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PHENOLOGY, POLLEN QUANTITY AND QUALITY OF VARIOUS EXOTIC CHERRY CULTIVARS IN JAMMU AND KASHMIR

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ABSTRACT
The objective of this study was to determine phenological, pollen quality and quantity of Stella, Sweet Heart and Lappins sweet cherry (*Prunus avium* L.) cultivars grown in Kashmir. During the study phenological stages *viz.* white tip, initial bloom, full blooming, petal fall and maturity dates were determined. Cultivar Lappins and Sweet Heart had earliest white tip stage (31st March) and full blooming (15th April) whereas in Stella, it was observed on (2nd April) and (16th April) respectively. Highest pollen viability (82.90%) was obtained in Stella and lowest in Lappins (78.10%). Maximum pollen germination was observed in Stella (58.66%) on a media containing 1% Agar + 15% sucrose + 0.025 g Boric acid where as lowest was observed in media containing 1% Agar + 5% Sucrose in Sweet Heart (26 %). A significant difference in pollen production rate was observed in all the cultivars with maximum pollen grains per flower in Stella (9764.75) and minimum in Lappins (6640.9). Maximum anther length and width was observed in Stella (852.4¼m and 745.60¼m) and minimum in Lappins (513.10¼m, 413.70¼m) respectively. Pollen length was recorded maximum in Stella (14.01¼m) and minimum in Sweet Heart (11.08¼m). Maximum pollen width was recorded in Stella (10.73¼m) and minimum in Sweet Heart (8.76¼m).

INTRODUCTION

Sweet Cherry (*Prunus avium* L.) occupies an important position among temperate fruits all over the world and is the season's first fruit crop to reach the market. Cherry belongs to family Rosaceae and genus *Prunus*. In India, Jammu and Kashmir, parts of Himachal Pradesh and Uttarakhand are main contributors of cherry production, among which Jammu and Kashmir contributes about 95 per cent of total production (Anonymous, 2013). Phenology determines the different phases of the life cycle of the plant and plant productivity. Phenology mainly focuses on time of flowering and cherry has received a considerable attention of the researchers as flowering time and production are highly correlated. Determining of the phenological stages and viability, germination capacity of pollen grains *in vitro* and fertility level of new cultivars are very important for breeders and growers. One of the important factors for fertilization success is pollen viability and germination. Pollen viability is an ability of a pollen grain to germinate and develop as a pollen tube (Prajapati and Jain, 2011). According to Thompson (2004), first requirement for economical fruit production is the availability of an adequate source of viable and compatible pollen. A good pollinizer should produce well developed pollen with both high viability and germination rates and having fertilization ability. Fruit set of different sweet cherry cultivars have been shown to be influenced by pollen donors (Sutyemez, 2011). Main and pollinizer cultivars should be cross compatible, have an overlapping blooming period and produce high amount of viable pollen. Pollen performance includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen competition and is an important component of fertilization success in fruit trees (Thompson, 2004; Hedley *et al*., 2004 and Koyuncu and Tosun, 2005). Recently some exotic cherry varieties namely Lappins, Sweet Heart and Stella have been introduced in Kashmir from Europe and planted in Zangam nursery at Pattan, Baramulla. The present investigation was carried out with the objective of studying the phenology, pollen quantity and quality of these exotic cherry cultivars in Kashmir conditions.

MATERIALS AND METHODS

The experimental site is situated at an elevation of 5266 ft at 34º08.927 N latitude 74º34.078 E longitudes in North Kashmir The experiment was conducted during 2014-2015 with an objective to determine the phenology of newly introduced sweet cherry cultivars, to determine the pollen viability, pollen germination, pollen production capacity and anther and pollen morphology. When about 10% of the flowers from the selected branches of the cultivars were open and rest of the flowers were either in white tip stage or in balloon stage, the date was recorded as date of initial bloom and the number of days taken up to initial bloom after 1st March (Reference Date) were worked out and when about

80% of the flowers were open, the date was deemed as full bloom and the number of days taken up to full bloom from the reference date was worked out. Similarly, date of maturity along with number of days from full bloom to harvesting date was determined when the cultivar acquired proper maturity standards as per colour, size and taste.

