Effect of pollination between commercial and wild strawberry

A thesis
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Uttar Banga Krishi Viswavidyalaya

In partial fulfillment of the requirements for the award of the degree of

MASTER OF SCIENCE (HORTICULTURE)
in
FRUIT SCIENCE

by

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(H-2021-010-M)

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WHERE WISDOM IS FREE

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FACULTY OF HORTICULTURE
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CERTIFICATE I

This is to certify that the work recorded in the thesis entitled, **"Effect of pollination between commercial and wild strawberry"** submitted by **Miss Diya Ghosal (H-2021-010-M)** in partial fulfillment of the requirements for the degree of **M.Sc. (Horticulture) in Fruit Science** of the Uttar Banga Krishi Viswavidyalaya, Pundibari, is the faithful and bonafide research work carried out under my personal supervision and guidance. Her thesis has been checked against plagiarism through **URKUND** software on **03-08-2023** and that the similarity index has been achieved as **0%** which is below the maximum tolerable range as per stipulation of this Viswavidyalaya. The thesis of Miss Diya Ghosal may be accepted for the award of the Master's Degree in Master of Science (Horticulture) in Fruit Science of Uttar Banga Krishi Viswavidyalaya. The results of the study reported in the thesis have not so far been submitted for any other degree or diploma.

The assistance and help received from various sources during the course of investigation have been duly acknowledged.

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ABSTRACT

Strawberry (*Fragaria* × *ananassa* Duch.) is a man-made hybrid of the Rosaceae family and is well known for their aroma, vivid red colour, juicy texture, excellent flavor and antioxidant properties. Wild strawberries tend to be small in size as compared to cultivated ones but are hardy and adaptable in nature. To get a strawberry that is big and hardy in nature, it is necessary to cross and hybridize them to improve their quality. Strawberry is benefited from the action of pollinators. However, insufficient pollination can lead to an increase in the percentage of misshapen or malformed fruits. Therefore, proper pollination of strawberries is necessary to get desirable attractive strawberries that are highly marketable. In this experiment, the aim was to determine the effect of modes pollination between commercial and wild strawberry. The experiment consisted of 6 treatments of different modes of pollination (open-, self- and cross-pollination) with 5 replications of each genotype (Winter Dawn and *Potentilla indica*) in Randomized Block Design (RBD). The plant and flower morphology parameters were analyzed prior to conduction of the pollination treatments. The results of the study indicate that the open-pollination method in cultivated strawberry yields high-quality fruit with a maximum fruit weight (20.16 g), fruit length (4.02 cm), fruit width (3.34 cm), weight of 100 achenes (0.09 g), TSS (7.58°B), reducing sugar (3.42%), total sugar (4.90%), ascorbic acid (52.64 mg/100g), anthocyanin (32.12mg/100ml) and minimum acidity (0.66%). Conversely, the self-pollination method in wild strawberry yields low quality fruit. The fruit obtained after cross-pollination of cultivated and wild strawberry showed intermediate characteristics, however, the reciprocal cross was not successful. Consequently, the results of this study are indicative of the quality fruit production by open-pollination in strawberries. Further research is necessary to understand the effect of pollen and development of hybrid of wild and commercial strawberry for varietal improvement as wild species of strawberry have good biotic and abiotic stress tolerance traits.

Keywords: Commercial, pollination, *Potentilla indica,* strawberry, wild, Winter Dawn.

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ABBREVATION

%	: Per cent
°C	: Degree Celsius
am	: Ante meridiem
CD	: Critical difference
cm	: Centimeter
DAP	: Days after planting
et al.	: Co-workers
g	: Gram
i.e.	: That is
kg	: Kilogram
m	: Meter
mm	: Millimiter
MT	: Metric tones
NPK	: Nitrogen phosphorous potassium
ns	: Non significant
PCR	: Polymeric Chain Reaction
pm	: Post meridiem
rpm	: revolution per minute
S.Em(±)	: Standard error of mean
TSS	: Total soluble solids
μ	: Micron

Chapter 1

INTRODUCTION

INTRODUCTION

The present day cultivated strawberry (*Fragaria ananassa* Duch.) is an octoploid hybrid of two native American species, *Fragaria chiloensis* and *Fragaria virginiana*, and belongs to the Rosaceae family (Hussain *et al.*, 2021). This hybrid species was discovered in the early 1700s in France, when the only form of strawberry available was the wild *Fragaria vesca*. This cultivar was little yet exceptionally sweet and aromatic. The French discovered that the native American strawberries were bigger in size (Sharma *et al.,* 2015). *F. virginiana* produces high-quality soft aromatic fruit that is tiny in size, but *F. chiloensis* produces large sized fruits. Crossing *F. chiloensis* (pistillate) with *F. virginiana* (staminate) yields larger, high-quality fruits known as *Fragaria* × *ananassa* (Sharma *et al.,* 2015).

Strawberry is a beautiful, sweet and refreshing fruit with a distinct aroma and delicate flavour. Fresh, ripe strawberries are a great source of vitamins and minerals. Aside from table use, it can be used for canning, confectionery making, jam and jelly making, and flavouring ice cream. Strawberry has the extra advantage of yielding early and high returns per unit area when compared to other fruits because its crop is ready for harvesting six months after planting. The FAO estimates that total global output in 2021 is up to 9175384.3 tonnes on 389665 hectares of land with a yield of 235468 hg/ha, with Colombia, Greece, Netherlands, Belarus, France, Iran, Ukraine, Australia, Belgium, and Peru as the key producing countries (Anon., 2021). In 2021–2022, India's production is 11,000 MT on 3,000 hectares of area (Anon., 2022). India's strawberries are produced in Haryana, Mahabaleshwar (Maharashtra), Himachal Pradesh, Uttar Pradesh, West Bengal, Delhi, Punjab, and Rajasthan (Hindocha, 2022).

Potentilla L. and *Duchesnea* Smith. are related genera of *Fragaria*, all belonging to the tribe Potentilleae (Sharma *et al*., 2015). *Potentilla indica* is an herbaceous plant with yellow blooms and small red fruits that look like strawberries; it is also known as Mock Strawberry or Indian Strawberry. It is endemic to Asia (India) and has adapted well to Indian soil and climate (Naruhashi *et al*., 1999*)*. *P. indica* is distinguished by its narrowly obovate petals, green, somewhat thick leaves, rhombic-oblong and acute centre leaflet, red fruiting receptacle, and red and virtually smooth achene surface (Soják 2012). Although viable intergeneric hybrids are created in certain combinations, the generic pair *Potentilla* and *Fragaria* may represent a situation where the hurdles to cross-ability between two evidently closely related genera are of a higher size (Asker, 1971). Mangelsdorf and East (1927) were the first to report intergeneric crosses between *Fragaria* and *Potentilla*. *P. (Duchesnea) indica* was the most successful pollinator in their studies.

Strawberry blossoms were mostly formed in compound cymose inflorescences with terminal growth and highly varied branch structure. Each inflorescence normally contains primary, secondary, tertiary and quaternary flowers; however, cultivar and growing conditions might influence this number (Madhuri *et al*., 2016). Strawberry is one of the most important fruit crops that benefits from pollinator activity, as poor pollination results in a higher percentage of damaged or malformed fruits (Sharma *et al*., 2015). Although the majority of commercial strawberry cultivars have hermaphrodite and self-compatible flowers, cross-pollination is promoted since stigmas open before anthers of the same flower and release pollen (Cao, 2022). Open-pollination enhances strawberry fruit weight, sweetness, firmness, and post-harvest life more than autogamous self-pollination, according to Klatt *et al.* (2014). Little is known, however, regarding how cross-pollination affects plant yield and a range of strawberry fruit quality characteristics. Strawberry farmers may be able to increase farm-gate revenues by better knowing how cross-pollination impacts fruit quality (Hokanson and Finn, 2000).

Banks (2015) defines wild strawberries as species that are cultivated without the interference of humans. These may not have a stronger and sweeter flavour, but they are perfectly adapted to their native environment. In nature, wild relatives are quite robust; they may grow well even in difficult conditions and provide consistent flowering. Many species of wild strawberries can flourish in rocky soils with little or no nitrogen (Taghavi, 2014). The cultivated strawberry has a small genetic base, yet there is significant variation in wild germplasm that may give solutions to production challenges. Wild plant species related to crop plants form an important source of useful traits for fruit quality improvement and biotic and abiotic stress tolerance. In recent fruit crop enhancement programmes, wide hybridization involving wild relatives and allied genus has gained attention. It removes the barrier for gene transfer and allows the genome of one species to be transferred to another, resulting in alterations in genotypes and phenotypes of progenies (Anushma *et al*., 2021). As a result, wild relatives have been used in several cross-breeding programmes to combine their hardiness with better fruit quality from commercial cultivars (Banks, 2015).

Considering the importance of pollination and crossing, the present experiment will be carried out with the following objectives:

1. To study the floral biology of both cultivated and wild strawberry
2. To study the cross compatibility between cultivated strawberry and wild species
3. To observe the quality parameter of fruits obtained from different pollination method.

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITRATURE

This chapter contains a thorough assessment of the pertinent experimental data for the current study, **"Effect of pollination between commercial and wild strawberry,"** under relevant headings and subheadings. There hasn't been enough research done on pollination between commercial and wild strawberries. In order to gain a comprehensive understanding of the impact of pollination on fruit crops, related research on other fruit crops has also been studied.

2.1. Effect of Pollination on Fruit Characteristics

2.1.1. Effect of Pollination on Strawberry

In a controlled environment, Dung *et al.* (2021) investigated the effects of cross-pollination on the fruit quality of the self-compatible strawberry cultivar Redlands Joy. Each of the four treatments, open-pollination, self-pollination by hand and cross-pollination with Sugarbaby (small-fruited) and Rubygem (large-fruited) cultivar, was applied to all flowers on each plant. Cross-pollination had no distinct impact on fruit mass, size, firmness, form, or shelf life, however, had an impact on the fruit's flavour and colour. Fruit produced by hand self-pollination included a greater proportion of fertilised seeds than fruit produced by autogamy, and it was also darker, redder, firmer, and longer.

In 16 strawberry cultivars, the impact of self-, open-, hand, and cross-pollination as well as the stamens' relative location to the receptacle in the flower on fruit set and deformity were studied. With self-pollination coming in first and open-pollination coming in second, the most deformed fruits were noted. It was discovered that cross-pollination and self-pollination both reduced the number of malformation fruit. Fern and Shasta produced the least malformation fruits during hand pollination. Pajaro × Catskill and Belrubi × Douglas produced the least deformed fruits during cross-pollination. Cross-pollination produced the most fruit with the best fruit size, followed by hand and open-pollination, and self-pollination produced the least fruit (Lata *et al*., 2018).

Zebrowska (1998) looked at how strawberry production components were affected by various pollination methods. Five strawberry genotypes - Dukat, Paula, Redgauntlet, Senga Sengana and B-302 had their yield components subjected to self-, wind, and open-pollination experiments. B-302 and Redgauntlet showed the largest effects of self-pollination, while Paulua showed the least effects of autogamy. In contrast, the later cultivar showed the largest entomophily effect, while Dukat showed the greatest wind pollination effect. The strongest evidence of autogamy was seen in genotypes that produced lengthy pollen grains with high size indices. Anther size, particularly length, was associated more strongly with yield components than breadth.

2.1.2. Effect of Pollination on other Fruits

To determine the impact of open cross-pollination and self-pollination on fruit set and fruit attributes in nine different varieties of litchi, Lal *et al*. (2021) carried an experiment in the active field gene bank of the ICAR-National Research Centre. Only three cultivars produced fruit under conditions of self-pollination, whereas all nine cultivars produced fruit at harvest under conditions of open cross-pollination, In open cross-pollination, the fruit weight was higher for all three cultivars: Dehradoon (21.46 g), Rose Scented (20.37 g) and Ajhauli (18.95 g), compared to Dehradoon (19.67 g), Rose Scented (18.89 g) and Ajhauli (17.35 g) that were self-pollinated.

The effects of jackfruit pollination techniques have been investigated in order to evaluate the physicochemical changes of jackfruit harvested at four different maturity stages and produced by natural and assisted pollination. By gently rubbing ripe male inflorescences against the surface of receptive female inflorescences, hand pollination was carried out. Female inflorescences were allowed to grow into syncarps on their own for natural pollination. Physical and chemical traits such colour, hardness, pH, titratable acidity, amount of lycopene and carotene, sugar content and antioxidant activity were assessed. The jackfruit flesh's suitability for eating was unaffected by the pollination method. Assisted pollination resulted in syncarp that was heavier in weight and uniform in shape (Mijin *et al.,* 2021).

A field experiment was done by Freihat (2018) to examine the effects of various pollination strategies on the quality and quantity of loquat fruit as well as fruit set. On the chosen trees, open, supplemental, rainy, and covered pollination treatments were applied. Results showed that when supplemental and open-pollination were used, fruit set was significantly higher than when rain and cover were used. Despite a substantial crop load, fruits produced through supplemental and open-pollination were also larger, heavier and having more sugar concentration.

Gawroski and Kaczmarska (2018) examined the effects of pollination mode on fruit set and seed formation in five cultivars and three breeding clones of blue honeysuckle. In comparison to isolated flowers (on average 8.6%), open-pollinated blooms produced a higher percentage of fruits (on average 94.7%).

In order to investigate the effects of self- and cross-pollination on fruit set and fruit quality of sour cherry cultivars, Ansari *et al.* (2010) conducted an experiment. Final fruit set averages revealed that open-pollination had a 14.6% fruit set advantage over artificial self-pollination's 13.0% and natural self-pollination's 4.4%. In comparison to self-pollination, cross-pollination results in fruits that are larger and have lower total soluble solids.

Patterson (1990) looked on the function of pollination in the partially self-fertile cultivar Apollo of the feijoa (*Acca sellowiana* (Berg) Burret) in terms of seed production and fruit development. Fruit from cross-, self-, and open-pollinated flowers differed noticeably in terms of growth and fruit quality. The highest fruit set, fruit weight, and pulp (endocarp) development were produced through cross-pollination. Fruit produced through cross-pollination contained more seeds than fruit produced by self-pollination. It is found that pollination of feijoa can significantly affect fruit uniformity through its impact on seed production.

2.2. Distant Hybridization

2.2.1. Distant Hybridization in Strawberry

Breeding obstacles between related wild germplasm and the domesticated strawberry, *Fragaria* × *ananassa*, were investigated by Marta *et al.* in 2004. Following an incomplete diallele mating design, 500 interspecific and intergeneric crosses were made between accessions of the wild strawberries *Fragaria vesca* (2x), *Duchesnea indica* (8x), *Potentilla tucumanensis* (2x) and 9 genotypes of the cultivated strawberry, *Fragaria* × *ananassa* (8x). When *D. indica* was employed as the female in crosses with *F.* × *ananassa*, numerous putative hybrids were formed, but when *D. indica* was used as the male, only a few achenes and plants were produced. For this reason, fluorescence microscopy was used to examine pollen-pistil compatibility relations in this direction of the cross. At the stigma level, 78.6% of the genotypic pairings were incompatible and

17.2% were so at the first third of the style. Only 14% of the 35 hybrid achenes produced by the *F. vesca* and *F.* × *ananassa* crossings germinated, leading to short-lived plants; histology investigations showed that inviable seeds had smaller embryos and less developed (or collapsed) endosperms than the plump control *F. vesca* seeds. Only male *P. tucumanensis* was employed, with unfavourable outcomes.

