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Study of pollen viability and pollen germination in different cultivars of litchi in sabour, Bhagalpur condition

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Abstract
The most important fruit of the Sapindaceae family is Litchi (*Litchi chinensis sonn*). It originated in China and is widely distributed in the tropics and subtropics (Knight, 1980). Litchi is an important sub-tropical evergreen fruit crop. Therefore, the present investigation entitled "Study of pollen viability and Pollen germination in different cultivars of litchi in sabour, Bhagalpur condition" was carried out during 2017-18 in the Department of Horticulture (Fruit and Fruit Technology), BAC, Sabour,. For this experiment four litchi cultivars *viz.*, Purbi, Bedana, Shahi and China were chosen for the present study. On the basis of data recorded on the pollen viability and germination percentage is maximum in cv. Purbi (87%, and 83.40%) and minimum was observed in cv. China (78%, and 74%). This might be due to genetic composition and environmental factor. The date of fruit-set in different cultivars of litchi under study varied from 13-03-2018 to 25-03-2018. The maximum duration of fruit set was noticed in Purbi (9.8 days) followed by Shahi (8.26 days). Among the four cultivars, fruit set was recorded to be highest in case of Purbi X Bedana (20) followed in Desi X Bedana (17).

Keywords: Pollen germination, pollen viability, sex ratio, Fruit set

Introduction
The litchi (*Litchi chinensis* Sonn.) is the most important fruit of family, sapindaceae. The chromosome number is 2n=30. Litchi is a subtropical fruit crop and is characterised by warm temperature. Litchi fruit is native of south China and grown in India since 18thcentury. Litchi is currently growing in about 30 countries in tropical and subtropical regions of the world. China is the largest producer of litchi in the world and followed by India. In India area under litchi is 91000ha, which gives a yield or production of about 578000 MT under well managed condition (FAO). Of the total production of litchi in India, about 40%is contributed by Bihar. The average productivity of litchi in Bihar is 8.0 t/ha (NHB 2016-17). The major Litchi producer district in Bihar are Muzaffarpur, Bhagalpur, Darbhanga, Khagaria and Hazipur.

This crop is highly specified to a particular climatic condition and probably due to this reason its cultivation is restricted to few countries in the world. In India for Litchi cultivation the temperature varies from 21 ºC to 37.8 ºC during flowering to fruiting. Vegetative growth is inhibited and restricted by temperature below 10ºC and above 35 ºC with maximum growth between 25-30 ºC depending on cultivars (Menzel *et al.* 1989) [33]. It is however, sensitive to cold and the crop is severely injured by temperatures below freezing but can withstand light frosts. Relatively high rainfall of 1,200mm per annum with high humidity is preferable (Tindall *et al.*1994). A certain degree of water stress is needed for flower initiation. A dry climate, free from rains for about two months before flowering induce flower bud differentiation, blossom and consequently give high production (Cohen *et al.* 1992) [15].

However, male (M_1 and M_2) and female (F) flowering stages may overlap on the same tree or between trees of the same cultivar, thereby providing an opportunity for self-pollination (Stern and Gazit, 2003). The results of pollination studies carried by Degani *et al.* (1995) [23] indicated that pollen parent can have an effect on fruit-set, fruit retention and quality of litchi fruits. Length of the flowering cycle varies with the genotype and weather, and, is much shorter under warm temperatures. After flower initiation, development of flower panicle and flower continues uninterrupted and leads to anthesis, which lasts about 4 to 6 weeks depending on the temperature and the cultivar (Menzel, *et al.* 2001) [32]. Litchi flowering follows the pattern of "male-female-male". The ratio of male to female flowers varies with the environment and among its various cultivars. High temperature during flower initiation reduces the proportion of female flowers (Cronje, 2009) [16].

