमुख स्फूटन अवधि रूबस निवियस के लिए सूबह 10.00 बजे से शाम 4.00 बजे तक दर्ज की गई थी, जबकि रूबस मेनिकुलेटस और रूबस पेनिकुलेटस में यह सुबह 10.00 बजे से शाम 6.00 बजे तक समाप्त हो गया था। सभी तीन प्रजातियों में सुबह 10.00बजे से दोपहर 12.00 बजे के मध्य सर्वाधिक परागकोष स्फुटन दर्ज किया गया, जोकि कमशः रूबस मेसिलेन्टस,रूबस निवियस, व रूबस पेनिकुलेटस मे अधिकतम क्रमशः 20.29प्रतिशत और 28.57 प्रतिशत दर्ज किया गया। परागो की अधिकतम मात्रा रूबस पेनिकुलेटस (15,9012 पराग/पुष्प) तदोपरान्त रूबस मेसिलेन्टस (96525 पराग/पुष्प) और रूबस निवियस में (57090 पराग/पुष्प) पाये गये। अध्ययन के तहत, हिंशालू प्रजातियों के लिए प्रयोग किये जाने वाले विभिन्न माध्यमों में पराग कणों का आकार (ल0×चौ0)शुष्क स्थिति में अधिकतम (35.87×17.73) पाया गया, जो कि रूबस पेनिकुलेटस में दर्ज किया गया। परागकणों का सर्वाधिक अंकुरण (90.54 प्रतिशत) एवं पराग नलियों की लम्बाई (1164.19 μ) रूबस मेसिलेन्टस में 48 घण्टों के बाद क्रमशः 10 प्रतिशत शंकरा विलयन + 2 पी0पी0एम0 जिबरेलिक ऐसिड एवं 5 प्रतिशत शंकरा विलयन + 1 पी0पी0एम0 GA_3 में दर्ज किया गया, तथा रूबस निवियस में अधिकतम पराग अंगकुरण (62.96 प्रतिशत) व पराग नली की लम्बाई (214.98 μ) 48 घण्टों के बाद 20 प्रतिशत शंकरा +4 पी0पी0एम0 GA_3 जबकि रूबस पेनिकुलेटस में 48 घण्टों के बाद 25 प्रतिशत शंकरा + 5 पी0पी0एम0 GA_3में अधिकतम पराग अंगकुरण (52.34 प्रतिशत) तथा पराग नली की लम्बाई (135.22 μ) दर्ज की गई। रूबस मेसिलेन्टस और रूबस निवियस में, वर्तिकाग्र की सर्वश्रेष्ठ ग्रहणशीलता, फल स्थापन विधि से क्रमशः 73.33 प्रतिशत और 86.66 पुष्प स्फुटन के दिन देखी गयी थी। जबकि रूबस पेनिकुलेटस में दृश्य विधि द्वारा इसकी पुष्टि की गयी। परपरागण के दौरान रूबस निवियस×रूबस मेसिलेन्टस में अधिकतम (80 प्रतिशत) फल स्थापन दर्ज किया गया था, जबकि रूबस पेनिकुलेटस में प्राकृतिक परागण करने पर उच्चतम (88.93 प्रतिशत) फलों का उत्पादन किया गया। वर्तमान शोध कार्य के परिणाम से यह निष्कर्ष निकलता है कि, उत्तराखण्ड की पहाड़ी स्थिति में हिंशालू के सबसे अच्छे उत्पादन एवं प्रजनन कार्य के लिए सर्वोत्तम समय एवं अवधि अप्रैल के मध्य से लेकर जून के अन्तिम सप्ताह तक है।

हेमावती हिरेगौडर
लेखिका

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सलाहकार

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CURRICULUM VITAE

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