Breeding to introduce a new aroma from a diploid wild strawberry *Fragaria nilgerrensis* was tried in cultivated strawberry, *F.* × *ananassa* (octoploid). All lines derived from the interspecifc hybridization of *F. nilgerrensis* var. Yunnan with *F.* × *ananassa* var. Toyonoka were sterile. After doubling the chromosome of the interspecific hybrid TN13 by colchicine treatment, 152 plants were regenerated. Among the progenies, 15 plants did not flower or set fruits (Group I), 28 bloomed but did not set fruits (Group II) and 109 lines (Group III) bloomed and fruit setting was also observed. Further, a superior progeny TN13-125 with peculiar peach like aroma was identified for future use in breeding programs (Noguchi *et al.*, 2002).

Many seeds were produced by intergeneric crosses between *Potentilla fruticosa* (2n = 14) and *Fragaria moschata* (2n = 42), but only nine plants were obtained from 554 seedlings. Four plants were healthy but sterile, while five perished without flowering. One plant had 21 chromosomes, four were aneuploid with 23, 24, 25, and 27 chromosomes, and four plants had the expected number of chromosomes (2n = 28). Evidence that two of the plants were hybrids included extended stem internodes and enlarged but not succulent receptacles as a response to growth factors. All nine plants may have developed from a typical fertilisation that, in some cases, was followed by chromosomal removal at an early stage of embryo development (Smith and Jones, 1985).

Jelenkovic *et al.* (1984) evaluated the intergeneric hybridization of *Fragaria* spp. and *Potentilla* spp. as a method of producing haploids. A total of 1345 fruits with an estimated 46089 seeds were produced via unidirectional intergeneric crossings employing diploid and tetraploid *Potentilla* species as the male parent and octoploid and hexaploid *Fragaria* species as the female parent. The *P. anserine* derived germinating seeds largely died at the cotyledon stage, with a few sprouting matroclinous octoploid seedlings. *P. fruiticosa* seeds resulted in the growth of matroclinous octoploids and true intergeneric hybrid seedlings, two different types of seedlings. Haploids have not been found. An odd phenotype was found in one of the *F.*

× *ananassa* cv. Tioga and *P. fruiticosa* seedlings, which, upon chromosomal analysis, revealed to be 2n = 9x = 63. This genotype showed total sterility in both males and females.

2.2.2. Distant Hybridization in other Fruit

Pujar *et al.* (2019) investigated the crossability of papaya cultivars (Arka Prabhath, Arka Surya, and Red Lady) with *Vasconcellea* species (*V. cauliflora, V. cundinamarcensis*, and *V. parviflora*). Arka Prabhath was discovered to be an excellent combiner with all three wild species (*V. cauliflora, V. cundinamarcensis*, and *V. parviflora*) with good fruit set and a higher mean number of fertile seeds per fruit. *V. cauliflora* was discovered to be an excellent combiner with all three female parents (Arka Prabhath, Arka Surya, and Red Lady) among male parents, resulting in improved fruit set and a higher mean number of viable seeds per fruit.

Citrus is among the most salt-sensitive perennial crops. One of the main rootstocks used worldwide is *Carrizo citrange* (CC) [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] which is tolerant to CTV but sensitive to iron chlorosis in alkaline soils. *C. macrophylla* is suited for saline soils because they restrict ion transport to the aerial part, whereas CC is sensitive to this condition as it quickly accumulates the ions and reaches toxic concentrations. Attempts to combine the beneficial characters of both rootstock species resulted in two somatic hybrids SMC-58 and SMC-73. The hybrids were tested for tolerance to iron chlorosis and salinity. Both hybrids were tolerant to iron chlorosis under chlorosis inducing treatment showing intermediate iron concentration in the leaves. The parameters viz., leaf greenness, increase in shoot biomass and iron concentration in the leaves were intermediate in the somatic hybrids as compared to their susceptible and resistant parents (Ruiz *et al.*, 2018).

Smith *et al.* (2013) attempted to produce intergeneic hybrids in Citrus, hybridization of *Citrus wakonai* P. I. Forst. & M. W. Sm. with a related genus *Citropsis gabunensis* (Engl.) Swing. & M. Kell. resulted in high rates of fruit set and seed formation.

For PRSV-P resistance breeding in papaya, *Carica papaya* when crossed with the PRSV-P immune species *Vasconcellea pubescens*, all progeny plants were infertile. *V. parviflora* is susceptible to PRSV-P but, when crossed to papaya, produced hybrids with some pollen fertility. Crosses between *V. pubescens* and *V. parviflora* have resulted in fertile F_1, F_2 and F_3 populations. Backcrosses to *V. parviflora* are producing a PRSV-P resistant *V. parviflora* which will be crossed with papaya. Thus *V. parviflora* can be effectively utilized as bridge species introgressing PRSV-P resistance from *V. pubescens* to cultivated *C. papaya* (O'Brien and Drew, 2010)

2.3. Effect of Pollen on Fruit Characteristics

Based on microscopic and transcriptome analyses, the effects of pollen sources on fruit set and fruit attributes of the Fengtangli plum (*Prunus salicina* Lindl.) were investigated. The pollinators utilised to pollinate the Fengtangli plum were Siyueli, Fenghuangli and Yinhongli. The Fengtangli fruit's size, weight, pulp thickness, soluble solids and sugar content were all impacted by the pollen sources used (Deng, 2022).

An investigation into artificially pollinating female Nam Hom flowers (H) with pollen from Nam Hom (H), Nam Wan (W) and Thai Tall Red (TTR) revealed that 2-acetyl-1-pyrroline (2AP) was discovered in all fruits, at 7 months after flowering, from all crosses. However, compared to the other crossing, fruit from H×W had the lowest 2AP content. Additionally, it was discovered that H×TTR's water had the highest level of turbidity and sugar concentration. These findings demonstrated that cross-pollination of Nam Hom coconuts involved the xenia effect. The smaller fruits and shells in HW, as well as the H×TTR crossing's larger fruits and shells relative to H×H, all indicate the presence of meta-xenia effects (Luckanatinvong and Siriphanich, 2016).

The date palm's physicochemical characteristics were significantly influenced by the pollen source. In 2015, Merwad *et al.* looked at the effects of different pollen grain sources on the Hayany date palm's fruit productivity and quality. Pollen grains from various places were used to pollinate female Hayany date palms. Each of the measured qualities was significantly impacted by the trial's diverse pollen grain origins. Rashied is the most suitable source of pollen grains to pollinate Hayany's female date palm since fruit quality has improved. To ascertain the impact of pollen sources and pollination frequency on fruit drop, yield, and quality of date palm cv. Dhakki, Shafique *et al.* (2011) conducted an experiment. The findings showed that the pollen source had a considerable impact on the percentage of fruit dropping. With pollination, the fruit's physical characteristics, such as fruit weight, flesh weight, and seed weight, dramatically improved. Pollinated fruits had the highest post-harvest chemical quality parameters like TSS and TSS:TA ratio, ascorbic acid concentrations, and reducing sugars. The amount of total phenols in the date palm fruit was not significantly impacted by the type of pollen or the quantity of pollinations. The productivity, maturity, and fruit quality of the Hayyani date palm were researched by Muhtaseb and Ghnaim in 2006. When trees were pollinated by Barakah male, the greatest fruit in terms of weight, length, and diameter were produced. Hayyani trees that were pollinated with Barakah pollen produced the fruit flesh percentage with the highest yield.

Sabir (2015) carried out a study to look into the effects of self-, free and cross-pollination on the characteristics of grape berries and seeds. Narince had five distinct pollination treatments. Berry set was substantially higher when free-pollination was used. The application of pollen from the Thompson Seedless and Cardinal cultivars by the pollinizers increased the percentage of berries that were set. The largest weight, length and width of the berry, as well as the quantity of seeds per berry, were also superior results of free pollination. These results showed that the examined grape cultivars had significant xenial and metaxenial effects.

In guava (*Psidium guajava*), an experiment was done to demonstrate how pollen parent improves fruit size and quality in intervarietal crosses. To investigate the impact of metaxenial on fruit quality, the crossed fruit was examined. As a pollen donor to cv. Pyriformed, results demonstrate that cv. Round improves the fruit's physical and biochemical quality, demonstrating a substantial metaxenial effect (Usman *et al.*, 2013).

Pollen quality, quantity and the impact of cross-pollination on pomegranate fruit set and quality were determined by Derin and Eti (2001). Both male (A type-unfertile) and bisexual (B type-fertile) flowers were used to assess the cultivars' pollen viability, pollen germination level, and pollen production capacity. In addition to these tests, the effects of open-, self- and cross-pollination (using pollen from both bisexual and male flowers) on fruit set and fruit quality were investigated. These tests demonstrated that the male flowers of Hicaz had the highest pollen viability and germination rate. Cross pollination rates were observed to be higher than those of fruit set in self and open pollination.

Chapter 3

MATERIALS AND METHODS

MATERIALS AND METHODS

The experiment entitled **"Effect of pollination between commercial and wild strawberry"** is conducted during 2022 – 2023 at Instructional Farm of Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya. The methods employed during the course of investigation and materials utilized have great significance in research program. The details of materials and methods adopted during the course and studies are enumerated below.

3.1. Location of experimental site:

The experiment was carried out at Instructional Farm of Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal 736165 during 2022-2023. The area lies under the Terai agro-climatic zone of West Bengal at an altitude of 43 m above the mean sea level at 26°39'83" N latitude and 89°38'64" E longitude (measured with geographic coordinate system).

3.2. Soil characteristics:

The soil is generally sandy loam in texture, acidic in nature having a pH of 4.0-6.8, high in raw humus content and having low water retention capacity. Nitrogen (272.83 kg/ha), phosphorus (24.24 kg/ha) and potash (102.56 kg/ha) content are medium to high with a low rate of nitrogen mineralization.

3.3. Climatic conditions:

The climate of this zone is subtropical humid with distinct features of high humidity and high rainfall. During the experimental period of time, the calculated weather reports are represented in table 3.1. The experimental site received average rainfall of 572.60 mm (October 2022 to May 2023) with average minimum and maximum temperature of 15.43°C and 29.17°C respectively during the period of investigation.

Table 3.1: Mean meteorological data during the period of field experimentation (2022-2023).

Month	Temperature		Relative humidity		Rainfall (mm)
	Maximum (°C)	Minimum (°C)	Maximum (%)	Minimum (%)	
October, 2022	31.00	18.00	82.00	69.00	218.60
November, 2022	30.00	11.00	71.00	51.00	0.00
December, 2022	27.00	8.00	84.00	52.00	0.00
January, 2023	24.20	8.60	91.70	54.00	0.00
February, 2023	26.70	13.40	82.80	54.90	0.00
March, 2023	29.60	17.00	74.30	51.60	104.00
April, 2023	32.00	26.20	71.50	57.10	78.80
May, 2023	32.90	21.30	75.90	59.50	171.20

3.4. Experimental design:

Two genotypes of strawberries, one commercial variety (C), i.e. Winter Dawn and another wild (W) were used in the experiment conducted during October 2022 to April 2023. Total 300 hundreds plants (150 plants of each genotype) were arranged in randomized block design (RBD) having six treatments combination with five replications each. Every replication is represented by a bed of size 2.5 m×1.5 m (3.75 m^2) having 10 plants with a spacing of 60 cm×50 cm. 60 cm of spacing is provided between two consecutive beds. Five flowers were selected from each plant in every month (January - March) and treatment was allocated to them. The six treatment combinations were explained in table 3.2.

Design of experiment = Randomized Block Design (RBD)

No. of treatments = 6

No. of replication = 5

Plant spacing = 60 cm × 50 cm on raised beds with 60 cm gap among the adjacent beds

Size of the individual bed = 2.5 m × 1.5 m (3.75 m^2)

No. of plots = 6 × 5 (30)

Plants per plots = 10

Total plants under the experiment = 30 × 10 = 300

No. of flowers per plants selected for each treatment = 15 (5 flowers in each month from January to March)

Duration of experiment = October 2022 to May 2023.

Table 3.2: Treatment combinations

Treatment	Treatment combinations	Description
T1	C × W	Cross pollination between commercial and wild strawberry
T2	W × C	Cross pollination between wild and commercial strawberry (reciprocal cross)
T3	C × C	Self pollination of commercial strawberry
T4	W × W	Self pollination of wild strawberry
T5	C	Open pollination in commercial strawberry
T6	W	Open pollination in wild strawberry

Figure 3.1: Experimental field layout:

T_1R_1	T_3R_1	T_5R_1	T_2R_1	T_4R_1	T_6R_1
T_3R_2	T_5R_2	T_1R_2	T_4R_2	T_6R_2	T_2R_2
T_5R_3	T_1R_3	T_3R_3	T_6R_3	T_2R_3	T_4R_3
T_1R_4	T_3R_4	T_5R_4	T_4R_4	T_6R_4	T_2R_4
T_3R_5	T_5R_5	T_1R_5	T_2R_5	T_4R_5	T_6R_5
Winter Dawn			Wild		

3.5. Cultural practices:

During field preparation, farmyard manure was applied as fertilizer at a rate of 3 kg per bed along with NPK dose of 45:52:40 g per bed. Additional foliar application of micronutrients was give at weekly interval. The field is kept weed free by applying black plastic sheets mulch. Irrigations were given at timely interval with the help of watering can during the whole experimental period. To prevent disease, insect and pest infestation, the recommended fungicides and pesticides were applied after visual appearance of infection symptoms.

Table 3.3: Cultural practices required for maintenances of genotypes.

Cultural practices	Cultivated	Wild
Availability of planting material	2nd week of October	3rd week of September
Planting date	10/11/22	10/11/22
Planting material	Runners	Runners
Foliar application of fertilizers	Application of micronutrients and NPK at weekly intervals.	Application of micronutrients and NPK at weekly intervals.
Affected by weeds	Yes	No
Weeding	Manually done at an interval of 20 days	Not applicable
Affected by disease	Yes (leaf spot, grey mold)	No
Application of fungicide	Application of fungicide at 20 and 60 DAP	Not applicable
Application of irrigation	Irrigated at 2 days interval	Irrigated at 5 days interval

Plate 1: Experimental field preparation

A: Beds preparation; B: Planting materials; C: Planting of Winter Dawn; D: Planting of *P. indica*

3.6. Nature of Work:

The following three types of pollination system were practiced

1) Restricted cross-pollination: pollination done by manually transferring of pollen from one genotype to another genotype.

2) Restricted self-pollination: pollination done by the pollen of same genotype.

3) Open cross-pollination: pollination done by any source or any pollinating agent.

3.7. Methodology:

3.7.1 Crossing:

For making crosses in this hybridization program the flowers that are in calyx splitting stage were selected and emasculated. During emasculation, while holding the flowers with hand, sepals and petals were removed with the help of a forceps. Then the anthers were removed with gentle but firm force. No injury was done to the stigmas. Immediately after emasculation the flowers were bagged (small parchment paper bags) to prevent random cross pollination, small perforations were made on the bag for better aeration. Pollination was done with the help of a soft paint brush within 24 hours after emasculation with freshly collected pollen and re-bagged. The flowers were tagged just after bagging. They were attached to the flower with the help of a thread with date of crossing and parentage written on tags. Bags were removed after 15-20 days of pollination (Cao, 2022).

3.7.2 Selfing:

The flowers were selected at calyx split stage and were bagged with parchment paper bag with small perforation for better aeration. Pollination was done by the pollen grain of same flower. Then tagging of the flower was done along with the date of selfing.

3.7.3 Open Pollination:

In open-pollination, pollination was done by natural source, so flowers at calyx split stage were selected and tagged.