In view of the above facts the work was carried out on commercial litchi cultivars grown in Bihar condition with following objectives:

Materials and Methods

The present investigation was carried out at the Horticultural Garden, Bihar Agricultural College, and Sabour during the year 2017-2018 with a view "study of pollen viability and Pollen germination in different cultivars of litchi in sabour, Bhagalpur condition". The details of materials used and experimentation adopted are discussed below:

The experiment was conducted in Horticulture Garden, Sabour, and the permanent experimental site of the Bihar Agricultural College, Sabour, and Bhagalpur. The experimental plot had well drained sandy loam soil of good fertility with levelled surface.

Location and climatic conditions

Bihar Agricultural College, Sabour is situated between 25°15'40" North longitudes of 45.72 meters above the mean sea level in the heart of the vast alluvial Gangetic plains of North India, South of River Ganga. The climate of Sabour is semi-arid, subtropical with hot desiccating summer, cold but frost less winter with an average annual rainfall of about 1150 mm precipitating mainly between middle of June to middle of October. Mainly three seasons influence the agricultural activities of this region, these are winter season (November to February), summer season (March to June) and rainy season (Mid June to October). Experimental Details

The experiment was conducted on uniform plants in respect of size, vigour and productivity of litchi hybrids and their parents in the orchard. The plants were 30 years old. All the trees received similar cultural practices and irrigation in the preceding years and during the experiment period. All details about materials used, experimental procedure followed and methods adopted for experiment are described below:-

Crop: Litchi
Number of treatments: 04
Number of replications: 05
Experimental design: Randomized block design

A. To study the pollen viability

1. Date of anther collection and pollen viability

The date of pollen collection was started during month of January and February in different flowers at different time period. Anthers were collected at dehiscence time between 8 and 10 a.m. by using forceps. After anther collection the pollen grains were distributed on the glass slide with the assistance of a brush and dyed with 1 or 2 drops of acetocarmine, thereafter left the slide for about 15-20 minutes for staining purpose. Then the pollen grains were observed under an optical microscope (compound microscope at 40× magnification) and the pollen grains which get fully stained got pink colour considered as viable pollen grains and which is not stained leave as transparent considered as non-viable pollen grains.

$$\text{Pollen viability\%} = \frac{\text{No. of viable pollen grains}}{\text{Total no of pollen grains}} \text{ X } 100$$

2. Pollen germination percentage

In vitro Pollen germination in liquid medium

In vitro germination test in liquid media was done by hanging drop technique. The liquid germination medium containing 150 g /1 sucrose, 100 mg/ l H_3BO_3, 1000 mg/ l $Ca(NO_3)_2$, 300 mg/l $MgSO_4$ and 100 mg/l KNO_3 at pH 5.5 was prepared. Therefore, for *in vitro* germination, pollen grains were thoroughly mixed in 1000ml of liquid germination medium to get uniform pollen samples. Fifteen micro liter of this mixture was placed on a glass slide having 8 mm diameter ring inside. Slides were inverted and placed on a rack in a polycarbonate sealed container lined with moistened blotting paper and incubated in dark at 26 ^{0}C temperature and observed them under optical microscope (compound microscope 40× magnifications).

$$\text{Pollen germination \%} = \frac{\text{No. of Germinated pollen grains}}{\text{Total no of pollen grains}} \text{X } 100$$

In vitro Pollen germination on solid medium

In vitro germination test was assessed in an agar solidifying medium containing 150g/sucrose, 100mg/l H_3BO_3, 300 mg /l $Ca(NO_3)_2$, 200 mg /l $MgSO_4$, 100 mg/ l KNO_3 and 10 g /l agar at pH 5.5. After preparation of this solidifying media, pollen grains were dusted uniformly on the thin layer of growing medium taken on the separate slide and incubated for 24 hours in dark at 26 ^{0}C temperature, and observed them under optical microscope (light microscope at 40× magnifications).

$$\text{Pollen germination \%} = \frac{\text{No. of Germinated pollen grains}}{\text{Total no of pollen grains}} \text{X } 100$$

