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# Pollen Storage Studies In *Brassica juncea* L. Czern and Coss

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**ABSTRACT**

Pollen is appropriately referred by some as 'Golden dust' extremely valuable on account of their tremendous applications in science, industries and public health. No other plant part even though extremely tiny in size is packed with so much information and power.

The retention of pollen viability after shedding varies significantly from species to species. Environmental factors, particularly humidity & temperature greatly affect viability. The relationship between air humidity and pollen longevity were investigated time to time. In the present studies pollen storage studies of *Brassica juncea* L. Czern. and Coss. was carried out by providing different temperatures and relative humidities as well storage in organic solvents.

On room temperature, in 0% RH 24 day's viability was recorded with 44%germination and 213 micron tube length. On freeze temperature, pollen was stored up to 28 days showing 20%, 18% and 9% maximum germination in 10%, 20% and 30% RH respectively with tube length of 185, 79 and13 microns respectively. While in organic solvents ***Brassica*** pollen gave best results in chloroform which was 284 micron length and percent germination with 28% and 25 days viability and least pollen storage was seen in Xylene, only 6 days viability with 102 micron maximum tube length.

**Keywords**: 3-celled pollen, ***Brassica juncea***, relative humidity, organic solvents.

## INTRODUCTION

***Oleiferous Brassica*** (rapeseed and mustard) has been an important conventional oil seed crop of the sub-continent. ***Brassica juncea*** L. (2n=AABB=36) is the most common source of edible oil in many Afro-Asian countries. In India is being grown extensively as oil seed. ***Brassica juncea*** is an amphidiploid as well as allopolyploid originated through natural crossing between ***B-nigra*** (2n=16) and ***B-compestis*** (2n=20). Availability of genetic variability is the prerequisite for any breeding programme.[1]

The artificial maintenance of the viability and fertilizing ability of pollen over a long period is an important problem from both the theoretical and practical point of view. Horticulturists and plant breeders having long been interested in crossing varieties species and even genera to produce new and improved types of plants better suited to human requirements. The pollen being a hereditary component has a predominant role in crop production programs. The disharmony in the flowering of the parent plants and their separation by distance is one of the several problems faced during carrying out hybridization. In such situation pollen storage is a tool in a hand of plant breeders in preserving the viability of pollen and using them as per their need.

Successful pollen storage is a very convenient tool in the hands of the breeders for improving trees by hybridization, occurring in different regions and also of those blooming in different seasons. The longevity of pollen grains enables the introduction of new character in plants by using stored living pollens.

The humidity of the air and the temperature affects the storage of pollen grains of different plant species; wherein success has been obtained in storage through manipulation of temperature and humidity. It is a universal fact that the pollen of several species remain viable for longer period at lower temperature than at higher temperature, and maximum longevity is obtained at low relative humidities (0-30%).

The present work deals with pollen storage of ***Brassica juncea* L. Czern. And Coss.** at low temperature i.e. on freeze temperature (5°C) and room temperature (25-30°C) on different relative humidities (0, 10, 20, 30 and 70 % RH). The viability of stored pollen was tested regularly after 24 hours.

## MATERIAL AND METHODS

### A) EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON POLLEN STORAGE

#### I) Pollen Collection

In ***Brassica***, anther dehiscence occurred soon after anthesis around 9-10 am. As there is high frequency of insect visit following anthesis most of the pollen is lost from the flowers. In these systems, the flower buds were collected early in the morning just before the anthesis for pollen collection. After removing the sepals and petals,

the flower buds are maintained under sunlight or desiccators with low humidity for 1-2hr. until anthesis. Hence, pollen can then easily be collected for further investigations.

Pollen were subjected to the room and freeze temperature at 0%,20%,20%,30% and 70% RH condition in 5 sets of each plant pollen and the mean of that readings was taken as a final readings of days stored, % of germination and tube length.

**II Storage and Preservation**

The pollen grains dried at room temperature for half an hour were equally divided & transferred to the glass vials & plugged with cotton wool. These glass vials were then kept in desiccators in which different relative humidities like 0%, 10%, 20, 30% and 70% were maintained. Procedure for maintaining humidity was followed as described by Wilson. [2] Concentrated Sulphuric Acid was used as the desiccant.

Thus, pollen samples were stored at different RH & temperature as follows,

Low (Fridge) temperature (5°C) – 0, 10, 20, 30, & 70% RH.

Room temperature (25 – 30°C) - 0, 10, 20, 30, & 70% RH.