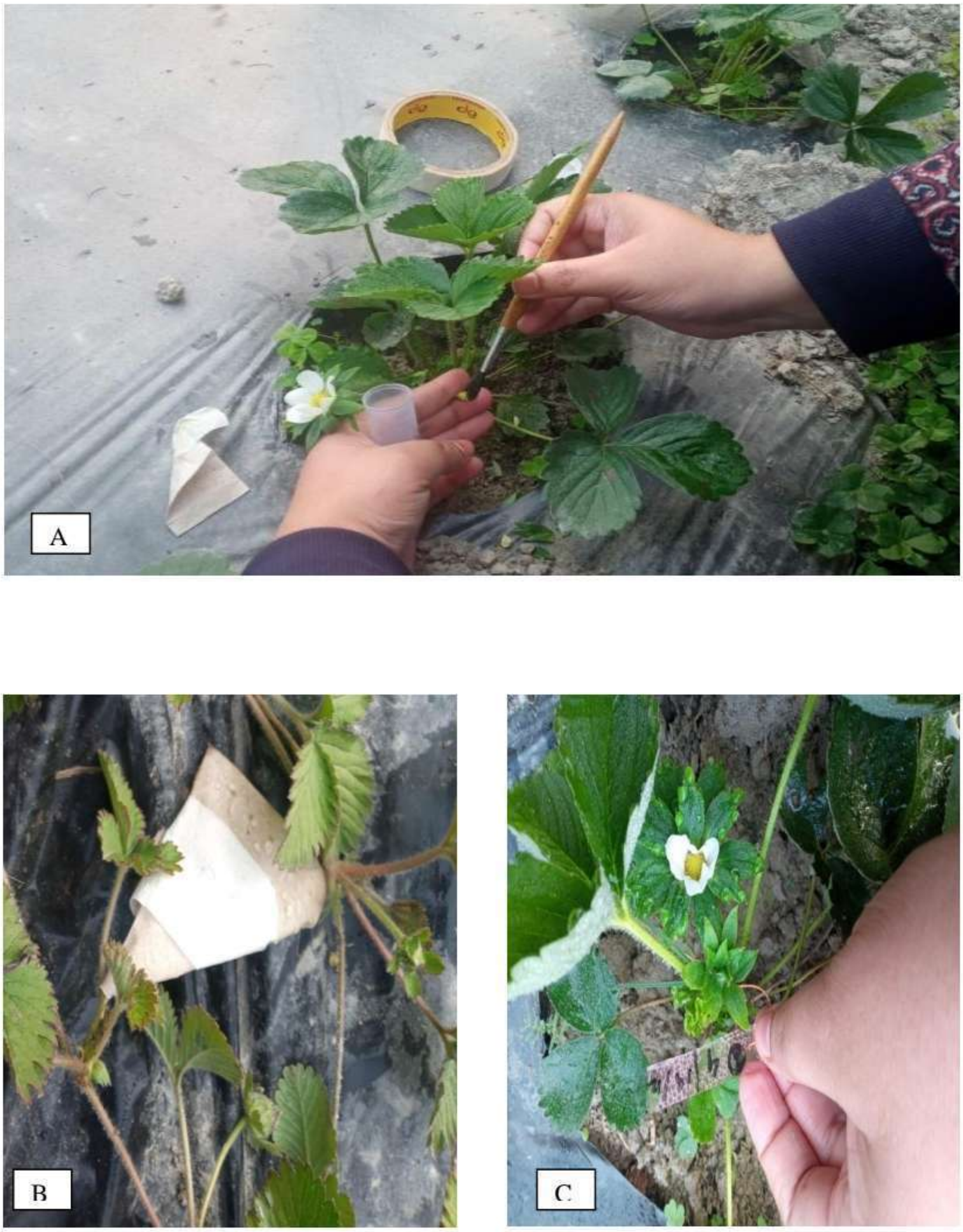


Plate 2: Application of different modes pollination as treatment

A: Cross-pollination; B: Bagging in self-pollination; C: Tagging in open-pollination

3.8. Observations Recorded:

3.8.1. Parent plant morphological parameter:

3.8.1.1. Plant height (cm):

Height of five tagged plants from both the genotypes was taken using a scale and then average of plant height were worked out and expressed in centimeters.

3.8.1.2. Plant Spread (cm):

Five plants were randomly tagged from both the genotypes and plant spread is recorded in E-W and N-S direction by measuring the total length and then average of plant spread were worked out and expressed in centimeters.

3.8.1.3. Total number of flower per plant:

Numbers of flowers per plant were recorded during every alternate day by observing the fully opened flowers from five tagged plants of both genotypes and then average was worked out.

3.8.1.4. Total number of fruits per plant:

Five different plants were chosen from both parent genotypes for the study, and the total numbers of fruits were counted at each picking. The results are shown as the number of fruits per plant.

3.8.1.5. Total number of runners per plant:

Number of runners was counted from five tagged plants of both and then average was worked out.

3.8.1.6. Leaf area (cm^2):

The areas of five randomly selected fully expanded leaves from five tagged plants were determined with the help of graph paper and then average was worked out and expressed in centimeters.

3.8.1.7. Length of petiole (cm):

Leaf petioles from five randomly selected fully expanded leaves from five tagged plants were selected and measured with a thread followed by the length of thread was determined with a scale and then average was worked out and expressed in centimeters.

3.8.1.8. Chlorophyll content (mg/100 g):

Fully matured leaves from five tagged plants were taken and prepared the sample with 2 g of leaf crush with 10 ml of 90% acetone. Prepared sample was stored for 2 days under cold dark condition. Sample was strained and centrifuge at 5000 rpm for 5 minutes. Volume made up to 10 ml with 90% acetone. The optical density of the sample was recorded with the help of spectrophotometer with wavelength of 663 nm and 645 nm against 90% acetone as reference (Ranganna, 1997).

$$\text{Chlorophyll a (mg/100 g)} = [(12.7\times A_{663}) - (2.63\times A_{645})] \times (D/10)$$

$$\text{Chlorophyll b (mg/100 g)} = [(22.9\times A_{663}) - (4.68\times A_{645})] \times (D/10)$$

$$\text{Chlorophyll a (mg/100 g)} = [(20.2\times A_{663}) + (18.02\times A_{645})] \times (D/10)$$

Where, A_{663} - Optical density at 663nm

A_{645} - Optical density at 645nm

D - Dilution factor

$$\text{Dilution factor} = \frac{\text{Volume of 90\% acetone x Volume made up}}{\text{Weight of sample x Volume of supernatant}}$$

3.8.2. Parent plant molecular characterization:

3.8.2.1. DNA isolation

DNA was extracted from leaf samples following the modified method of Mandal *et al*. (2013).Fresh 80 mg leaf material was washed in distilled water and rinsed with 80% ethanol. Washed leaf sample was ground finely with 500 μl extraction buffer and 20 μl of B-mercapto ethanol. Then 300 μl of extraction buffer was added and the sample was ground once more. Ground material was transferred to 2 ml centrifuge tube and added 75 μl 10% SDS. Entire content was taken in to the tubes and kept for incubation in water bath at 65°C for 10 minutes. After incubation 200 μl of 0.5 M potassium acetate was added and thoroughly mixed. Then the tubes were incubated at

0°C for 20 min. It was centrifuged at 10000 rpm for 10 minutes at 26°C.Supernatant was collected in fresh tubes and ice-cold isopropanol (60 % volume of supernatant) was added at room temperature and mixed thoroughly. Again tubes were centrifuged at 10000rpm for 10 minutes at 26°C. The supernatant was discarded and 500 µl wash solution was added in the eppendrof. After centrifugation the DNA pellet was collected and air dried up to complete disappearance of alcoholic smell. Collected DNA pellets were then suspended in 100 µl of TE buffer. Suspended DNA was treated with 1 µl of RNase (1.43 µg/ul, Sigma) and incubated at 37°C for30 min. The extracted DNA samples were stored at -20°C until use.

3.8.2.2. PCR amplification and sequencing

PCR amplification was performed in a total of 25 µl reaction mixture that contained 2µl of gDNA,0.2 µl of 5U/µl Taq DNA polymerase (Xcelris.), 2.5 µl of 10X PCR Buffer, 1 µl of 2.5 mM dNTPs mixture, 1 µl of each primer (100 ng/µl) and 16.8 µl of HPLC grade water. The PCR protocol used was as follows: initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation for 1min at 94°C, annealing for 30 s at 54.15°C (MatK) and 54.4°C (rbcL) and extension for 1 min at 72°C with a final extension for 5 min at 72°C and a 4°C holding temperature. PCR products were resolved on a2% agarose gel containing 0.05 mg/ml ethidium bromide and visualized under UV light. Gel photographs were scanned using Gel Doc System. After confirmation of single fragment appearance PCR product was sent for sequencing to Unipath Specialty Laboratory Ltd., Ahmedabad, Gujarat.

3.8.3. Flower parameters:

3.8.3.1. Days taken for flowering:

The numbers of days were counted from the date of planting to generation of first flower and 50% flowering was determined when 50% plants in a plot had at least one open flower of the parent genotypes and the average was worked out.

3.8.3.2. Flowering duration:

From the initial flowering through the last flowering, the lengths flowering phase were noted of the five tagged parent genotypes. It was expressed in terms of days.

3.8.3.3. Anthesis time:

The mode of flower opening was observed from the moment the petals started unfolding till the flowers were fully opened. 15 buds that were anticipated to open the following day were marked the night before to track the timing of anthesis in both the parent genotypes. Observations for number of fully opened flowers were made from 5 am at intervals of 30 minutes from the next morning. The period during which maximum number of flowers opened was determined.

3.8.3.4. Stigma receptivity:

To study the receptivity of stigma by fruit set method, 5 emasculated flower buds were hand self-pollinated at different times varying as two hour prior anthesis to two hours after anthesis. The pollinated buds were covered with paper bags and tagged as usual. The fruit set was observed 10 days after pollination when ovaries started swelling. The period during which maximum numbers of fruits were set was determined.

3.8.3.5. Fruiting duration:

From the initial fruit set to the last fruit setting, the length of each fruiting period were documented for the five tagged plants from both parent genotypes. It was stated as a number of days.

3.8.3.6. Days taken for fruit maturity:

For each treatment, the dates from pollination in cross-pollination, bagging in self-pollination, and tagging in open-pollination up until the date the plant reached edible maturity stage were noted.

3.8.3.7. Percentage of successful fruit setting:

The number of crossings produced and the number of successful fruit sets were recorded for each treatment. Using the data, the percentages of successful fruit setting were calculated using the given formula (Kidmos *et al.*, 1996).

$$\text{Percentage of fruit} = \frac{\text{Number of successful fruit set}}{\text{Total number of cross made}} \times 100$$

3.8.4. Pollen parameters:

3.8.4.1. Pollen size (µm):

Using a microscope with a 40x magnification, the average length and breadth of pollens in each genotype were measured.

3.8.4.2. Pollen viability (By Acetocarmine):

Pollen viability assessments were used to ascertain the reproductive state of the pollen. With 1% acetocarmine, pollen vitality was examined. A clean glass slide was used for this test. By gently crushing and pressing the strawberry anthers, the fresh pollens were allowed on slide. After that, a drop of 1% acetocarmine was added, followed by a cover slip. After observation under a microscope, pollen grains were categorised as viable or non-viable depending on whether they stain well and appear plump and typical (Heslop-Harrison, 1992).

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

3.8.4.3. Pollen germination percentage:

The pollen grains were cultivated in media containing 0.4g agar and 30mg of boron, along with sucrose concentrations of 5%, 10%, 15% and 20% to determine the germination percentage. Sterilized petriplates were filled with freshly produced medium, which were then autoclaved at 121°C for 15-20 minutes at 15 psi. This is followed by keeping it within the growth chamber. From both the genotype, five flower buds at the calyx split stage were collected in a small glass beaker filled with water and placed next to the window in the same lab, facing the light. The pollens were sprinkled into the petriplates within the laminar air flow the very next day. After that, intermittent observations of the pollen tube's growth under a microscope at a magnification of 40x and 100x were made. The length of the pollen tube and the amount of time needed for pollen germination were noted (Heslop-Harrison, 1992).

3.8.5. Fruit parameters:

3.8.5.1. Physical parameters:

3.8.5.1.1. Fruit shape:

The following types of fruit forms were noted in accordance with IBPGR strawberry descriptor (1986):

a) Kidney shape	d) Oblate	g) Round
b) Conical	e) Bi-conical	h) Almost cylindrical
c) Wedged	f) Ovoid	i) Cordate

3.8.5.1.2. Fruit weight (g):

Ten mature fruits were weighed using a balance, and the results were recorded.

3.8.5.1.3. Fruit length (cm):

Vernier Calliper was used to measure the length of 10 ripe fruits.

3.8.5.1.4. Fruit width (cm):

The diameter of 10 mature fruits will be measured with the help of Vernier Caliper at their shoulder region.

3.8.5.1.5. Number of achene per fruit:

Achenes were removed from each fruit separately and then manually counted after the harvest of cross-pollinated, self-pollinated, and open-pollinated fruits.

3.8.5.1.6. Weight of 100 achene (g):

A hundred manually counted achenes' weight is recorded using an electronic balance.

3.8.5.2. Bio-chemical Parameters:

3.8.5.2.1. Total Soluble Solids (°Brix):

A digital hand refractometer was used to measure the brix value of completely developed fruits. A drop of fruit juice is placed on the prism of the refractometer. The reading was used to determine the TSS percentage (Ranganna, 1997).

3.8.5.2.2. Reducing sugar (%):

Freshly harvested fruit samples weighing 10 g were gathered and ground with a mortar and pestle. Take the sample in a volumetric flask with a 100 ml capacity. In the burette, a sample was obtained. Add 2 ml of Fehling solution A and 2 ml of Fehling solution B to the 100 ml conical flask to make a volume of 50 ml, and then add 1-2 drops of the methylene blue indicator. The flask was heated continuously while the sample was slowly poured from the burette during titration. Brick red serves as the conclusion in the end (Ranganna, 1986).

3.8.5.2.3. Total sugar (%):

Freshly harvested fruit samples weighing 10 g were crushed with a mortar and pestle. Take a volumetric flask with a 100 ml minimum volume. Then, 2–3 ml of concentrated hydrochloric acid was added to 20 ml of juice in a conical flask. A drop of phenolphthalein indicator was put to it the following day. In the burette, a sample was obtained. Then, in a 100 ml conical flask, combine 2 ml Fehling solution A and 2 ml Fehling solution B to make a volume of 50 ml, then add 1- 2 drops of methylene blue indicator. The flask was heated continuously while the sample was slowly poured from the burette during titration. Brick red serves as the conclusion in the end (Ranganna, 1986).

3.8.5.2.4. Non-Reducing sugar (%):

Non-reducing sugar were calculated by subtracting the values of reducing sugar from the total sugar.

3.8.5.2.5. Total acidity (%):

Citric acid, which is present in strawberry juice and may be tested against common bases like sodium hydroxide, is easily neutralised by strong bases. 10 g of sample was taken. The material was diluted with distilled water to a volume of 100 ml. The sample was titrated to the end point with 0.1 N sodium hydroxide standard solutions. The sample was mixed with 2-3 drops of phenolphthalein indicator, which was then titrated to a light pink end point. It was noted how much sodium hydroxide was utilized (Ranganna, 1997).

3.8.5.2.6. Ascorbic acid content (mg/100 g):

Using the 2, 6-Dichlorophenol-Indophenol visual Titration method, the ascorbic content of fruit pulp was measured (Ranganna, 1986). Fruit samples weighing 20g were taken, crushed with 3% meta phosphoric acid, and filtered. 25 ml of 3% meta phosphoric acid and 5 ml of that juice were combined. Then, an aliquot of 5 ml was obtained from it and titrated against dye. The end point was characterized by a slight pink hue that lasted.