Preparation of specimens for microscopic observation

For this Palynology investigation, the specimens were studied under light microscopy. The pollen samples for LM were acetolysed following the technique developed by Erdtman (1960) modified by Takahashi (1987). The anthers were soaked overnight in acetic acid for softening in 2 ml polyethylene centrifuge tube and were crushed prior to acetolysis. The outmost care was taken to remove the debris and/or unwanted material e.g., fractions of floral parts or anther, filament, etc. The acetic acid acid was then decanted and acetolysis mixture (9 ml acetic anhydride: 1 ml conc. sulphuric acid) was added to the centrifuge tube. The acetolysis took place at 100ºC for 3-5 min. A glass rod was inserted into each tube to stir the pollen sample within acetolysis mixture for the completion acetolysis process evenly. After acetolysis grains became yellow-brown to brown in colour. The acetolysed materials were washed with distilled water, dehydrated in ethanol series (70%, 80%, 90%, 95%, 99.5% and 100%) and transferred in the benzene. A drop of silicon oil (viscosity 3000 cs.) was mixed with the material left in the benzene. The tube containing the material was left stand overnight at 75 ºC until the benzene had evaporated completely. The slides sealed with paraffin wax. At least four slides per specimens were made. All slides were investigated and photographed.

To study the effect of pollen parameters on fruit set observation to be taken

Crossing was conducted with the pollen from all these four varieties and Deshi and Purbi was taken as female parent for crossing purpose.

1. No. of flowers crossed
2. Number of fruit retained after 26 days of completion of fruit set.

The crossing is conducted by removing all the male flowers from those cultivars which is selected as female with the help of forceps and then dusting the pollen of four cultivars which is selected for study and then bagged the panicles for 10 days

thereafter counting the initial fruit set and after 26 days recorded the fruit retained.

$$\text{Fruit retention (\%)} = \frac{\text{Number of Fruits per panicles}}{\text{Number of initial fruit set}} \text{X } 100$$

3. Sex ratio

Sex ratio of different cultivars is calculated by using this formula

$$\text{Sex ratio} = \frac{\text{Total number of male flowers}}{\text{Total number of female flowers}} \text{X100}$$

Results and Discussion

The observations has been taken on Pollen viability and Pollen germination, fruiting characteristics along with crossing effect and fruit set were recorded during the course of present investigation. In order to get complete picture of these parameters, the data collected were put to statistical analysis and their interpretation have been presented in this chapter. The results of the present investigation were elaborated in the preceding chapter. In this chapter scientific and logical interpretation of result obtained are discussed for clear and better understanding. Efforts have also been made to support the results with the previous findings.

Pollen viability and germination

The pollen viability and germination percentage is maximum in cv. Purbi (87%, and 83.40%) and minimum was observed in cv. China (78%, and 74%).This might be due to genetic composition and environmental factor. Amma and Kulkarni (1979) reported that for successful germination, pollen grains of different species require different growth component like water, sugar solution, inorganic salts, vitamins *etc*. at varying ranges and the composition and pH of the growth media are equally important factors for emergence of pollen tube from the grains on the media. Robbertse *et al.* (1992). Found that the obturator plays a vital role in the fertilization process of the litchi by facilitating the growth of pollen tubes around the broad base of the ovule towards the micropyle and embryo sac. Kozai *et al.,* (2004). Previous studies on peach demonstrated that elevated temperatures during flower development can significantly reduce fruit set.

Sex-ratio

Sex-ratio was significantly higher in China 3.17 followed by Bedana 2.84. The sex ratio is a variable component within panicles, trees and among genotypes. Sahay *et al*. (2005) reported that Bedana had significantly the maximum sex ratio followed by Late Bedana and Lal Bombai while the minimum was obtained in Ahjouli which was remained *at par* with China and Deshi. According to Pathak *et al*. (2013) sex ratio of flowers varied between 2.74:1 in cv. China and 5.8:1 in cv. Piazi. Similar observation was also recorded by Sarkar and Bandopadhyay (1989).

Fruit set

The date of fruit-set in different cultivars of litchi under study varied from 13-03-2018 to 25-03-2018. The maximum duration of fruit set was noticed in Purbi (9.8 days) followed by Shahi (8.26 days). The question of fruit set is of utmost importance in the production of any fruit crop. The variation observed in terms of fruit-set might be due to the differences in genetic composition of different litchi genotypes. Sharma and Roy (1987) reported that fruit se has occurred within 7-18 days of flowering. Mc Conchie and Batten (1991) suggested that the most appropriate time for fruit to be considered set is when most fruitlets on a panicle reach maturity.