**III Prehumidification of the stored pollen grains.**

The stored pollen grains were hydrated before the viability test. The pollen grains were placed on a glass slide kept in a covered Petri dish (6") lined with moist fitter paper. The pollen grains were humidified for 10 minutes.

**IV Viability test (*in Vitro*).**

The viability of the pollen was tested *in vitro* by sitting drop technique. [3] In this method, a drop of the culture medium was placed on the slides, & pollen grains were cultured in these drops. The slides were then placed in Petri plates lined with moist fitter paper to prevent evaporation. A large no. of replicates could be conveniently raised by this method. The cultured medium used was the best suited medium for germination of pollen *in vitro*. For ***Brassica*** 15 % sucrose was used as a culture medium to check the viability of stored pollen grains as this medium gave maximum germination *in vitro* experiment.

**B) POLLEN STORAGE IN ORGANIC SOLVENTS**

**a) Storage and Preservation**

Storage of pollen grains in organic solvent is probably the simplest method so far.[4] The pollen grains were collected as described previously in the morning time just after the dehiscence of anthers. The collected pollen grains were then dried over silica gel at 0°C, and then 5ml of each organic solvent such as Benzene, Isopropyl alcohol, Chloroform Acetone, Xylene was taken separated in the glass vials, plugged tightly and kept in an ice container of refrigerator which has a temperature near about 4 °C.

**b) Prehumidification of the stored pollen**

The stored pollen grains were removed from the glass vials at an interval of 48 hrs. with glass dropper on a slide. The organic solvent left on the slide was allowed to evaporate. The slides were then kept in a Petri dish lined with wet filter paper for humidification before culturing the pollen grains.

**c) Viability test**

The viability of the hydrated stored pollen was tested *in vitro* by culturing the pollen grains in standardized solution. The % of germination and the viability of pollen in days were monitored under the microscope.

In the experimental period the atmospheric relative humidity was high during the months of June to September while in the dry months it was low. In month of July and August it went up to 88% (average monthly mean) while in month of April and May it decreases up to 20.16 % (average monthly mean). The temperature of Nagpur city was less in monsoon and winter but it increased as the dry summer season approached. The highest mean temperature recorded in 2004-2005 was 42 °C while minimum was 12.6 °C and in the year 2005-2006 the maximum mean temperature recorded was 43.4 °C and minimum mean temperature was 11.6 °C. During the experimental period as per meteorological data the monthly average maximum temperature, minimum temperature, relative humidity and total rainfall are as shown in the table 1.

**RESULTS**

In case of ***Brassica***, as the pollen is 3 celled, the metabolic rate of the pollen is quite high and so they showed very less viability on room as well as on freeze temperature. On room temperature, in 0% RH 24 days viability of pollen was recorded with 44 percent germination and 213 micron tube length. 10 days storage was recorded on 10% RH with 125 microns length on 20 % and 30% RH 11 and 5 days viability of stored pollen was recorded respectively with 12% and 5%germination. On freeze temperature, pollen was stored up to 28 days with 23 percent germination with 25 microns tube length in 10%, 20% and 30%. Pollen showed 2%, 18% and 9% germination respectively with tube length of 185 microns, 79 microns and 13 microns respectively (Table 2).

Effect of different organic solvents such as benzene, isopropyl alcohol, chloroform acetone,

xylene on the viability of ***Brassica*** pollen grains at 4 ° C are given as follows;

The maximum viability, tube length and highest maximum percent of germination were found in Chloroform which was 25 days, 284 µ tube lengths and 28% germination respectively. Benzene showed 23 days viability with 34% of germination and maximum tube length of 246 µ. In case of Isopropyl alcohol, 168 µ tube lengths were found with maximum 26 % germination. In Xylene only 6 days viability was observed with 102 µ maximum length. Acetone showed 17 days viability with 31 % of germination and maximum tube length of 178 µ (Table 3).