3.8.5.2.7. Anthocyanin contain of fruit (mg/100 ml):

For the entire sample, estimation of anthocyanin was performed by the procedure prescribed by Ranganna (1997). Ethanolic HCl was prepared by mixing 95% ethanol and 1.5 N HCl in the ratio of 85:15 i.e 85 ml of ethanol was mixed with 15 ml of 1.5N HCl.

$$\text{1.5N HCl} = \frac{\text{Equivalent weight of HCl} \times \text{Normality (required)} \times 100}{\text{Purity of HCl} \times \text{Specific gravity of HCl}}$$

2.5 ml of the sample was obtained and combined with 10 ml of ethanolic HCl in a volumetric flask. The combination was then kept in a refrigerator overnight at a temperature of 4°C. The mixture was filtered using a Whattman no. 1 paper the following day. The filtered mixture was then transferred to a fresh 25 ml volumetric flask, where ethanolic HCl was used to make up the volume. Anthocyanin was measured in a UV spectrophotometer at 535 nm against an ethanolic HCl as a blank. The following formula was used to estimate the anthocyanins, which were displayed as mg/100 ml:

$$\text{Total OD/100ml/g} = \frac{\text{OD} \times \text{Volume made up} \times 100}{\text{Volume/Weight of sample}}$$

$$\text{Total Anthocyanin (mg/100ml/g)} = \frac{\text{Total OD/100ml/g}}{\text{98.2 (extinction factor)}}$$

3.9. Statistical Analysis:

For statistical interpretation, analysis of variance for each parameter was performed using GenStat Eleventh Edition. Means separations for different parameter were performed using Critical Difference (CD) test ($P \leq 0.05$). To find the mean that are significantly different from each other, Tukey's test has been done using GenStat Eleventh Edition.

3.9.1. Data Transformation:

Data transformation is the most successful corrective technique when the variation and the mean are functionally connected. With this method, the original data are transformed onto anew scale, creating a new data set that is intended to meet the variance homogeneity requirements. The guidelines shown below were helpful to choose the transformation scale for percentage statistics derived from recent data that is most appropriate.

Rule1: Percentage data that falls between the ranges of 30 to 70% need not be modified.

Rule 2: Only percentage data in the ranges of 0–30% or 70–100%, but not both, should be transformed using the square root method.

Rule 3: For percentage data that does not fall within any of the ranges mentioned in Rules 1or 2, the arcsine transformation or transformation should be employed (Gomez and Gomez, 1984).

Chapter 4

RESULT AND DISSCUSSION

RESULT AND DISSCUSSION

The present research work entitled **"Effect of pollination between commercial and wild strawberry"** were carried out Instructional Farm of Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari during 2022- 2023. The results obtained are presented as under:

4.1. Parent Plant morphological parameter:

4.1.1. Plant height:

The data pertaining to the plant height is presented in Table 4.1.1. According to the data, average plant height of Winter Dawn is 17.16 cm, whereas, average height of *P. indica* is 10.30 cm. The current results are in correspondence with the findings of Ullah *et al.* (2022) and Khound *et al.* (2021).

4.1.2. Plant Spread:

Plant spread determines the size of the plants in different directions. The data given in Table 4.1.1 revealed that the *P. indica* (19.91 cm) has almost half plant spread then the cultivated strawberry Winter Dawn (36.38 cm). The increase in plant spread might be due to increased length and upright growth of leaf petioles which lean outwards resulting in higher plant spread. The research results are in conformity with the findings of Ullah *et al.* (2022) and Rathod *et al*. (2021) in strawberry

4.1.3. Total number of flower per plant:

Data recorded on total number of flowers per plant as depicted in Table 4.1.1 revealed that the flowers per plant was less in *P. indica* (22.00) whereas, Winter Dawn has 29.20 flowers on an average. The research results are in line with the findings of Todeschini *et al.* (2018) and Khound *et al.* (2021).

4.1.4. Total number of fruits per plant:

There was significant difference among the number of fruits produced per plant which is presented in Table 4.1.1. On an average, Winter Dawn has 24.20 fruits per plant and *P. indica* has 17.40 fruits per plant. These results are in conformity with the findings of

Todeschini *et al.* (2018) and Khound *et al.* (2021).

4.1.5. Total number of runners per plant:

Production of runners was very high and good in *P. indica* (6.80) than Winter Dawn (1.60), as they grow more vigorously (table 4.1.1). Similar results had been reported by Ullah *et al.* (2022) and Khound *et al.* (2021) during performance analysis of strawberry cultivars.

4.1.6. Leaf area:

The average leaf area of Winter Dawn (63.08 cm^2) was double of the average leaf area of *P. indica* (30.82 cm^2) as given in table 4.1.2. These results are in conformity with the findings of Ullah *et al.* (2022) and Rathod *et al.* (2021) during analysis of performance of strawberry cultivar Winter Dawn.

4.1.7. Length of petiole:

Similary like leaf area, petiole length of Winter Dawn (15.20 cm) was double of *P. indica* (7.18 cm) from the data given in table 4.1.2. Sharma and Godara (2019) and Rathod *et al.* (2021) found similar result of petiole length in control treatment in strawberry variety Sweet Charlie and Winter Dawn respectively.

4.1.8. Chlorophyll content:

Fully mature leaves of Winter Dawn were dark green in colour, while, leaves of *P. indica* were greenish yellow in colour. Total chlorophyll content was found to be 150.51 mg/100 g and 76.66 mg/100 g in Winter Dawn and *P. indica* respectively. In Winter Dawn, chlorophyll a and b are 57.60 mg/100 g and 103.29 mg/100 g respectively. Chlorophyll a content in *P. indica* was found to be 28.21 mg/100 g, while chlorophyll b content is 50.94 mg/100 g (table 4.1.2).

Santin *et al.* (2017) had reported similar findings of chlorophyll content in case of strawberry variety Camarosa and Camino Real.

Table 4.1.1: Mean table of plant morphological characteristics (plant height, plant spread, flower/plant, fruits/plant and runners/plant)

Observation	Plant height (cm)		Plant Spread (cm)		Flower/plant		Fruits/plant		Runners/plant	
	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*
P1	17.84	10.06	37.99	20.01	28.00	23.00	24.00	17.00	2.00	6.00
P2	16.98	11.23	34.75	21.09	27.00	22.00	23.00	19.00	1.00	6.00
P3	17.02	10.65	35.95	20.43	30.00	21.00	25.00	16.00	2.00	7.00
P4	15.96	9.87	36.23	19.02	29.00	23.00	24.00	20.00	2.00	8.00
P5	18.01	9.68	36.98	18.98	32.00	21.00	25.00	15.00	1.00	7.00
Mean	**17.16**	**10.30**	**36.38**	**19.91**	**29.20**	**22.00**	**24.20**	**17.40**	**1.60**	**6.80**
Variance	0.67	0.40	1.45	0.83	3.70	1.00	0.70	4.30	0.30	0.70
T score	14.81***		24.36***		7.42***		6.80***		-11.62***	
P value	2.11×10^{-7}		2.5×10^{-8}		0.00015		0.00052		3.92×10^{-6}	
Mean difference	6.86		16.47		7.20		6.80		5.20	

*Significant at 5% level, ** Significant at 1% level, *** Significant at 0.01% level

Table 4.1.2: Mean table of plant morphological characteristics (leaf area, length of petiole, chlorophyll a, b and total)

Observation	Leaf area (cm^2)		Length of petiole (cm)		Chlorophyll a (mg/100g)		Chlorophyll b (mg/100g)		Total chlorophyll (mg/100g)	
	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*
P1	74.30	32.50	15.16	7.80	57.41	28.21	103.68	50.95	150.48	76.62
P2	51.00	27.30	16.64	6.82	58.06	27.96	101.98	49.87	151.34	78.12
P3	59.20	30.10	14.12	7.01	59.01	26.98	104.25	52.64	149.68	77
P4	61.50	29.60	15.30	6.95	57.51	28.92	102.96	49.99	148.99	76.58
P5	69.40	34.60	14.79	7.33	56.01	29.01	103.59	51.23	152.04	74.96
Mean	**63.08**	**30.82**	**15.20**	**7.18**	**57.60**	**28.22**	**103.29**	**50.94**	**150.51**	**76.66**
Variance	82.37	7.87	0.85	0.15	1.18	0.67	0.74	1.25	1.51	1.29
T score	7.59***		17.84***		48.11***		82.72***		98.79***	
P value	0.00031		5.06×10^{-6}		2.19×10^{-10}		2.54×10^{-13}		6.15×10^{-14}	
Mean difference	32.26		8.02		29.38		52.36		73.85	

*Significant at 5% level, ** Significant at 1% level, *** Significant at 0.01% level

4.2. Parent plant molecular characterization:

After DNA isolation from the genotypes, PCR amplification was done to get the DNA bands. For both the genotypes, the bands were very close, hence for further assessment, sequencing was done. After sequencing, the cultivated genotype had shown 99.52% match with *Fragaria* × *ananassa* and the wild genotype have 99.58% similarity with *Potentilla indica* with matK gene. With rbcL gene, cultivated genotype showed 98.61% similarity while, wild genotype showed 98.15% similarity (Table 4.2).

Table 4.2: BLAST score data and species identification

Sample Id	Query Cove (%)	E-Value	Identity (%)	Target Accessions	Species	Gene
W	100.00	0.00	99.52	HM850689	*Potentilla indica*	matK
C	100.00	0.00	99.58	OP764691	*Fragaria* × *ananassa*	matK
W	99.00	0.00	98.61	MF695003	*Potentilla indica*	rbcL
C	100.00	0.00	98.15	OP764691	*Fragaria* × *ananassa*	rbcL

After morphological and molecular comparison, both the parent genotypes were found very distinct from each other. Hence they are suitable for being used in distant hybridization program. Winter Dawn has maximum plant height (17.16 cm), plant spread (36.38 cm), flowers/plant (29.20), fruits/plant (24.20), leaf area (63.08 cm^2), petiole length (15.20 cm) and chlorophyll content (150.51 mg/100g) but runners/plant was maximum in *P. indica* (6.80).

Potentilla species have been used in traditional medicine in the treatment of different ailment, disease or malady (Tomovic *et al.*, 2015). Plants exhibit antioxidant, hypoglycemic, anti-inflammatory, antitumor and anti-ulcerogenic potential properties (Tomczyk *et al.*, 2008). The aqueous extracts from selected *Potentilla* spp. showed the strongest antimicrobial activity (Tomczyk *et al.*, 2008), due to which no attack of disease and pest had been observed during the experimental period.

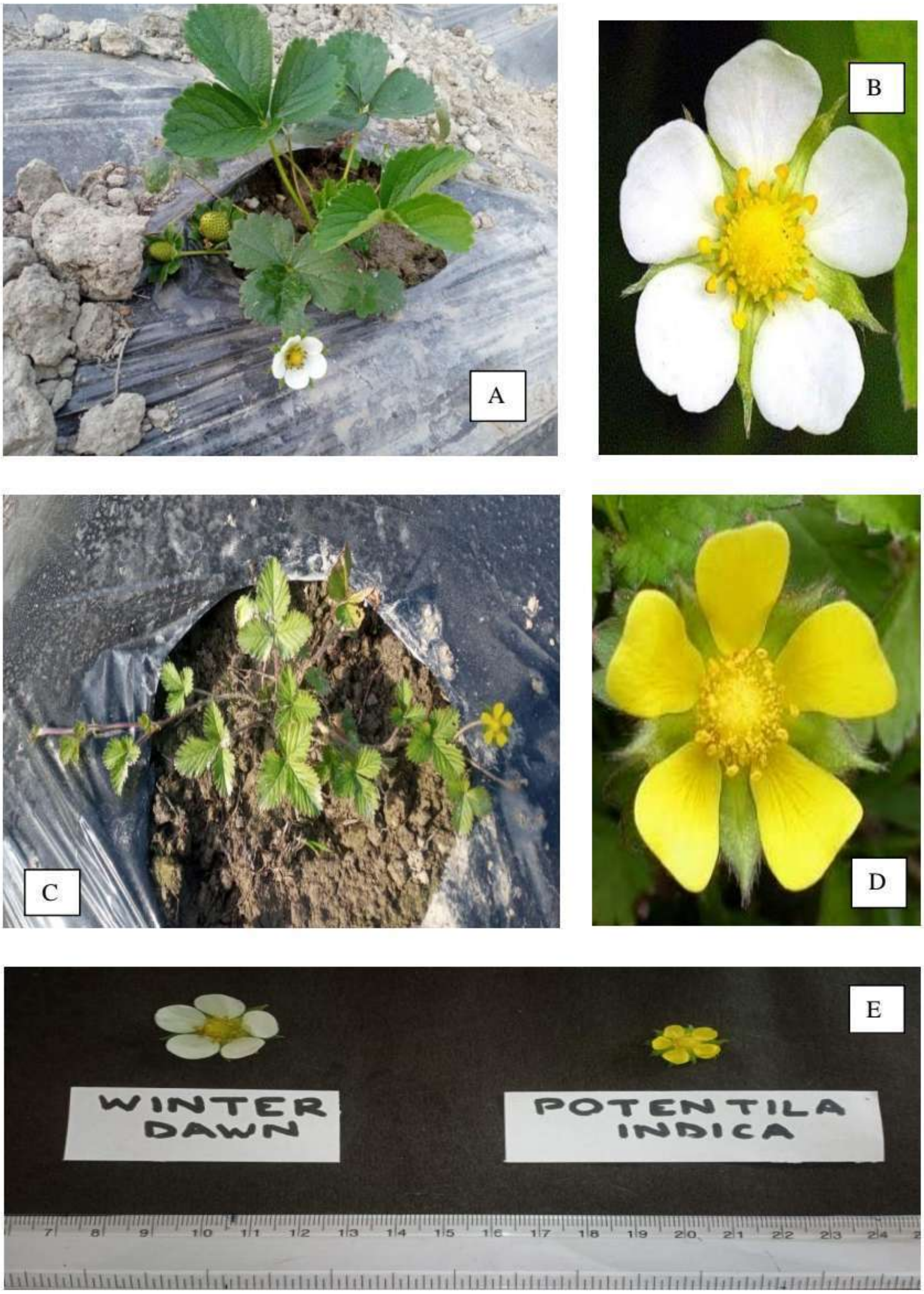
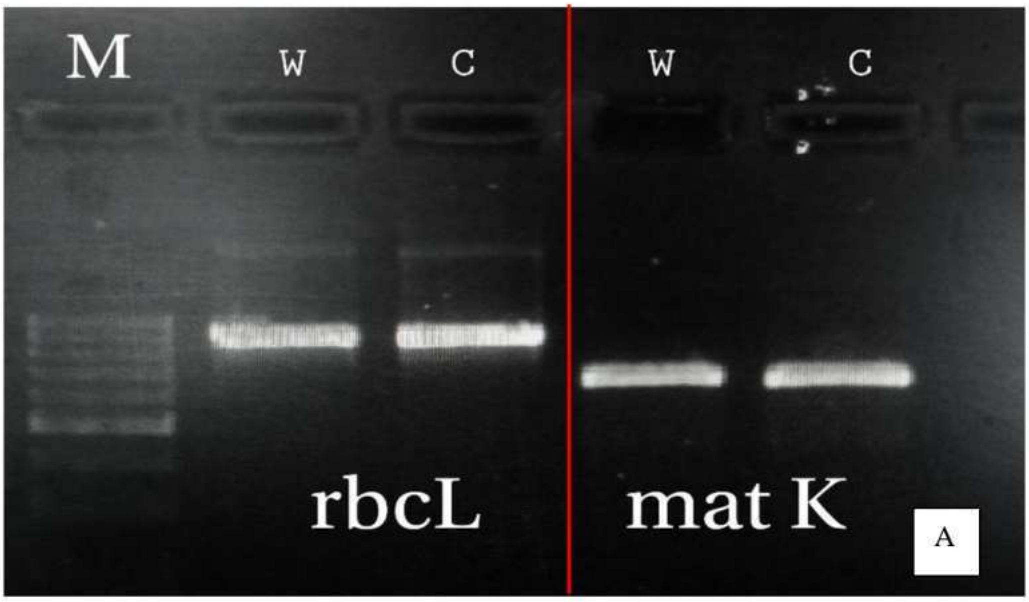