Effect of crossing method on fruit set

Among the four cultivars, fruit set was recorded to be highest in case of Purbi X Bedana (20) followed in Desi X Bedana (17). There was poor fruit set by selfing. In contrary to this result Brijwal *et al.* (2016) [8] had reported that initial fruit set under self-pollination was significantly higher than all crosses and open-pollination methods. Forneman *et al.* (2012) also reported the lower initial fruit set in all cross-pollination as compared to self-pollination in 'Wai Chee' litchi cultivar. Mc Conchie and Batten (1991) had suggested that the most appropriate time for fruit to be considered set is when most fruit left on a panicle reach maturity.

Table 1: Pollen viability and germination percentage of litchi cultivars

Cultivars	Pollen viability (%)	Pollen germination (%)
Purbi	87.00	83.40
Bedana	80.00	74.60
Shahi	83.00	74.40
China	78.00	74.60
C.D at 5%	5.29	4.53
C.V	4.63	4.24

Table 2: Date of fruit set initiation and duration of fruit set.

Cultivars	Date of initial fruit set	Duration of initial fruit set (Days)	No of fruit set per panicle
Purbi	21-03-2018	9.80	6
Bedana	15-03-2018	8.20	5
Shahi	20-03-2018	8.26	4
China	28-03-2018	7.40	7

Table 3: Flower crossed and no of initial fruit sets

Cultivars	No. of flower crossed	No. of initial fruit sets	Fruit %	No. of fruit retained after 26 days	Fruit %
Desi X Bedana	60	17	28.33	12	70.58
Desi X Shahi	28	14	50.00	07	50.00
Desi X Purbi	24	15	62.50	12	80.00
Purbi X Bedana	30	18	60.00	08	44.44
Purbi X Shahi	28	17	60.71	07	14.17
Purbi X Deshi	80	12	15.00	08	66.66