The flowers at balloon stage were transferred to the laboratory immediately. Anthers were removed and placed into a dark coloured bottle to promote dehiscence at room temperature. The pollen was collected and stored in refrigerator for further study. Pollen germination test was performed according to Eti (1990). Fresh pollen grains were used for *in-vitro* germination tests. The pollen grains were dusted on petri plates containing agar power (1%) solidified with different concentrations of sucrose (5, 10, 15, and 20%) alone and in combinations with boric acid (0.025g). After the pollen dusting, the petri plates were incubated at 25 ± 1ºC for 24 hours. After 24 hours of incubation, the pollen grains were observed under the microscope at magnification of 10 x 40 X. The germinated pollen grains (pollen grain was considered as germinated when the length of the pollen tube was equal or exceeded its diameter) were also counted. Pollen viability was tested according to Eti (1990). The viability of pollen grains was determined by 2, 3, 5- triphenyl tetrazolium chloride (TTC) stain test. One per cent of tetrazolium salt soulution was prepared by dissolving 1 gm of tetrazolium salt in 100 ml distilled water. The pH of the solution was adjusted to 7.0 for proper staining. Drops of TTC solution were placed on glass slides and were evenly dusted with fresh pollen and kept at room temperature for 2 hours under day ligh. Pollen with blood red color was counted as viable and with yellowish color or colorless as non-viable.

For determining pollen production rate of selected cultivars, twenty flowers collected randomly from each replicate were evaluated for number of anthers per flower (AF), number of pollen grains per flower (PF) and number of pollen grains per anther (PA). The pollen grains per anther were calculated using haemocytometer (Fein-Optic, Blankenburg) (Eti, 1990). Ten anthers from the male parent were selected and collected in the test tube and were completely shaken so that the pollen gets dehisced from anthers. Distilled water was added to the pollen collected in the test tube and a volume was made upto 1ml. This solution was then shaken and a homogenous pollen suspension was prepared. A drop of the homogenous pollen suspension was placed into one of two counting chambers and then it was covered with a cover slip and observed under microscope. The pollen was counted in A-square (0.1mm³). The calculations were made as flows:

n = number of pollen grains in A-square No of pollen of pollen in 1ml = n x 10000

In the present study, 10 anthers were selected, so pollen grains per anther were calculated.

$$\text{No. of pollen grains per anther} = \frac{\text{No of pollen grains in 1ml}}{10}$$

The pollen grains per flower (PF) were calculated as: PF = PA x AF

Besides this, pollen and anther morphology (using Ocular microscope) was also determined. The data generated during an experiment was analyzed in a completely randomized and block randomized design. To satisfy module, assumptions of experiment were subjected to critical difference.

RESULTS AND DISCUSSION

Phenology

The phenological data is presented below in Table 1 and (plate I to IV) showing different phenological stages in cherry. The white tip stage in sweet cherry cultivars under study commenced from 31st of March and completed on 2nd April. The first white tip was recorded on 31st March (31 days DFRD) in Sweet Heart and Lappins followed by Stella on 2nd April. The days to white tip stage ranged from 31 to 33 days. The initial bloom (10% flowers open) was observed from 6th to 8th April. The initial bloom was observed on 6th April in Sweet Heart and Lappins whereas, initial bloom in Stella was observed on 8th April. The full bloom (80%) in cherry cultivars commenced from 15th to 16th April. The full bloom was observed on 15th of April in all cultivars except Stella which was observed on 16th April. The days to full bloom ranged from 46 to 47 days from the reference date. The maximum number of days to full bloom was observed in Stella (47 days) and minimum in Sweet Heart and Lappins (46 days). The petal fall was observed in Sweet Heart and Lappins on 22nd

Table 1: Phenological stages of exotic sweet cherry cultivars

S.No	Name of the cultivar	Date of White Tip	Days towhitetip	Date of initial bloom (10%)	Days toinitial bloom	Date of full bloom (80%)	Days to Full Bloom	Date of petalfall	Days topetal fall	Date of Fruit Maturity	Days from Full bloom
1	Stella	2/4	33	8/4	39	16/4	47	25/4	56	11/6	66
2	Sweet Heart	31/3	31	6/4	37	15/4	46	22/4	53	30/6	76
3	Lappins	31/3	31	6/4	37	15/4	46	22/4	53	02/7	78

Reference date : 1st March

Table 2: Pollen viability (%) of sweet cherry cultivars determined by TTC (2,3-5 Triphenyl tetrazolium chloride) stain test