Plate 3: Comparison of parent plants

A: Winter Dawn plant; B: Winter Dawn flower; C: *P. indica* plant; D: *P. indica* flower; E: Comparison of size of flower of Winter Dawn and *P. indica*



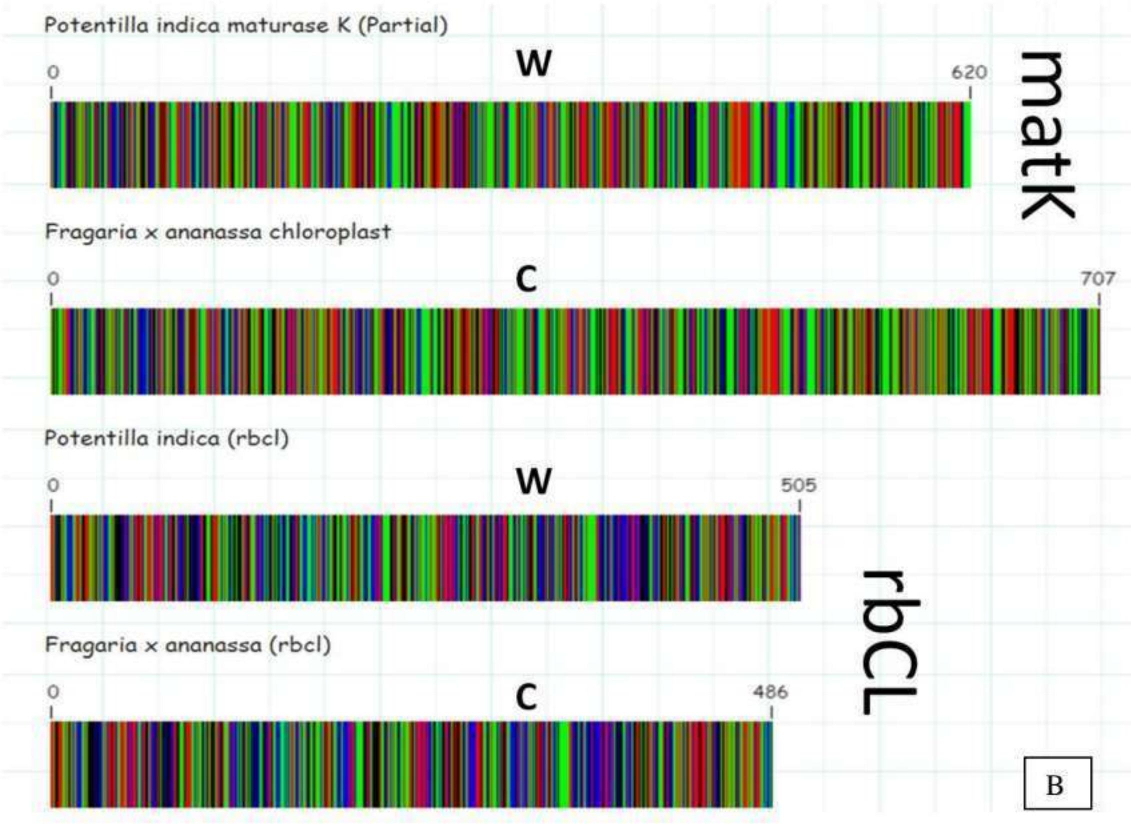


Plate 4: Molecular analysis result of parent plants

A: DNA bands; B: DNA barcodes

4.3. Flower parameter:

4.3.1. Days taken for flowering:

Flowering phase were noted for both the genotypes. As mentioned in table 4.3, in Winter Dawn first flower was seen within 19 days, 50% flowering occurs within 30 days and in case of *P. indica,* flower emergence started 10 days after planting. 50% flowering occurs within 20 days.

4.3.2. Flowering duration:

The data pertaining on duration of flowering (days) of strawberry genotypes are given in Table 4.3. Flowering duration of Winter Dawn was longer than *P. indica*. In Winter Dawn flowering lasted till 122 days, whereas in case of *P. indica*, flowering lasted till 103 days.

The research results are in conformity with the findings of Kandwal (2016) and Gireesha (2017) in strawberry var. Camrosa and Chandler respectively. Desai (1963) has reported that the first flower of strawberry appeared in January and the fruit matured in about three weeks. Ali (1962) and Chauhan (1966) have reported that flowering season varies not only from place to place but also from year to year.

Table 4.3: Parent plant flowering data

Plant	**Days taken to emergence after planting**				**Flowering duration**		**Fruiting duration**	
	1st flower		**50% flowering**					
	Winter Dawn	***P. indica***	**Winter Dawn**	***P. indica***	**Winter Dawn**	***P. indica***	**Winter Dawn**	***P. indica***
P1	19.00	11.00	32.00	21.00	131.00	107.00	98.00	90.00
P1	21.00	9.00	35.00	17.00	110.00	97.00	87.00	88.00
P3	17.00	13.00	29.00	24.00	127.00	98.00	92.00	89.00
P4	19.00	12.00	28.00	19.00	119.00	101.00	95.00	92.00
P5	20.00	8.00	30.00	20.00	124.00	112.00	86.00	86.00
MEAN	**19.20**	**10.60**	**30.80**	**20.20**	**122.20**	**103.00**	**91.60**	**89.00**

4.3.3. Anthesis time:

The process of opening of flowers in strawberry took place rather quickly. The calyx segments were noted to separate out gradually due to the inner pressure of the protruding corolla. The corolla made its appearance through the slightly split sepals at about one to two days before full opening of the flowers. Petals opened out exposing the gynocium and anthers became visible from the top. The petals along with the sepals and the epicalyx gradually stretched out and the anthesis was complete. It took about two to two and half hours for the buds to accomplish anthesis. Observations on the rate of anthesis were recorded at half hours interval daily. The results are presented in Table 4.4. The peak time of anthesis with maximum flower opening was observed between 9:00 am to 9:30 am (24.01%) in Winter Dawn and from 10:30 am to 11:00 am (34.67%) in *Potentilla indica*.

Similar results had been reported by Kandwal (2016) in strawberry var. Camrosa, where maximum anthesis period was from 6 am to 12 noon with peak anthesis from 10 am to 12 noon.

4.3.4. Stigma receptivity:

To ascertain stigma receptivity by fruit set method flower buds were emasculated and pollinated with fresh pollen at hourly intervals. It is evident from Table 4.5 that at the time anthesis i.e., from 9:00 am to 10:00 am for Winter Dawn, fruit set percentage (88%) was highest and in *P. indica,* 10:00 am to 11:00 am had maximum fruit set percentage (80%).

Kandwal (2016), Lata *et al.* (2018) and Darrow (1937) also reported that stigma receptivity is higher on the day of anthesis in strawberry. Hopper (1918) however has reported that in strawberry the anthers mature later than the stigma. Chauhan (1966) reported similar findings in the cultivars Pusa Early Dwarf and Kalimpong Local under Delhi condition.

4.3.5. Duration of fruiting:

From the table 4.3, it is seen that the fruiting duration was observed to be of 91 days in Winter Dawn and 89 days in *P. indica*.

4.3.6. Days taken for fruit maturity:

From the table 4.6, it can be seen that the time taken in cross pollination for fruit set was 22-25 days. After bagging in self pollinated fruits, it took 17-24 days in Winter Dawn and 15-20 days in *P. indica*. Open pollination set fruit within 15-21 days in Winter Dawn and 12-18 days in case of *P. indica*.

Table 4.4: Time of anthesis

DATE		Time														
		5:00 AM	5:30 AM	6:00 AM	6:30 AM	7:00 AM	7:30 AM	8:00 AM	8:30 AM	9:00 AM	9:30 AM	10:00 AM	10:30 AM	11:00 AM	11:30 AM	12:00 PM
11/12/22	Winter Dawn				1	-	1	2	2	4	5					
	Anthesis %				6.67 %		6.67 %	13.34 %	13.34 %	26.67 %	33.34 %					
	P. indica							1	-	1	2	3	5	3		
	Anthesis %							6.67 %		6.67%	13.34 %	20.00%	33.34%	20.00%		
12/12/22	Winter Dawn					1	1	2	-	4	5	2				
	Anthesis %					6.67%	6.67 %	13.34 %		26.67 %		13.34%				
	P. indica								1	1	2	2	4	5		
	Anthesis %								6.67%	6.67%	13.34 %	13.34%	26.67%	33.34%		
13/12/22	Winter Dawn				1	1	1	2	2	2	5	1				
	Anthesis %				6.67 %	6.67%	6.67 %	13.34 %	13.34 %	13.34 %		6.67%				
	P. indica								1	2	-	1	5	5	1	
	Anthesis %								6.67%	13.34 %		6.67%	33.34%	33.34%	6.67%	
14/12/22	Winter Dawn					1	1	1	3	4	5					
	Anthesis %					6.67%	6.67 %	6.67 %	20.00 %	26.67 %	33.34 %					
	P. indica							1	-	1	1	2	6	4		
	Anthesis %							6.67 %		6.67%	6.67%	13.34%	40.00%	26.6%		
15/12/22	Winter Dawn					1	-	2	2	4	6					
	Anthesis %					6.67%		13.34 %	13.34 %	26.67 %	40.00 %					
	P. indica									1	1	2	6	3	2	
	Anthesis %									6.67%	6.67%	13.34%	40.00%	20.00%	13.34%	
Average anthesis %	Winter Dawn				2.69 %	5.34%	5.34 %	12.01 %	12.00 %	**24.01 %**	21.34 %	4.00%				
	P. indica							2.67 %	8.01%	8.01%	8.01%	13.34%	**34.67%**	26.67%	4.01%	

Table 4.5: Stigma receptivity

DATE		TIME					
		7:00 AM	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM
23/12/22	Winter Dawn		2	5	1		
	Fruit set %		40%	100%	20%		
	P. indica			1	3	4	1
	Fruit set %			20%	60%	80%	20%
24/12/22	Winter Dawn		3	4	1		
	Fruit set %		60%	80%	20%		
	P. indica				5	3	
	Fruit set %				100%	60%	
25/12/22	Winter Dawn		2	5	2		
	Fruit set %		40%	100%	40%		
	P. indica			2	4	4	1
	Fruit set %			40%	80%	80%	20%
26/12/22	Winter Dawn		3	4			
	Fruit set %		60%	80%			
	P. indica				5	4	
	Fruit set %				100%	80%	
27/12/22	Winter Dawn		3	2	1		
	Fruit set %		60%	80%	20%		
	P. indica			1	3	2	
	Fruit set %			20%	60%	40%	
Average fruit set %	Winter Dawn		52%	**88%**	20%		
	P. indica			16%	**80%**	68%	8%

Table 4.6: Total number of successful fruit setting

Treatment	Month												Mean success fruit set %
	January				February				March				
	Fruit set %			Days to mature	Fruit set %			Days to mature	Fruit set %			Days to mature	
	Initial	10 days later	At harvest		Initial	10 days later	At harvest		Initial	10 days later	At harvest		
V×W	3.40 (10.50)	1.60 (7.060	0.60 (2.77)	25	2.20 (8.22)	0.80 (3.92)	0.40 (2.29)	22	1.20 (5.540	0.40 (2.29)	0.00 (0.00)	-	0.34 (2.69)
W×V	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	-	0.00 (0.00)
V×V	93.40 (75.67)	92.40 (74.59)	91.00 (73.01)	24	90.00 (72.52)	89.00 (71.26)	87.40 (69.67)	20	87.80 (69.70)	85.60 (67.75)	83.20 (65.85)	17	87.20 (69.18)
W×W	81.80 (65.12)	79.80 (63.45)	78.20 (62.30)	20	83.00 (65.85)	82.00 (65.02)	80.80 (64.12)	17	78.00 (62.11)	76.00 (60.71)	73.00 (58.72)	15	77.34 (61.62)
V	98.60 (84.91)	97.80 (81.77)	97.00 (80.13)	21	97.80 (82.64)	96.20 (78.92)	94.40 (76.36)	17	93.60 (75.94)	90.40 (72.21)	88.00 (69.90)	15	93.13 (75.35)
W	91.00 (73.03)	90.40 (72.27)	89.20 (71.03)	18	92.00 (74.08)	90.80 (72.83)	89.20 (71.08)	14	85.00 (67.32)	82.80 (65.55)	80.60 (63.89)	12	86.34 (68.49)
S.Em (±)	2.00	1.61	1.56		1.76	1.63	1.46		1.38	1.14	0.86		1.14
C.D. at 5%	5.95	4.78	4.64		5.25	4.86	4.36		4.10	3.40	2.58		3.65

*Figures in parentheses indicate arc sign transformation

The research results are in conformity with the findings of Praveena (2018) where cross pollinated fruits took longer time to mature whereas, open pollinated fruit required least number of days.

4.3.7. Percentage of successful fruit setting:

The fruit setting percentage under different treatments were noted. According to the data given in table 4.6, it can be seen that the fruit setting percentage in the month of January was higher for Winter Dawn and for *P. indica*, fruit setting percentage was higher in February. Under open pollination, Winter Dawn set maximum (93.13%) fruits, followed by *P. indica* (86.34%). On selfing, Winter Dawn gave higher fruit set (87.20%) then *P. indica* (77.34%).

The research results are in line with the findings of Klatt *et al.* (2013) and Anees *et al.* (2022), in strawberry; Lal *et al*. (2021) in litchi and Ansari *et al.* (2010) in sour cherry.

After crossing both the genotypes only 0.34% of fruit set was observed while no fruit setting has been seen in reciprocal cross. The ovary of *P. indica* dried 10 days after pollinating with Winter Dawn pollen.

Marta *et al.* (2004) stated that direct crosses between octoploid and lower ploidy species are often unsuccessful. Mangelsdorf & East (1927) reported the first successful intergeneric *Fragaria* × *Potentilla* cross that produced two short-lived seedlings; they also obtained hybrids between *F. vesca* (2x) and *D. indica* (8x) that were weak and died before reaching the adult stage. Several cross combinations were tried by Jones (1955), who pointed out that crosses with *Potentilla* as female parent were entirely unsuccessful. Ellis (1962) reported the production of hybrids with different ploidy levels in crosses between species of *Fragaria* and *Potentilla*, most of which died and some of the few that survived were sterile.

4.4. Pollen parameter:

4.4.1. Pollen size:

Freshly dehisced pollen grains looked dirty yellow in a mass to the naked eye and golden yellow when viewed under the microscope. The size of pollen in both the genotype was quite similar. The average size of Winter Dawn pollen is 21.98 μm whereas average size of *P. indica* pollen was found to be 24.31 μm.

Similar result had been reported by Kandwal (2016) in Camarosa variety of strawberry. Ali (1962), Chauhan (1966) and Rajput and Singh (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 (Table 4.7).