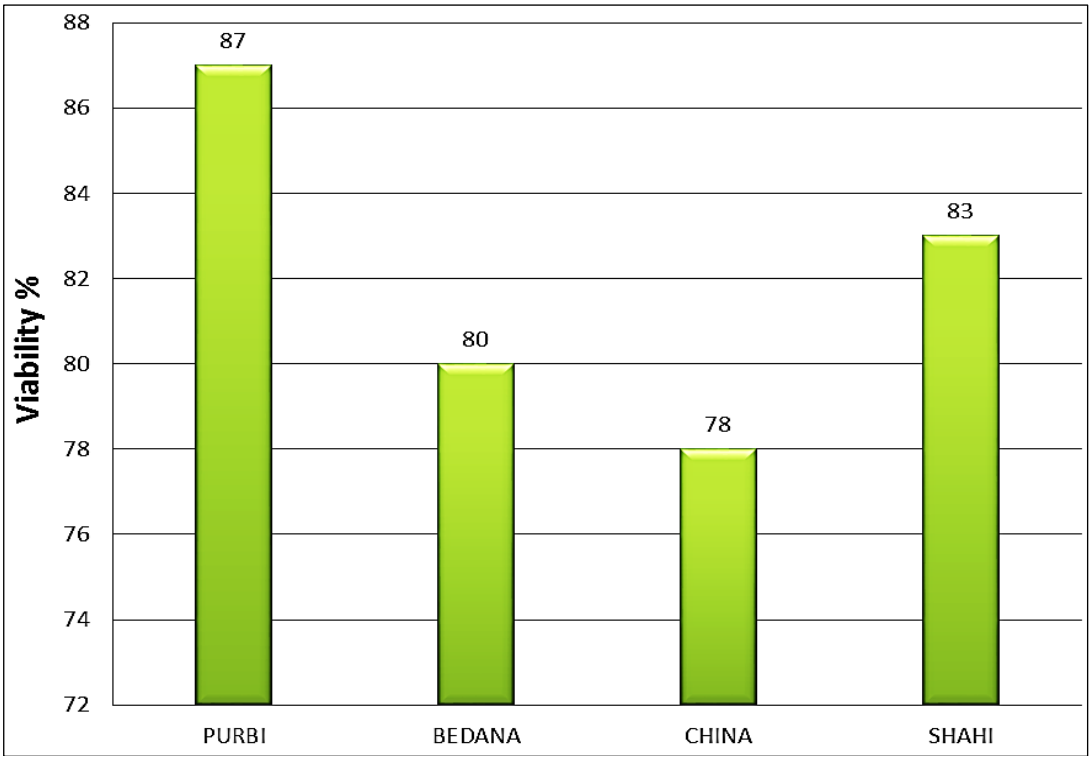

Fig1: Pollen viability % of different cultivars

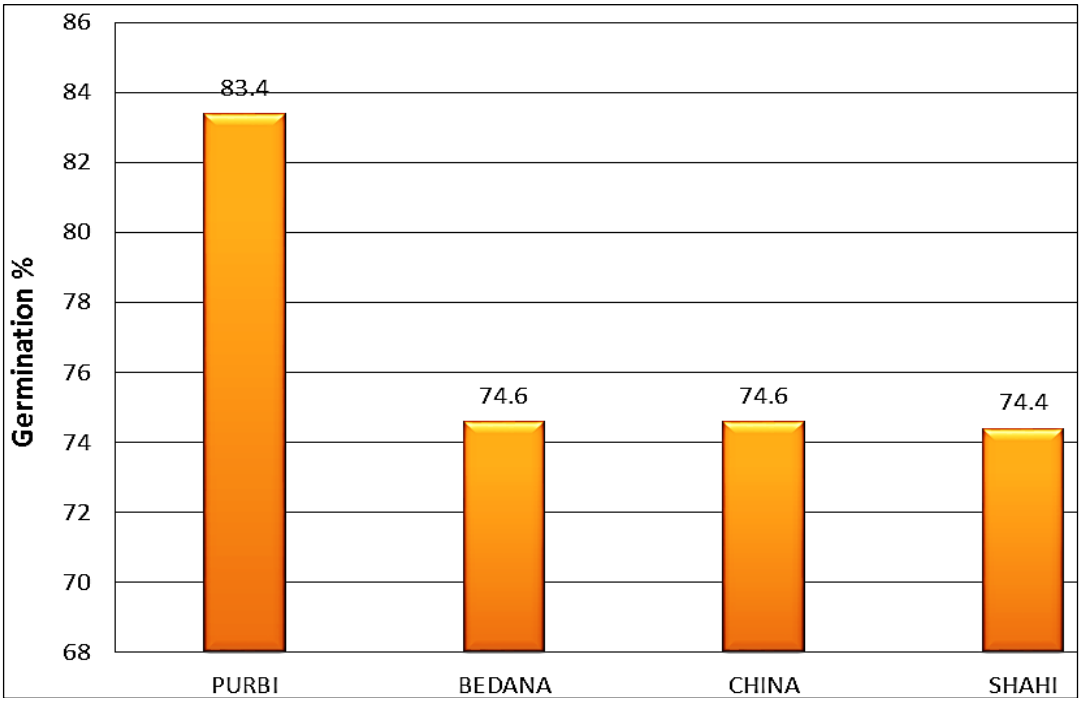

Fig 2: Pollen germination % of different cultivars

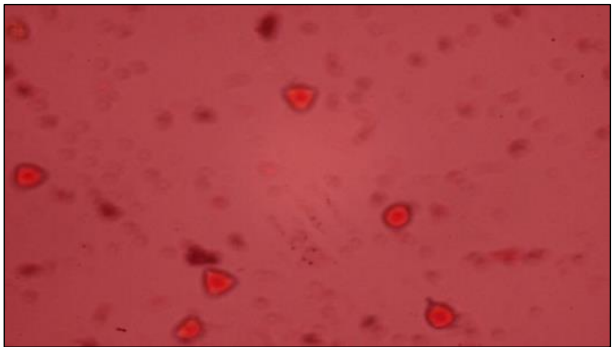

Fig 3: Pollen view of Bedana at 10X

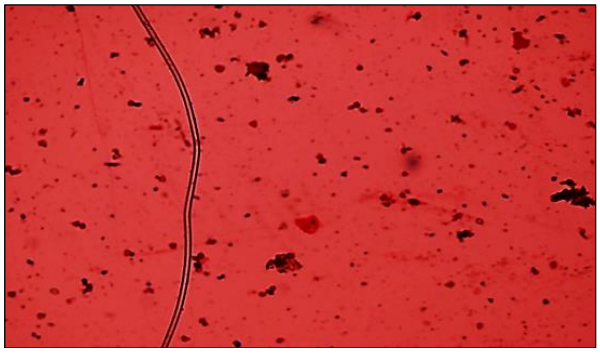

Fig 4: Pollen view of Purbi at 10X

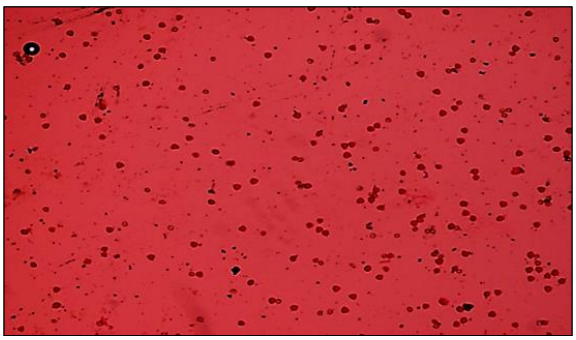

Fig 5: Pollen view of Shahi at 10X