Genotype	Viable (%)Mean ± S.D	Dead (%)Mean ± S.D
Stella	82.90 b ±2.92	17.10 b ±1.63
Sweet Heart	80.20 b ±2.39	19.10 c ±2.28
Lappins	78.10 b ±2.42	22.10d ± 1.85
CD (p ≤0.05)	2.378	1.781

Table 3: Pollen production parameters of sweet cherry cultivars

Genotypes	AFMean ± S.D	PFMean ± S.D	PAMean ± S.D
Stella	38.5 a ± 0.527	9764.75 a± 22.28	253.63 a ± 3.29
Sweet Heart	38.0 b ±1.054	9268. 25 b ± 23.72	243.90 b ± 3.73
Lappins	37.1 c ±0.875	6640.90 c ± 27.93	179 c ± 4.454
CD 0.05	0.77	152	38.54

AF = Number of Anthers per flower ; PF = Number of pollen grains per flower; PA = Number of pollen grains per Anther = PF/ AF

Table 4: Anther and pollen morphology of studied cultivars

Genotype	Anther Character Length	Width	Shape	Pollen Character Length	Width	Shape
Stella	852.4a±10.67	745.6a±7.73	ProlateSpheriodal	14.01a ± 0.92	10.73a± 0.61	Sub Prolate
Sweet Heart	773.6b±10.69	654.3b± 14.55	Sub Prolate	11.08b ± 0.98	8.76 b± 0.62	Sub Prolate
Lappins	513.1c ±22.75	413.7c ±6.37	Sub Prolate	12.11b ± 0.49	10.36 a ±0.60	Sub Prolate
CD (p ≤ 0.05)	13.47	10.70		0.69	0.46	

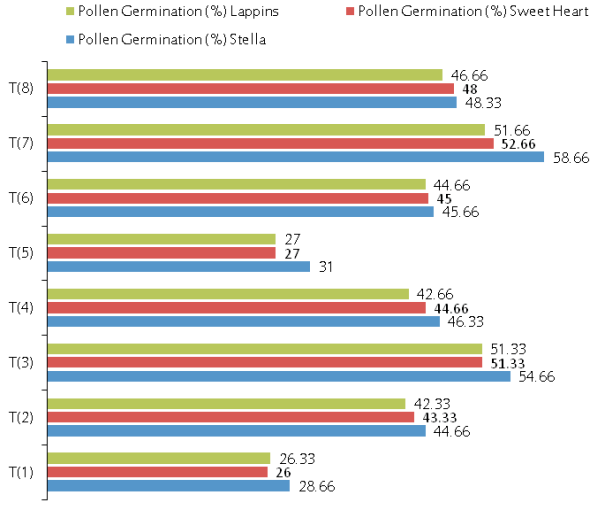
Shapes on the Basis of (Length: Width; < 1.0 = (Oblate Spheriodal); 1.0-1.14 = (Prolate Spheriodal); – 1.33 = (Sub Prolate)

Plate I: First bloom

Plate II: Initial Bloom

Plate III: Full Bloom

Plate IV: Fruit set



T(1) 1 % Agar + 5% Sucrose
T(2) 1 % Agar + 10% Sucrose
T(3) 1 % Agar + 15% Sucrose
T(4) 1 % Agar + 20% Sucrose
T(5) 1 % Agar + 5 % Sucrose + 0.025 g Boric acid
T(6) 1 % Agar + 10 % Sucrose + 0.025 g Boric acid
T(7) 1 % Agar + 15 % Sucrose + 0.025 g Boric acid
T(8) 1 % Agar + 20 % Sucrose + 0.025 g Boric acid