**Table 1** Meteorological data for the period from Feb. 2004-Jan. 2006

| Month | Year | Average mean Temperature °C | | Average mean relative humidity (%) | | Total rainfall (mm) |
|---|---|---|---|---|---|---|
| | | Max. | Min. | 8.30 Hrs. | 17.30 Hrs. | |
| **February** | 2004 | 30.16 | 14.8 | 55.55 | 33.31 | Nil |
| | 2005 | 31.9 | 16.1 | 55.59 | 31.96 | 3 |
| **March** | 2004 | 38.8 | 20.2 | 36.09 | 21.22 | Nil |
| | 2005 | 36.2 | 20.1 | 46.16 | 24.67 | 14.2 |
| **April** | 2004 | 41.7 | 25.3 | 37.4 | 22.2 | 5.3 |
| | 2005 | 40.2 | 22.8 | 35.06 | 20.16 | 6.3 |
| **May** | 2004 | 42 | 27.7 | 45.54 | 29.48 | 9.7 |
| | 2005 | 43.4 | 27.4 | 30.87 | 22.87 | 10.2 |
| **June** | 2004 | 37.6 | 26.1 | 63.13 | 50.76 | 135.1 |
| | 2005 | 41 | 28.1 | 50.83 | 40.4 | 231 |
| **July** | 2004 | 33.5 | 24.5 | 79.03 | 67.35 | 305.7 |
| | 2005 | 31.2 | 24.1 | 85.7 | 78.06 | 282.5 |
| **August** | 2004 | 29.7 | 23 | 86.32 | 78.64 | 233.6 |
| | 2005 | 30.6 | 23.6 | 88.06 | 78.32 | 195.8 |
| **September** | 2004 | 33.3 | 23.3 | 81.4 | 73.56 | 59.5 |
| | 2005 | 32.1 | 23.5 | 84.93 | 76.23 | 310.5 |
| **October** | 2004 | 33.5 | 17.8 | 69.06 | 60.77 | Nil |
| | 2005 | 32.5 | 20.5 | 74.93 | 66.7 | 98.4 |
| **November** | 2004 | 31.9 | 15.5 | 65.56 | 51.4 | 134.4 |
| | 2005 | 31.4 | 14.1 | 63.86 | 55.53 | Nil |
| **December** | 2004 | 29.9 | 12.6 | 60.32 | 43.51 | Nil |
| | 2005 | 29.1 | 11.6 | 65.43 | 51.64 | Nil |
| **January** | 2005 | 28.9 | 13.7 | 65.51 | 46.09 | 129.3 |
| | 2006 | 30.4 | 12 | 61.58 | 36.93 | 37 |

* Source: - Regional meteorological centre, Nagpur

**Table 2:** Effect of Temperature and Relative Humidity on Storage of Brassica *juncea* pollen

| No. of days storage | Temp. | % of relative humidity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0% RH | | 10% RH | | 20% RH | | 30% RH | | 70% RH | |
| | | % of Ger. | PT (µ) | % of Ger. | PT (µ) | % of Ger. | PT (µ) | % of Ger. | PT (µ) | % of Ger. | PT (µ) |
| 1 | Room | 44 | 213 | 28 | 125 | 12 | 80 | 05 | 26 | -- | -- |
| | Freeze | 23 | 205 | 26 | 185 | 18 | 79 | 09 | 13 | -- | -- |
| 3 | Room | 35 | 116 | 19 | 93 | 10 | 55 | 03 | 13 | -- | -- |
| | Freeze | 20 | 160 | 20 | 163 | 13 | 66 | 06 | 10 | -- | -- |
| 5 | Room | 38 | 139 | 21 | 80 | 08 | 16 | 02 | 08 | -- | -- |
| | Freeze | 21 | 123 | 23 | 177 | 13 | 45 | 05 | 08 | -- | -- |
| 8 | Room | 22 | 143 | 15 | 41 | 03 | 13 | 00 | 00 | -- | -- |
| | Freeze | 18 | 138 | 21 | 148 | 11 | 36 | 01 | 03 | -- | -- |
| 11 | Room | 20 | 123 | 13 | 20 | 02 | 09 | -- | -- | -- | -- |
| | Freeze | 15 | 113 | 20 | 159 | 10 | 32 | 00 | 00 | -- | -- |
| 13 | Room | 20 | 98 | 08 | 13 | 00 | 00 | -- | -- | -- | -- |
| | Freeze | 13 | 83 | 17 | 122 | 08 | 25 | -- | -- | -- | -- |
| 16 | Room | 17 | 63 | 01 | 10 | -- | -- | -- | -- | -- | -- |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Freeze | 17 | 43 | 18 | 85 | 03 | 18 | -- | -- | -- | -- |
| 19 | Room | 12 | 53 | 00 | 00 | -- | -- | -- | -- | -- | -- |
| | Freeze | 10 | 55 | 15 | 89 | 01 | 08 | -- | -- | -- | -- |
| 21 | Room | 06 | 18 | -- | -- | -- | -- | -- | -- | -- | -- |
| | Freeze | 05 | 22 | 08 | 63 | 00 | 00 | -- | -- | -- | -- |
| 24 | Room | 02 | 08 | -- | -- | -- | -- | -- | -- | -- | -- |
| | Freeze | 03 | 13 | 05 | 50 | -- | -- | -- | -- | -- | -- |
| 28 | Room | 00 | 00 | -- | -- | -- | -- | -- | -- | -- | -- |
| | Freeze | 01 | 06 | 03 | 19 | -- | -- | -- | -- | -- | -- |
| 30 | Room | 00 | 00 | -- | -- | -- | -- | -- | -- | -- | -- |
| | Freeze | 00 | 00 | 02 | 10 | -- | -- | -- | -- | -- | -- |
| 31 | Room | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | Freeze | -- | -- | 00 | 00 | -- | -- | -- | -- | -- | -- |