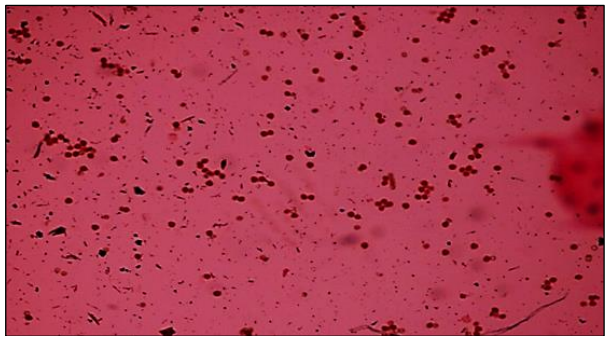

Fig 6: Pollen view of China at 10X

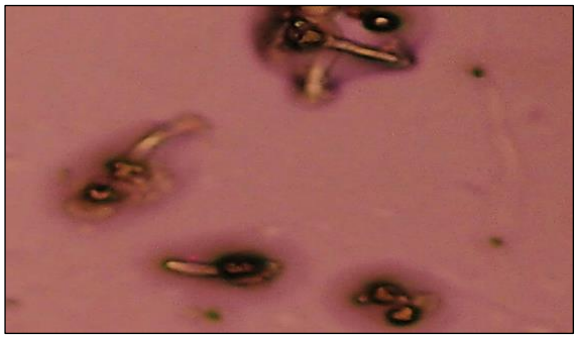

Fig 7: Pollen germination of Shahi at 40X

Fig 8: Pollen germination of Purbi at 40X

Fig 9: Pollen germination of Bedana at 40X

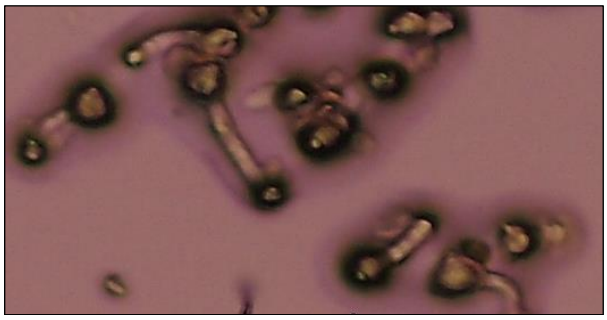

Fig 10: Pollen germination of China at 40X

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