Figure 1: Pollen germination of selected cherry cultivars

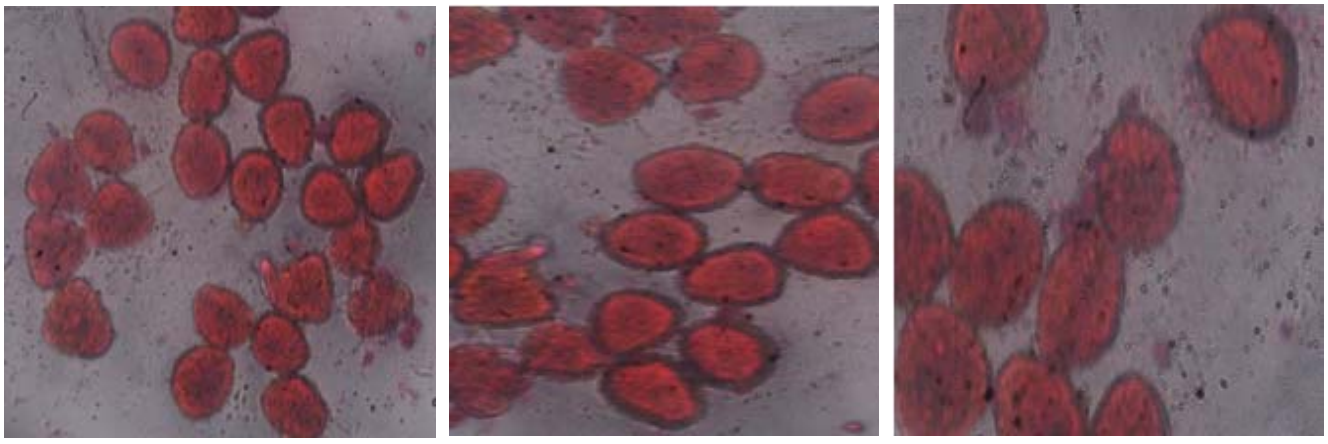
April followed by Stella on 25th April. The maximum numbers of days to petal fall from reference date were observed in Stella (56 days) and minimum in Sweet Heart and Lappins (53 days). The date of fruit set in cherry cultivars was observed from 3rd May to 4th May. In all cultivars, fruit set was observed in 64 days, however in Stella it was observed in 65 days from the reference date. Date of fruit maturity: Date of fruit maturity in the various cultivars studied varied between mid-June to 1st week of July. Stella took only 65 days while as Sweet Heart and Lappins took 76 and 78 days respectively from full bloom to maturity.

The results of present study are supported by the results of Glowacka and Rozpara (2014) who observed that all cherry trees began flowering in the last week of April and finished in the first week of May. The period of flowering lasted from 10 - 14 days. The results of the present investigation with respect to blooming and fruit set are in agreement with previous reported work in different sweet cherry cultivars (Tosun and Koyuncu, 2007, Gratacose and Cortes, 2008 and Moghadam *et al.*, 2009).

Pollen viability

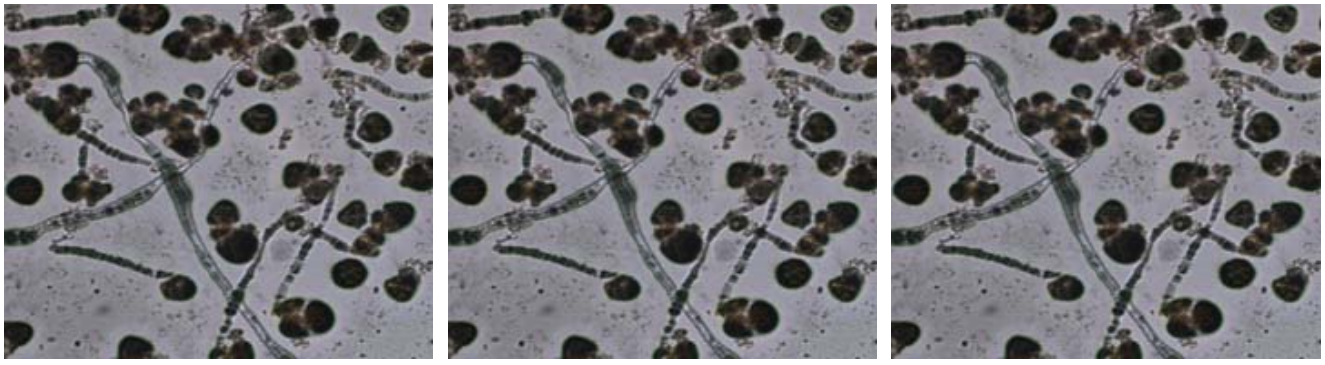
Pollen germination

A significant difference in pollen germination was recorded among various cultivars in different media (Fig.1). Pollen germination percentage increased in all cultivars (Plate VI) upon increasing sucrose concentration upto 15% and decreased at higher concentration. Pollen germination percentage of 54.66 was recorded in medium containing 1% agar+15% sucrose in Stella (Plate VI.A) followed by 51.33 per cent both in Sweet Heart (Plate VI.B) and Lappins (Plate VI.C). The lowest (26.00%) germination percentage was recorded in Sweet Heart in medium containing 1% Agar + 5% sucrose. Pollen germination percentage increased in combination with boron and decreased at higher concentrations of sucrose. Highest (58.66%) germination