**Table 3:** Pollen viability of *Brassica juncea* in different organic solvents

| | **Organic solvent** | **Viability (in days)** | **Maximum percent of germination** | **Maximum tube length (in μ )** |
|---|---|---|---|---|
| 1 | Benzene | 23 | 34 | 246 |
| 2 | Isopropyl alcohol | 18 | 26 | 168 |
| 3 | Chloroform | 25 | 28 | 284 |
| 4 | Acetone | 17 | 31 | 178 |
| 5 | Xylene | 6 | 16 | 102 |

**DISCUSSION**

The retention of pollen viability after shedding varies significantly from species to species. Environmental factors, particularly humidity & temperature greatly affect viability. With regard to storage temperature above zero (between 0° – 10°C). Metabolic processes are severely retarded and respiration is reduced as a consequence of the low water content in mature pollen. The pattern of inhibition of biochemical reactions involved in pollen respiration can also be explained on the basis of low water content. Dehydration of proteins would reduce enzyme activity [11]; likewise, ordinary dehydration of trinucleate pollen would probably damage the male cell nuclear component & thus reduce viability. By comparative studies on pollen morphology & physiology [9,12-15]showed that the binucleate and trinucleate pollen grains shows difference in their physiological & structural characters at the time of pollen dispersal. Under natural condition, two-celled pollen grains have a much longer life span because of their protective structure low plasma water content, & reduced metabolic activity, whereas the trinucleate pollens are short-lived due to their less resistant wall construction & high moisture content, which can be easily lost by desiccation. This type of pollen has a high rate of metabolism.

Pollen storage in different organic solvents was first demonstrated by Iwanami [4 and 16] and Nakamura [17]. The organic solvents include benzene, petroleum, diethyl ether, acetone, chloroform etc. whose efficiency varies greatly for different plant species. Pollen grains stored in non-polar organic solvents like benzene, diethyl ether and cyclohexane retained viability and showed very little leaching of phospholipids, sugars and amino acids into the solvent. On the other hand extensive leaching of substance and loss of viability was seen in polar organic solvents [18-19], thus establishing a correlation between the polarity of the solvents and its potency for pollen storage.

The temperature which is also one of the important factors has a major role in the pollen longevity. McGuire [20] has reported that the viability can be substantially extended at a temperature of about 0°C. Khan et.al. [21] , Bernabas and Rajki [22] on ***Zea mays***, Nandeshwar [23] on various cotton varieties, Rudra [24] on ***Punica granatum***, Aronne [25] studied ***Cistus incamus*** and ***Hyotus communis*** ,Rewatkar [26] on ***Oryza sativa*** and Rangari[27] has also made similar observations.

Relative humidity is of the most important factor influencing pollen storage. Pollen grains are usually shed under dehydrated condition (water content < 20%), and the metabolism is very low. The water content of ***Brassica*** is quite high and metabolism is also high due to 3 cell structured pollen grains. The pollen grains shed in the morning have about 20% higher water content than those shed at noon [27-29].

In the present investigation, ***Brassica*** pollen studied show germination in sucrose solution, since respiration is the metabolic activity taking place in the stored pollen; during the process

stored sugar is converted to organic acids. This may be the reason for the want of high concentration of sugar for germination is case of stored pollen grains.

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**CONCLUSION**

Pollen storage is an important technique not only for its use in controlled pollination but also in supplementation of pollen in many varieties for obtaining good fruit set therefore it has got a commercial application in hybrid production and also to increase fruit yield . The stored pollen can be used for tissue culture which has many uses similarly artificial seeds can be produced from stored pollen. In the light of at available evidences, it is obvious that temperature alone cannot be the ideal storage condition, unless coupled with suitable levels of relative humidity. At room temperature & 100% RH pollen lost viability within 24 hrs. Relative humidity between 0 – 20% had been reported to retain viability for several days. In present study it is found that lower the storage temperature longer is the pollen viability. Benzene, Isopropyl alcohol, Chlorophyll, Acetone and Xylene used as a storage medium of pollen grains. Xylene is not suitable for storing the pollen grains.

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