4.4.2. Pollen viability (By Acetocarmine):

The viability of pollen grains of both parent strawberry genotypes under study was high. The fresh pollen grains show 100% viability on the day of flower opening (anthesis).

Kandwal (2016) and Rajput and Singh (1967) reported 100% viability of strawberry pollen grain on the day of anthesis (Table 4.7).

Table 4.7: Pollen size and viability

Observations	Pollen size (µm)		Pollen viability (%)	
	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*
1	21.76	24.56	100	100
2	22.08	23.97	100	100
3	21.98	24.28	100	100
4	21.96	24.76	100	100
5	22.12	23.98	100	100
Mean	**21.98**	**24.31**	**100**	**100**

4.4.3. Pollen germination:

The data on pollen germination and pollen tube length of both the genotypes are presented in Table 4.8. The data revealed that the maximum pollen germination percentage at 6 hours was recorded with 15% sucrose media for both Winter Dawn (57.00%) and *P. indica* (47.33%) while 5% sucrose media with 25.66% pollen germination show the lowest pollen germination in Winter Dawn. But in *P. indica,* minimum pollen germination (1.67%) was shown at 20% sucrose media. The maximum pollen tube length in Winter Dawn (39.33 µm) and *P. indica* (27 µm) was recorded with 20% sucrose media and 5% sucrose media gave least length of pollen tube in Winter Dawn (11.00 µm) and *P. indica* (0.33 µm). Similarly, at 12 hours maximum pollen germination percentage in Winter Dawn (91.66%) and *P. indica* (82%) was seen in 15% sucrose media while minimum was seen in 5% sucrose media for Winter Dawn (51.66%) and in *P. indica* (18.00%), it was shown at 20% sucrose media.

Table 4.8: Pollen germination

Treatments	Pollen germination percentage				Pollen tube growth (μ)			
	6hrs		12hrs		6hrs		12hrs	
	Winter Dawn	***P. indica***	**Winter Dawn**	***P. indica***	**Winter Dawn**	***P. indica***	**Winter Dawn**	***P. indica***
5% sucrose	25.66	12.00	51.66	34.66	11.00	0.33	33.33	1.66
10% sucrose	50.00	38.66	82.00	66.66	26.6	1.80	37.33	5.33
15% sucrose	57.00	47.33	91.66	82.00	32.00	14.00	45.33	21.33
20% sucrose	41.00	1.67	67.33	18.00	39.33	27.00	58.66	36.00
S.Em	3.07	2.67	2.08	3.07	0.89	1.09	1.61	1.68
C.D. at 5%	10.17	8.60	6.89	10.19	2.97	3.62	5.35	5.57

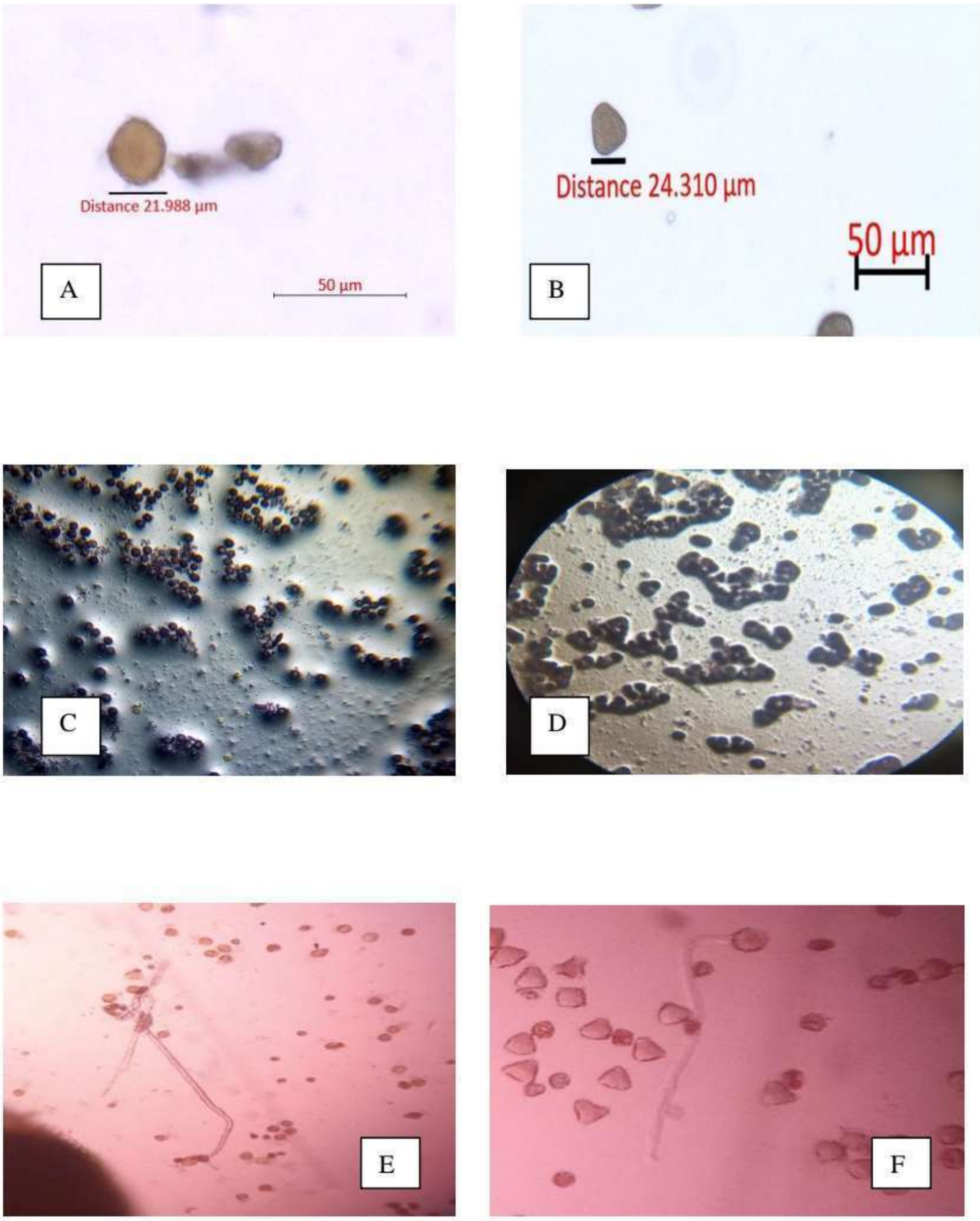


Plate 5: Pollen parameter comparison

A: Pollen size estimation of Winter Dawn; B: Pollen size estimation of *P. indica;* C: Pollen viability of Winter Dawn; D: Pollen viability of *P. indica*; E: Pollen tube growth in Winter Dawn; F: Pollen tube growth in *P. indica*

Pollen tube length was maximum in 20% sucrose media with 58.66 µm in Winter Dawn and 36 µm in *P. indica* and 5% sucrose media gave least length of pollen tube in Winter Dawn (33.33 µm) and *P. indica* (1.66 µm).

Similar results had been reported by Rawat *et al*. (2003) and Kandwal (2016), where increasing sucrose concentration resulted in increase of pollen germination percentage and increase of pollen tube length in strawberry.

4.5. Fruit parameter:

4.5.1. Physical parameters:

4.5.1.1. Fruit shape:

The fruits of Winter Dawn were conical in shape whereas the fruits of *P. indica* were oblate. The fruits produced after crossing were misshapen with oblate to round in shape (Table 4.9). Deformations in strawberry fruits are due to pollination limitation. Carew *et al.* (2003) explained that misshapen fruits may be due to stamen and carpel viability, effects of the environment, improper pollination and lack of nutrition must. The distribution of pollen grains across the receptive stigmas seems to be important for regular fruit development resulting in non-deformed, well marketable fruits. Beside even pollen distribution, Winfree *et al.* (2011) found that a sufficient amount of pollen grains per flower/stigma is crucial for full fruit set, e.g. in watermelon (*Citrullus lanatus*). Thus, malformation in fruits can possibly be explained by an interactive effect of even pollen distribution and meeting a threshold of pollen grains per flower/stigma (Khatt *et al.*, 2013).

4.5.1.2. Fruit weight (g):

In general, the fruits of Winter Dawn were larger in size compare to *P. indica* which were small. Pollination had significant effect in fruit weight of strawberry. Hence, from the table 4.9, the maximum fruit weight was obtained was in T_5 (20.16 g) and minimum weighted fruits were obtained from T_4 (0.74 g), which is statistically at par with T_6 (0.87 g). The cross product, i.e. T_1 was intermediate in weight (9.76 g). The fruits obtained in selfing were slightly smaller than open-pollinated fruits.

4.5.1.3. Fruit length (cm):

Like fruit weight, fruit length was also significantly affected by mode of pollination. Maximum fruit length was obtained in T_5 (4.02 cm) which was statistically equal to T_3 (3.77 cm) and minimum size fruits were obtained from T_4 (0.52 cm), which was statistically at par with T_6 (0.78 cm). The cross-pollinated fruit (T_1) is having intermediate length of 1.37 cm. Open pollinated fruits have more length than self-pollinated fruits (Table 4.9).

4.5.1.4. Fruit width (cm):

The result (Table 4.9) demonstrates that the pollination had a significant effect in fruit width, with maximum width in open-pollinated fruit from T_5 (3.34 cm) and minimum in self-pollinated fruit of T_4 (0.86 cm).

4.5.1.5. Number of achene per fruit:

Pollination had significant effect in decreasing the number of achenes, as from table 4.9, it is clear that the cross-pollination (T_1) produced least number of achenes (60) compare to open-pollination, as T_6 has maximum number of achenes (212).

4.5.1.6. Weight of 100 achenes (g):

From table 4.9, it can be seen that minimum weight of 100 achenes was obtained in T_1 (0.009 g) and maximum was obtained in T_5 (0.094 g). Hence mode of pollination had significant effect, as open-pollinated fruits had more weight of achene than cross-pollinated fruits.

4.5.2. Bio-chemical Parameters:

4.5.2.1. Total Soluble Solids (^{o}Brix):

Pollination effects the total soluble solids (TSS) of the fruits significantly, as from the table 4.9, the maximum TSS had been obtained in T_5 (7.58°B) which was open-pollinated fruit and minimum was found in T_4 (3.58°B) which is statistically equal to T_6 (3.96°B).

4.5.2.2. Reducing sugar (%):

The data from table 4.9 showed that the pollination had a significant effect on reducing sugar. Fruits from T_5 (3.42%) had maximum reducing sugar whereas, fruits from T_4 (1.81%) had minimum reducing sugar content. Statistically, T_3& T_5 and T_4& T_6 are similar.

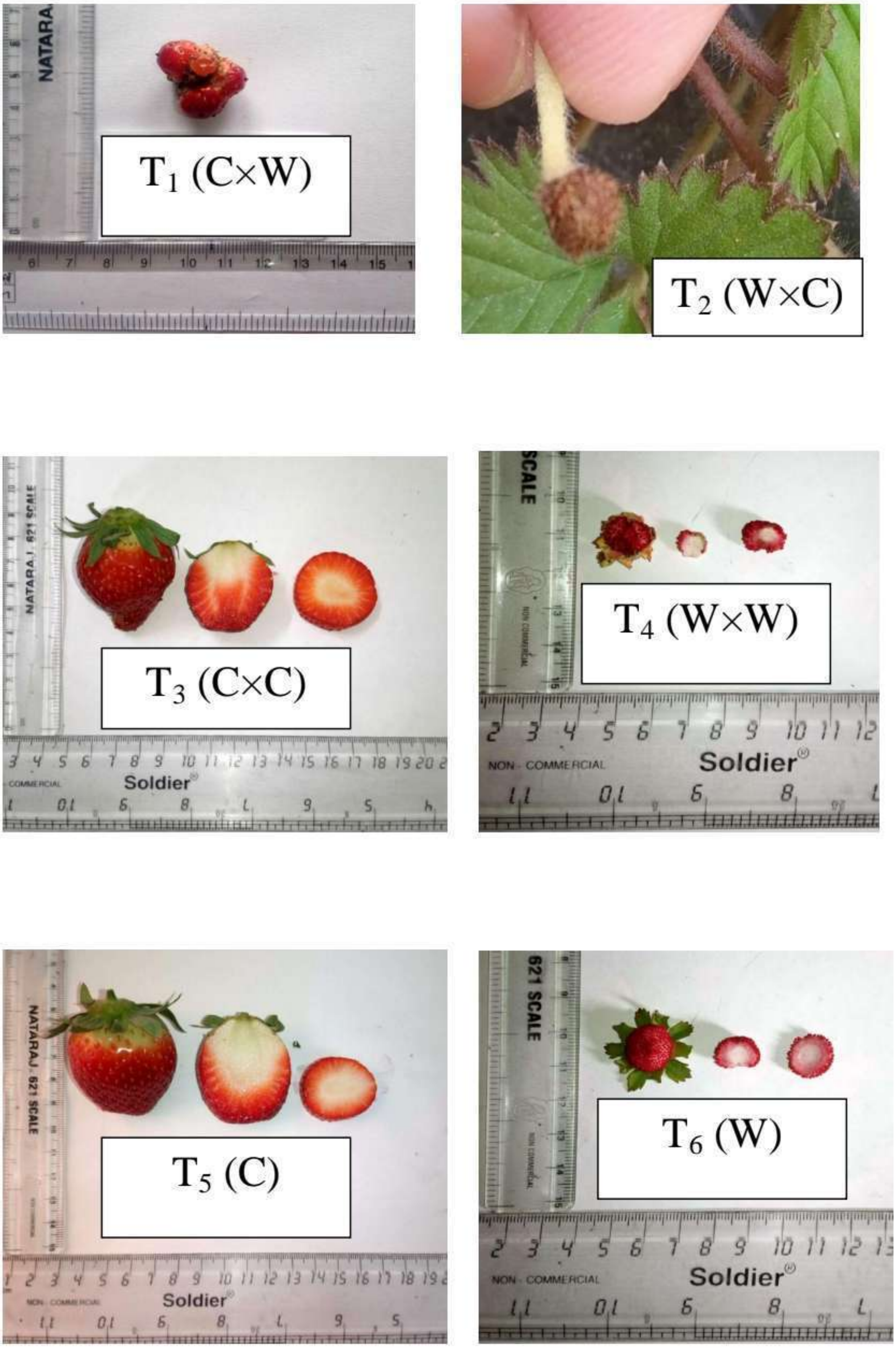


Plate 6: Fruits obtained after different pollination treatment

4.5.2.3. Total sugar (%):

Similarly like reducing sugar, mode of pollination significantly affect total sugar. From table 4.9, T_5 (4.90%) had maximum and T_4 (2.83%) had minimum total sugar content.

4.5.2.4. Non-reducing sugar (%):

Data from table 4.9 showed that T_5 (1.48%) had maximum and T_3 (0.83%) had minimum non-reducing sugar content.

4.5.2.5. Total acidity (%):

When different mode of pollination applied to strawberry, the total acidity was recorded minimum in T_5 (0.66%) and maximum in T_4 (1.02%). Additionally, T_1, T_3 and T_5 were statistically similar (Table 4.9).

4.5.2.6. Ascorbic acid content (mg/100 g):

The data in Table 4.9 showed that ascorbic acid content was significantly influenced by different mode of pollination. T_5 (52.64 mg/100 g) recorded maximum ascorbic acid content and T_4 (21.65mg/100g) had minimum ascorbic acid. Statistically, T_1 and T_2 are at par; similarly, T_3 and T_5 are also similar.