A: Stella **B: Sweet Heart** **C: Lappin's**

Plate V: Pollen viability of sweet cherry cvs. cvs.cultivars



A: Stella **B: Sweet Heart** **C: Lappin's**

Plate VI: Pollen germination of sweet cherry cvs.

percentage was recorded in Stella in the media 1% Agar + 15% sucrose + 0.025g boric acid in Stella followed by Sweet Heart (52.66%). The lowest (27.00%) pollen germination was recorded in Sweet Heart and Lappins in media containing 1% agar + 5% sucrose + 0.025gm boric acid.

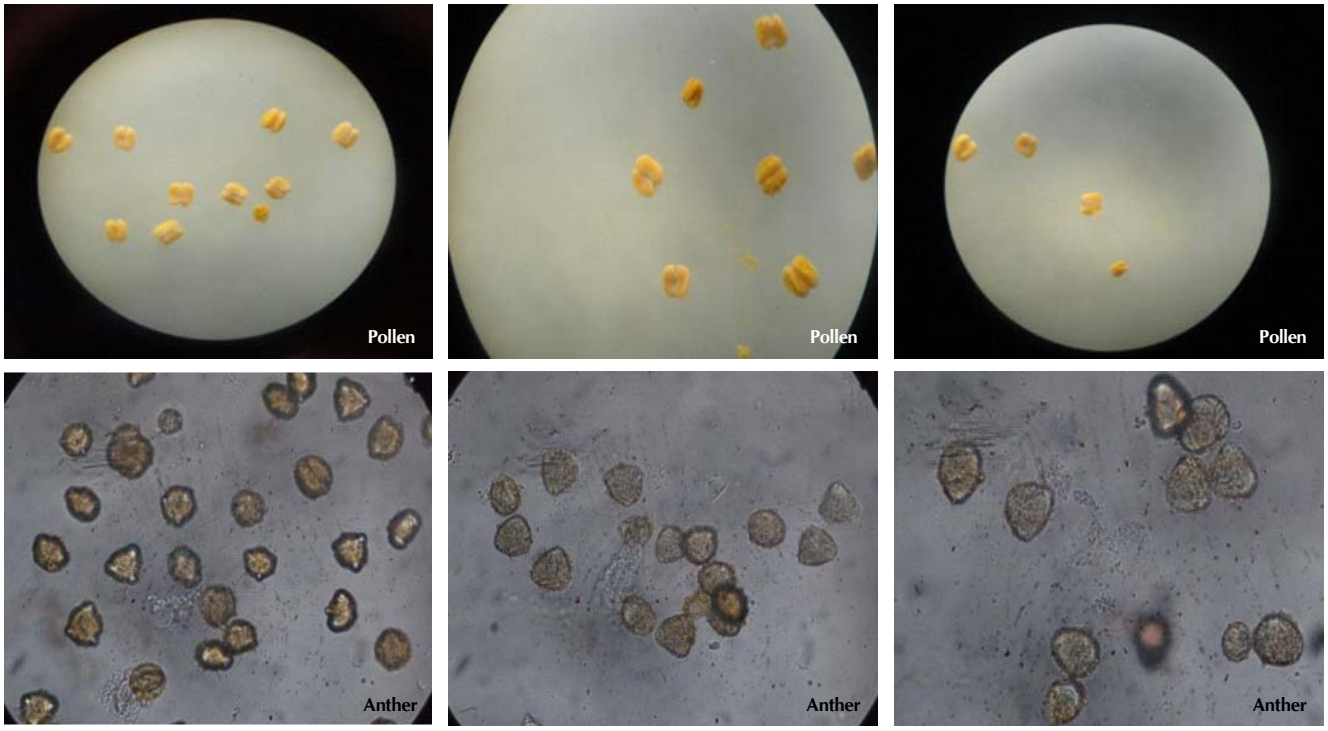
The above results indicated that the germination was improved by higher sucrose concentration of 15% and with the addition of Boric acid the germination percentage increased further. The pronounced effect of sucrose and boric acid on increasing pollen germination is in accordance with Johri and Vasil (1961) and Shivanna and Johri (1989), who stated that the externally supplied sucrose maintains the osmotic pressure and acts as a substrate for the pollen metabolism. Boron is believed to promote pollen germination by affecting Hz -ATPase activity, which initiates pollen germination and pollen tube growth. Stanley and Loewus (1964) reported that boron is directly involved in pectin synthesis and thus indirectly involved in the development of pollen tube membrane. Boron helps in the growth of pollen and growing pollen tubes in vascular plants (Sidhu and Malik 1986). Likewise various plant extracts enhance pollen germination and pollen tube growth (Sarika and Mary, 2012).The results of the present investigation are supported by work carried by various workers namely Hormaza and Herrero (1999), Paydas *et al*. (1998) in different sweet cherry cultivars.

Pollen Viabilty

Viability of pollen grains was determined through Triphenyl Tetrazolium Chloride (TTC) staining test (Plate V). A significant difference in viability was observed among various cultivars studied (Table 2). The highest pollen viability (82.90%) was recorded in Stella (Plate VA), followed by Sweet Heart (80.20%){Plate VB} and lowest (78.10%) was recorded in Lappins (Plate VC), while as the dead pollen percentage were highest in Lappins (21.90%). Tosun and Koyuncu (2007) and Sutyemez (2011) estimated pollen viability in various sweet cherry *cvs*. using TTC and FDA stains and reported that pollen viability ranged between 79 to 91 per cent using TTC test. The pollen viability ratios of the studied cherry cultivars were over 75 per cent). The findings of the present study are in accordance with the previous studies (Sutyemez and Eti, 1999 and Davarynejad *et al.*, 2008).

Pollen production rate

The data on pollen production rate of Stella, Sweet Heart and Lappins is given in the Table 3.The highest number of anthers (38.5) per flower were recorded in Stella followed by (38.0) in Sweet Heart. The lowest numbers of anthers (37.1) were observed in Lappins. The highest (9764.75) number of pollen grains was observed in Stella followed by Sweet Heart (9268.25) and lowest (6640.93) number of pollen grains per



A: Stella **B: Sweet Heart** **C: Lappins**

Plate VII: Anther and pollen morphology

flower were observed in Lappins. A significant difference in respect of pollen grains per flower was observed among various cultivars. Significant difference in the pollen grains per anther was recorded among the genotypes (Table 3,). The highest number of pollen grains (253.63) per anther was recorded in Stella followed by (243.98) per anther in Sweet Heart and the lowest numbers of pollen grains (179) per anther were recorded in Lappins . For a cultivar to be used as a pollinizer, in addition to high pollen viability and pollen germination rates, it is important that its anther produce high amount of pollen. The highest pollen production and pollen viability are important for fertilization because not all pollens germinated on stigma reach the carpels, thus for successful fertilization, pollen cultivars producing high amount of pollens are desired (Stosser, 1984). Almost similar results with respect to number of anthers per flower, number of pollen grains per flower and number of pollen grains per anther were reported by Tosun and Koyuncu, (2007) and Sutymez (2011) while studying fertilization biology of different sweet cherry cultivars.

Anther and pollen morphology

The data on anther and pollen morphology of Stella, Sweet Heart and Lappins is given in the Table 4, Plate VIII. The highest anther length (852.40μm) was recorded in genotype Stella followed by Sweet Heart (773.60ìm). The lowest anther length was observed in male genotype Lappins (513.10μm) as shown in the Table 4. In Stella, maximum anther width (745.60ìm) was observed followed by Sweet Heart (654.30ìm). The minimum anther length was recorded in Lappins (413.70μm). Prolate spheriodal anther shape was observed in Stella , whereas, Sweet Heart and Lappins had Sub-prolate shapes on the basis of Length: Width. The highest pollen length (14.01μm) was observed in Stella followed by Lappins (12.11μm). The lowest anther length was recorded in Sweet Heart (11.08m). A significant difference in pollen widths was observed in aμll cultivars under study. Maximum pollen width (10.73μm) was observed in Stella followed by Lappins (10.36μm) and the minimum pollen width (8.76μm) was recorded in Sweet Heart. Pollen shapes were observed on the basis of Length: width ratio. Stella, Sweet Heart and Lappins had similar shape *i.e.* Sub- prolate (Table 4). These finding with respect to pollen and anther morphology are in agreement with Tosun and Koyuncu (2007) and Sutymez (2011).

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