4.5.2.7. Anthocyanin contain of fruit (mg/100 ml):

Pollination effect anthocyanin content of fruit significantly. Maximum anthocyanin content is recorded in T_5 (32.12 mg/100 ml) and minimum is recorded in T_4 (18.38 mg/100 ml) from table 4.9.

Open pollinated fruits had fewer deformities, more fruit weight, and a longer shelf life, resulting in a higher economic value as well as superior post-harvest quality due to more intense red colour and a lower acid and higher sugar percentage than cross and self pollinated fruits. The process underlying the benefits of strawberry pollination in open conditions is based on the fertilisation of the strawberry's actual 'nut' fruits, the achenes (Csukasi *et al.,* 2011). While unfertilized achenes have little physiological functionality due to insufficient pollination, fertilised achenes generate the plant hormone auxin, which mediates the buildup of gibberellic acid (Csukasi *et al.*, 2011). These plant hormones work together to promote fruit growth by increasing cell proliferation and size, increasing the weight of strawberry fruits. (Klatt *et al.,* 2013). This enhances fruit quality and thus commercial grades by reducing deformities produced by unfertilized and so physiologically inactive achenes (Ariza *et al*., 2006). Higher amounts of both plant hormones improve strawberry post-harvest quality. Although auxin alone inhibits anthocyanin accumulation, high amounts of both auxin and gibberellic acid can promote anthocyanin accumulation (Klatt *et al*., 2013). In contrast to firmness and colour changes, auxin and gibberellic acid have no direct effect on strawberry sugar and acid levels (Klatt *et al*., 2013). However, greater fruit firmness is related with more stable cell walls, which may lower respiration, which has been shown to inhibit metabolic processes impacting sugar and acid concentration during storage. (Caner *et al*., 2008).

Similar findings have been reported by Klatt *et al.* (2013), Wietzke *et al*. (2018), Abrol *et al.* (2019) and Anees *et al.* (2022), in strawberry; Chauhan *et al.* (2017) in persimmon, Ahmad *et al.* (2021) in loquat and Lal *et al*. (2021) in litchi.

4.6. Correlation coefficient for fruit characters:

In the present experiment, correlation coefficients among ten fruit quality parameters were estimated to study how these characters influence fruit weight. The estimates of correlation coefficients have been presented in figure 4.1 to figure 4.4.

4.6.1. Correlation coefficient for fruit characters of T_3 (self pollination in Winter Dawn):

An overview of the figure 4.1 revealed that reducing sugar is showing high positive significance with TSS (0.92), ascorbic acid (0.89), weight of 100 achenes (0.91) and number of achenes per fruit (0.95) indicating higher contribution of this character towards fruit weight.

TSS is also positively significant with anthocyanin (0.84). However acidity and ascorbic acid were positively non significant with TSS. Weight of 100 achenes is positively correlated with number of achenes per fruit (0.89).

Negative correlation was observed for fruit weight and reducing sugar; weight of 100 achenes with fruit length and anthocyanin with ascorbic acid and acidity.

4.6.2. Correlation coefficient for fruit characters of T_4 (self pollination in *P. indica*):

In self pollinated fruits of *P. indica*, fruit weight is positively significant with ascorbic acid (0.98) and weight of 100 achenes (0.88) as revealed in figure 4.2, showing high contribution of ascorbic acid and weight of 100 achenes to increase the fruit weight.

Reducing sugar is positively significant with TSS (0.89) and positively non significant with number of achenes per fruit. Ascorbic acid is showing positive non significant relation with weight of 100 achenes.

Few characters had shown negative correlation such as fruit length with number of achenes per fruit and reducing sugar; acidity with ascorbic acid, fruit weight and weight of 100 achenes; fruit width with number of achenes per fruit.

4.6.3. Correlation coefficient for fruit characters of T_5 (open pollination in Winter Dawn):

The data in figure 4.3 revealed that the TSS is positively significant with reducing sugar (0.88) and number of achenes per fruit (0.96) however it is positively non significant with ascorbic acid and acidity.

Acidity had significantly positive correlation with fruit weight, fruit width and weight of 100 achenes. Fruit weight positively correlated with fruit length, fruit width, weight of 100 achenes, acidity and reducing sugar.

However, ascorbic acid had shown high negative significant correlation with fruit weight (-0.96). Fruit width was negatively significant with number of achenes per fruit and TSS. Acidity and reducing sugar are negatively correlated with number of achenes per frui.

4.6.4. Correlation coefficient for fruit characters of T_5 (open pollination in *P. indica*):

An overview of the figure 4.4 revealed that fruit weight was found to be positively ad significantly correlated with ascorbic acid (0.93), indicating higher contribution of this character towards fruit weight. However anthocyanin, reducing sugar, acidity, TSS, number of achenes per fruit, fruit width ad fruit length were positively correlated with fruit weight.

Fruit length was positively ad significantly associated with fruit width (0.91), whereas positive and non significant correlation of fruit length with TSS, weight of 100 achenes, number of achenes per fruit was found. TSS of the fruit has positive significant correlation with reducing sugar (0.89).

Significantly negative correlation was observed for frit weight with weight of 100 achenes; fruit length with anthocyanin, ascorbic acid, reducing sugar, acidity; fruit width with anthocyanin, ascorbic acid, reducing sugar; weight of 100 v with anthocyanin, ascorbic acid, acidity ad TSS; acidity with reducing sugar and reducing sugar with anthocyanin.

Negative association may arise primarily from developmentally induced relationship (Adam, 1967). A developing structure may struggle for a common factor with minimum availability of nutrient and if one structure is more favored than the other, in that case negative correlation may arise between them. Pleiotropy and/or linkage may also be the genetic reasons for these types of negativity.

Similar results reported by Lal *et al*. (2018) in litchi, Pandey *et al.* (2016) in Indian gooseberry and Kumar and Dudi (2011) and Mishra and Nandi (2018) in tomato.

.

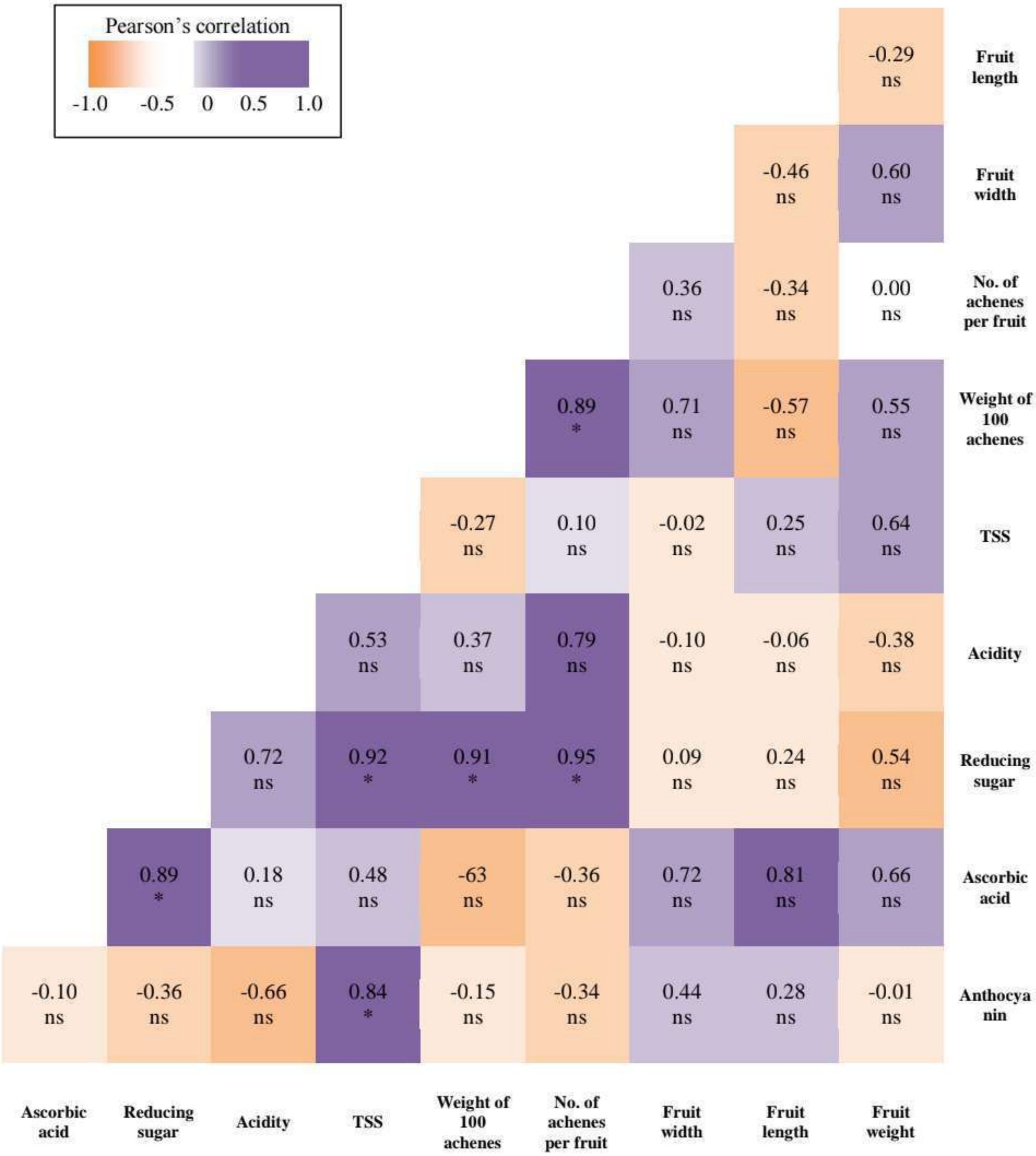
Table 4.9: Mean table of fruit physical and biochemical parameters

Treatment	Fruit Shape	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Number of Achenes per Fruit	Weight of 100 Achenes (g)	TSS (°Brix)	Reducing Sugar (%)	Total Sugar (%)	Non-Reducing Sugar (%)	Total Acidity (%)	Ascorbic Acid (mg/100g)	Anthocyanin (mg/100g)
T_1 (C×W)	Oblate	9.76^{b}	1.37^{b}	1.016^{a}	60.00^{a}	0.009^{a}	5.44^{b}	2.81^{b}	3.69^{bc}	0.87^{a}	0.85^{b}	43.42^{b}	25.05^{bc}
T_3 (C×C)	Conical	17.04^{c}	3.77^{c}	2.754^{b}	190.00^{b}	0.086^{c}	6.20^{b}	3.26^{c}	4.09^{c}	0.83^{ab}	0.81^{b}	46.20^{b}	28.68^{cd}
T_4 (W×W)	Oblate	0.74^{a}	0.52^{a}	0.862^{a}	196.20^{b}	0.024^{b}	3.58^{a}	1.82^{a}	2.83^{a}	1.01^{abc}	1.03^{c}	21.65^{a}	18.38^{a}
T_5 (C)	Conical	20.16^{d}	4.02^{c}	3.346^{b}	200.40^{b}	0.094^{d}	7.58^{c}	3.42^{c}	4.91^{d}	1.49^{c}	0.66^{a}	52.64^{c}	32.12^{d}
T_6 (W)	Oblate	0.87^{a}	0.78^{a}	1.234^{a}	212.60^{b}	0.027^{b}	3.96^{a}	1.90^{a}	3.30^{ab}	1.41^{bc}	0.90^{b}	25.50^{a}	20.20^{ab}
Grand Mean		**9.72**	**2.09**	**1.84**	**171.84**	**0.05**	**5.35**	**2.64**	**3.77**	**1.12**	**0.85**	**37.88**	**24.89**
S.Em (±)		0.42	0.07	0.18	6.94	0.002	0.18	0.06	0.11	0.13	0.02	1.20	1.16
CD at 5%		1.26	0.22	0.54	20.79	0.005	0.54	0.20	0.33	0.39	0.07	3.61	3.48

*Means with same letter are not significantly differs from each other

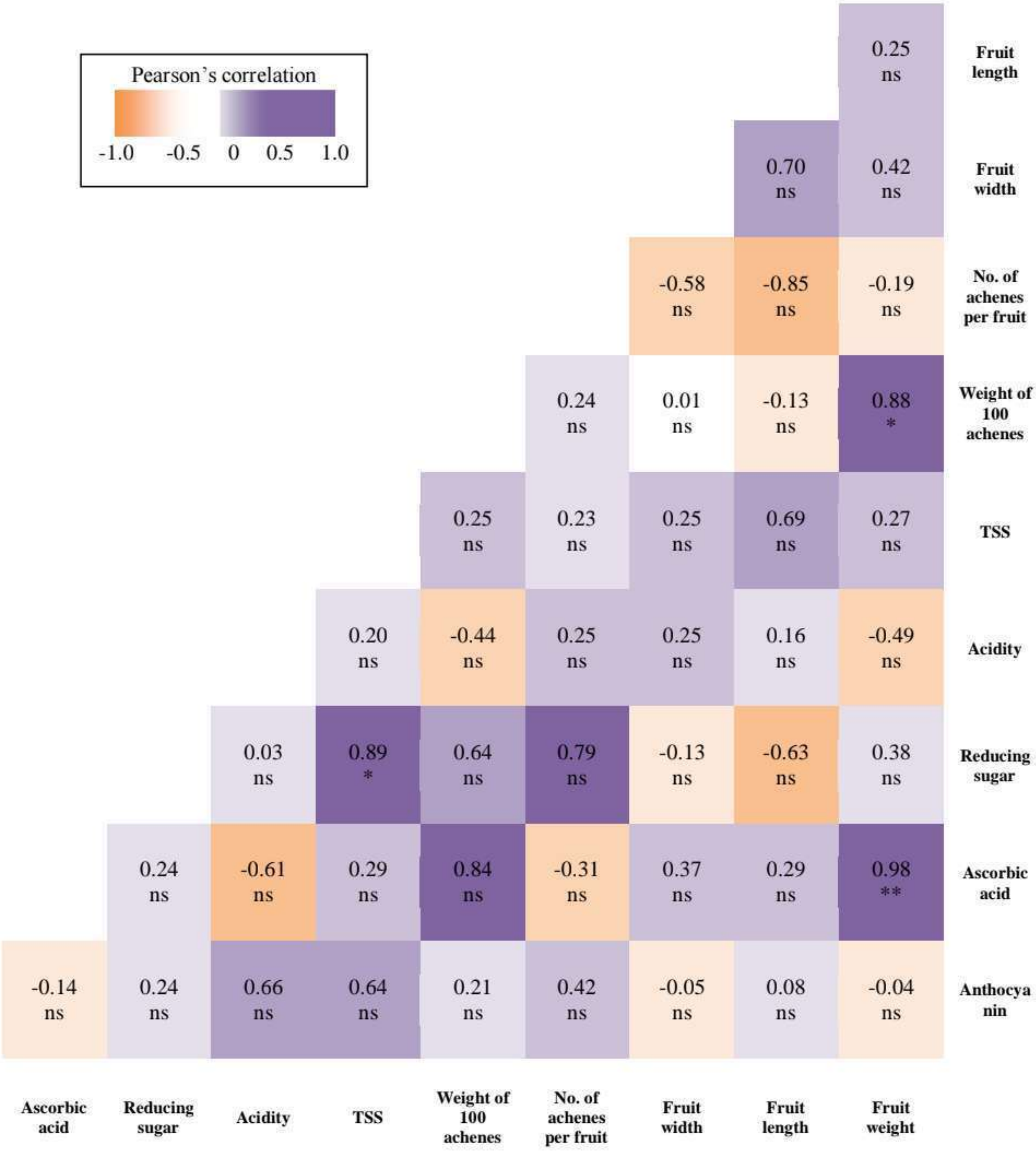
T_2 (W×C) was the reciprocal cross in which fruits failed to set.

Figure 4.1: Correlation coefficient for fruit characters of T_3 (self pollination in Winter Dawn)



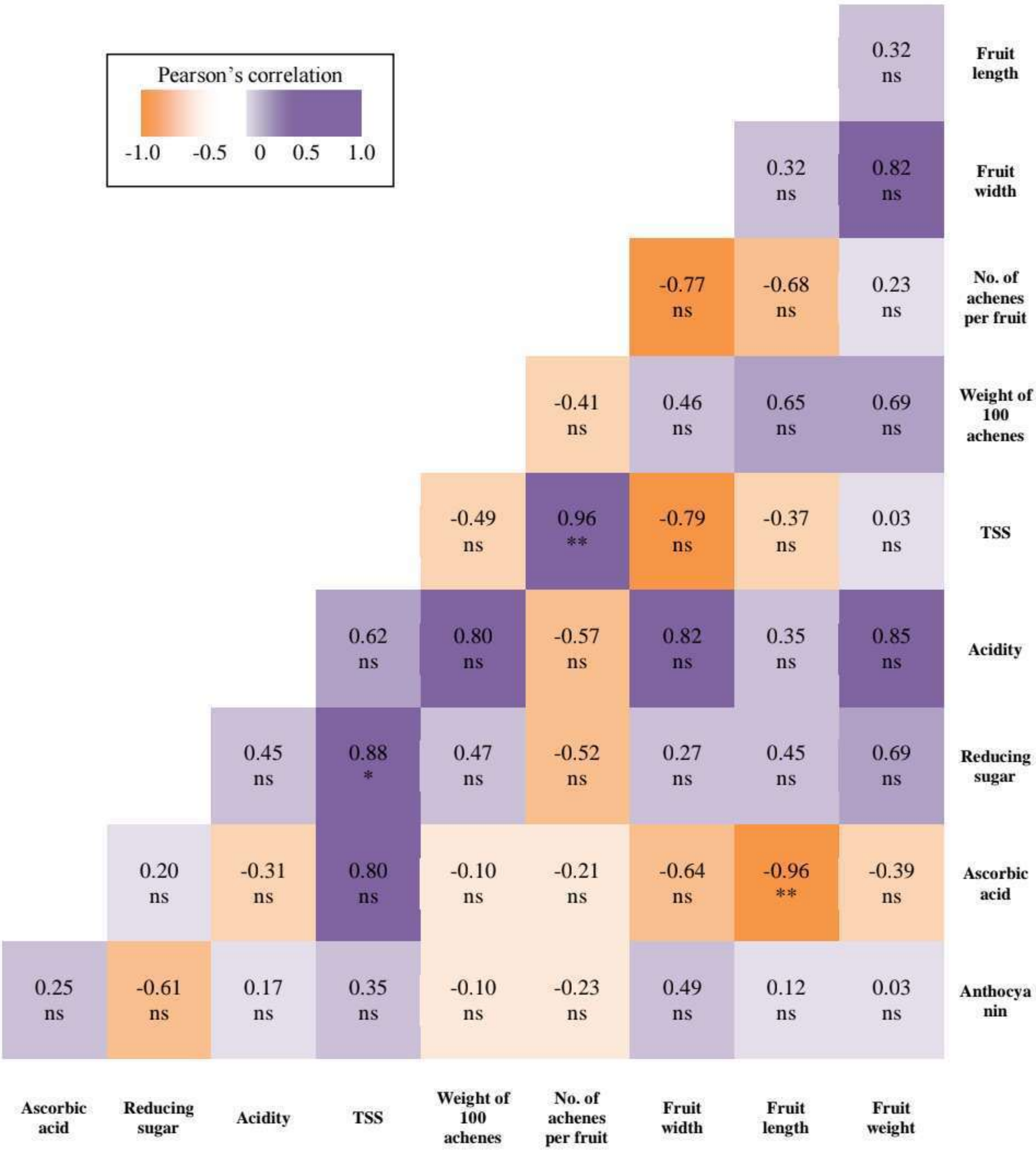
ns p>=0.05, * p<0.05, ** p<0.01, *** p<0.001

Figure 4.2: Correlation coefficient for fruit characters of T_4 (self pollination in *P. indica*)



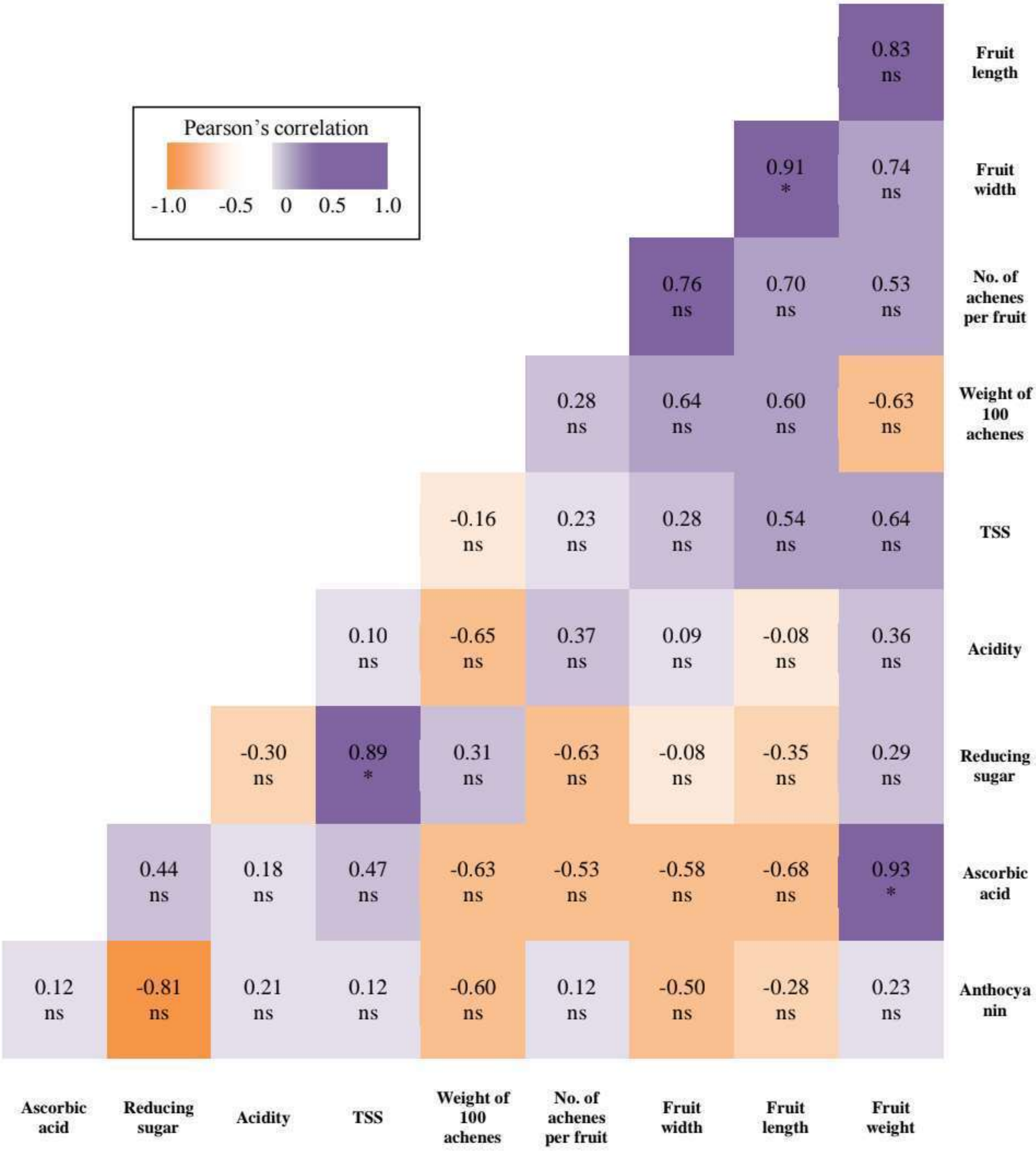
ns p>=0.05, * p<0.05, ** p<0.01, *** p<0.001

Figure 4.3: Correlation coefficient for fruit characters of T_5 (open pollination in Winter Dawn)



ns p>=0.05, * p<0.05, ** p<0.01, *** p<0.001

Figure 4.4: Correlation coefficient for fruit characters of T_6 (open pollination in *P. indica*)



ns p>=0.05, * p<0.05, ** p<0.01, *** p<0.001

Chapter 5

SUMMARY AND CONCLUTION

SUMMARY AND CONCLUTION

Strawberry is significant due to its high nutritional value, vitamin C concentration, and appropriateness for a variety of goods and uses. According to reviews of the literature, numerous aspects of floral biology, pollination and fruit set are the most significant pre-requisites for any improvement and hybridization effort. The wild native strawberry, which is well adapted to the climatic conditions of West Bengal's Teri zone, has gotten little attention.

The present investigation on the aspect of effect of modes of different pollination on fruit quality has been carried out in Instructional Farm of Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari during 2022- 2023. The salient finding of the study are being summarized in the following paragraphs:

The plant molecular and morphological parameters analysis showed that both the genotypes were distinctly different from each other, selection as parent materials for distant hybridization was highly significant. Molecular analysis conformed the identification of commercial and wild genotypes as *Fragaria × ananassa* and *Potentilla indica*. Plant morphological studied showed that the Winter Dawn has maximum plant height (17.16 cm), plant spread (36.38 cm), flowers/plant (29.20), fruits/plant (24.2), leaf area (63.08 cm^2), petiole length (15.20 cm) and chlorophyll content (150.50 mg/100g) but runners/plant is maximum in *P. indica* (6.80).

Both flowering duration (122 days) and fruiting duration (91 days) were longer in Winter Dawn; however, flower emergence was earlier in *P. indica* (10 days). Stigma receptivity was higher during anthesis time. Anthesis and stigma receptivity of Winter Dawn were between 9:00 am to 10:00 am and for *P. indica* it was from 10:00 am to 11:00 am.

The fruit setting percentage in the month of January was higher for Winter Dawn and for *P. indica*, fruit setting percentage was higher in February. Under open pollination, Winter Dawn set maximum fruits in both open (93.13%) and self pollination (87.20%). After crossing both the genotypes, using Winter Dawn as mother plant, only 0.34% of fruit set was observed while no fruit setting has been seen in reciprocal cross.

The average size of Winter Dawn pollen is 21.98 µm whereas average size of *P. indica* pollen is found to be 24.31 µm. Maximum pollen germination percentage was seen at 12 hours in Winter Dawn (91.66%) and *P. indica* (82%) was seen in 15% sucrose medium while pollen tube length was maximum in 20% sucrose medium with 58.66 µm in Winter Dawn and 36 µm in *P. indica*. Pollen vialbility was recorded 100% in both the genotypes.

After crossing, open pollination in cultivated strawberry (T_5) gives high quality fruit with maximum fruit weight (20.16 g), fruit length (4.02 cm), fruit width (3.34 cm), weight of 100 achenes (0.09 g), TSS (7.58°B), reducing sugar (3.42%), total sugar (4.90%), ascorbic acid (52.64 mg/100g), anthocyanin (32.12mg/100ml) and minimum acidity (0.66%). Self pollination in wild strawberry (T_4) gave poor quality fruit. Fruit obtained after crossing in T_1 (cross pollination of cultivated and wild strawberry) showed intermediate characteristics. In crossing, fruits produced least number of achenes (60), which were germinated but died after few days. The reciprocal cross was unsuccessful.

Overall it can be concluded that open pollination produces good quality fruits, due to proper pollination of each and every stigmatic head. During experimental period, *P. indica* had showed some immense potentiality to resist disease and pest attack which can be further studied and exploit for distant hybridization. Intensive crossing should be done with other commercial strawberry varieties for production of F_1. Since seed production is less after crossing and seedling are unable to survive, hence embryo rescue protocol should be standardized for getting F_1 through distant hybridization and for future crop improvement program.

Chapter 6

FUTURE SCOPE OF THE RESEARCH

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- The conclusions drawn from the present experiment investigation are based on single season in a particular location, which might have wide implications. The experiment needs to be repeated over year, season and location before drawing any definite conclusions.

- The present experiment was conducted by using only one variety of strawberry, i.e., Winter Dawn. Furthermore varieties are needed to be crossed with *P. indica* to study the effect of pollination.

- Seeds of crossed fruit germinated, but seedlings failed to survive after few days, hence standardization of embryo rescue protocols needed to be done to obtain F_1 generation for further study.

- Intensive study of *Potentilla indica* is needed to be done to exploit its biotic and abiotic stress resistance traits, which can be incorporated in the commercial cultivars for better quatily and marktable fruit production.

Chapter 7

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APPENDIX

Table A.1: Analysis of variance of fruit setting percentage

Source	Degrees of freedom	Mean sum of square								
		January			February			March		
		Initial	50%	Harvest	Initial	50%	Harvest	Initial	50%	Harvest
Replication	4	6.46	3.72	7.42	41.95	22.91	12.43	11.87	4.04	2.64
Treatment	5	6683.07	6635.19	6743.29	6647.72	6,648.74	6,456.98	5,921.50	5,775.78	5,628.09
Error	20	20.07	12.99	12.99	15.63	13.39	10.77	9.56	6.56	3.77

Table A.2: Analysis of variance of pollen germination

Source of variance	Degrees of freedom	Mean sum of square							
		Pollen germination percentage				Pollen tube growth			
		6hrs		12hrs		6hrs		12hrs	
		Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*	Winter Dawn	*P. indica*
Treatment	3	548.75	1398.97	916.55	2560.44	432.97	463.23	374.66	747.63
Error	8	28.33	21.50	13	28.41	2.41	3.60	7.83	8.50

Table A.3: Analysis of variance of fruit physical and biochemical parameters

Source	Degrees of Freedom	Mean Sum of Square											
		Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Number of Achenes per Fruit	Weight of 100 Achenes (g)	TSS (°Brix)	Reducing Sugar (%)	Non-Reducing Sugar (%)	Total Sugar (%)	Total Acidity (%)	Ascorbic Acid (mg/100g)	Anthocyanin (mg/100g)
Treatment	4	401.97	14.07	6.38	19885.50	0.007	13.46	2.81	0.46	3.13	0.089	917.99	163.77
Replication	4	0.22	0.01	0.03	147.20	0.000	0.06	0.01	0.15	0.13	0.006	10.55	3.61
Error	16	0.89	0.02	0.16	240.50	0.000	0.16	0.02	0.09	0.06	0.002	7.23	6.72

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ABSTRACT Strawberry (Fragaria × ananassa Duch.) is a man-made hybrid of the Rosaceae family and is well known for their aroma, vivid red colour, juicy texture, excellent flavor and antioxidant properties. Wild strawberries tend to be small in size as compared to cultivated ones but are hardy and adaptable in nature. To get a strawberry that is big and hardy in nature, it is necessary to cross and hybridize them to improve their quality. Strawberry is benefited from the action of pollinators. However, insufficient pollination can lead to an increase in the percentage of misshapen or malformed fruits. Therefore, proper pollination of strawberries is necessary to get desirable attractive strawberries that are highly marketable. In this experiment, the aim was to determine the effect of modes pollination between commercial and wild strawberry. The experiment consisted of 6 treatments of different modes of pollination (open, self and cross pollination) with 5 replications of each genotype (Winter Dawn and Potentilla indica) in Randomized Block Design (RBD). The plant and flower

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