Received: 16 Jul 2019 | **Revised: 16 Mar 2020** | Accepted: 19 Mar 2020

Electronic Journal of Plant Breeding

Research Note

Assessing the viability and storage life in pollen grains of rice (*Oryza sativa* L.)

P. R. Navitha[1] and P. Sindhumole[2*]

[1]MES Kallady College, Mannarkkad, Palakkad.
[2]Dept. of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, KAU, Thrissur - 680 656, Kerala, India
***E-Mail**: sindhumole.p@kau.in

Abstract

In rice (*Oryza sativa* L.), the major cereal and staple food of millions in the world, hybridization programmes are very crucial for crop improvement. When the parents involved in crossing programme are varying in their flowering duration, storage of pollen grains for long time is highly advantageous for producing desirable hybrid combinations at any time. A study was undertaken to assess the viability and storage life of rice pollen grains through different storage methods. The anthers with pollen grains of the rice variety Uma, were collected from the field lab and transferred to various storage media. Aluminium foil and polythene bag were used as storage materials along with/without desiccators. Silica gel, Rice flour and raw rice were used as the desiccators. Samples packed in various storage media were kept under different conditions like Ambient, Refrigerator and Freezer. Afterwards, viability (%) of each sample was observed on 5th, 10th, 15th, 20th, 25th and 30th day of the storage using Acetocarmine dye method. The results after one month of storage indicated that pollen kept in Aluminium foil with silica in all the conditions showed better viability percentage, and the highest (87.24%) was in Freezer. Pollen grains had low viability in normal storage (without any media/dessicator) in all cases except ambient condition wherein, the lowest percentage of viability was noticed in Polythene bag without silica. This result indicates that the pollen storage in the Aluminium foil with silica gel in freezer condition is a highly effective and promising strategy for plant breeders and farmers, involved in plant breeding, hybridisation and genetic conservation programmes.

Keywords

Rice, Pollengrain, storage, viability

For more than half of humanity, 'rice is life'. Rice (*Oryza sativa* L.) has shaped the culture, diet, and economy of millions of people all over the globe. Rice has a great role in maintaining the life. It is a nutritional staple food which provides instant energy as its most important component is carbohydrate (starch). In rice, flowering occurs about 25 days after booting. Pollen grains are 3-celled and short lived: so it is very essential to maintain their viability for longer time in order to accelerate the efforts for higher seed production especially in hybrids.

Plant breeders have long been interested in crossing varieties/species of rice to produce new and improved rice types better suited to human requirements. However, a major problem is that the chosen parents may vary in their flowering duration so that the pollination process will be hindered. In hybridization programmes, successful transfer of pollen grains to the stigmatic surface for fertilisation, before the pollen loses its viability, is the most crucial step. Moreover, mostly the parents selected for crossing may not be having the same flowering duration. Hence, to conduct the hybridization programmes effectively, appropriate storage methods of pollen grains with sufficient viability over a long time must be standardised. Moreover, the pollen storage is useful for breeding programmes, artificial pollination in self-incompatible lines and genetic conservation. Longevity of pollen varies greatly with plant species and storage conditions.

In rice, varieties vary widely in their flowering duration thereby forming distinct groups like early, medium, long *etc*. Hence it is very difficult to obtain synchronous flowering in parents, if hybridization between varieties belonging to these different classes is to be conducted for hybrid production. Nowadays, staggered sowing of parents is followed to overcome this. However, it is very difficult, laborious and often without assured success. Hence there is an urgent need to identify suitable methods for storing the pollen grains of male parent and also to determine their storage life with sufficient viability for an effective pollination. The most efficient technique to overcome this barrier, imposed by time and space, is the use of stored pollen. Pollen storage also helps to avoid the staggered sowing of various batches of parental types. Thus, it will provide greater flexibility in experimental studies on pollen and allows the use of a wide array of rice lines for hybridization programmes.

Thus, the present investigation is proposed with the following objectives:

1. To identify appropriate method for storing the pollen grains of rice
2. To find out the method for extending the shelf life of stored pollen for effective seed set.

The present study was carried out at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, Thrissur during April to May, 2018. The mature anthers along with pollen grains were collected from Uma rice variety, in the morning (between 9 am to 10 am) in petridish lined with tissue paper to provide a sufficient moisture and to keeping away the contaminants like dust, spores *etc*. These anthers were stored under various conditions as detailed in Table 1.

Table 1. Storage conditions of rice pollen grains

Tr .No.	Medium / Condition
T1	Normal Room Temperature
T2	Polythene Bag with Raw rice
T3	Polythene Bag with Rice flour
T4	Aluminium Foil with Raw Rice
T5	Aluminium Foil with Rice Flour
T6	Polythene Bag without silica
T7	Polythene Bag with silica
T8	Aluminium Foil without Silica
T9	Aluminium Foil with Silica

All these treatments were repeated under Ambient, Refrigerator and Freezer conditions. In order to avoid the mild changes in temperature and humidity occurring whenever the refrigerator/freezer was opened frequently, Aluminium foils and Polythene bags, with pollen grains and desiccators, were placed in closed petridish and closed non-transparent plastic box, respectively, before keeping them in the freezer.Various treatments were properly labeled with details like date of filling, name of desiccator *etc*.

Viability of the samples were observed on 5th, 10th, 15th, 20th, 25th, and 30th day of storage. Each packet was taken out, the required quantity of pollen grains was taken out and the packet was immediately closed and replaced. Then, the pollen grains to be tested were allowed to warm to room temperature, before conducting the viability test using Acetocarmine dye.

In this experiment, pollen viability was estimated using Acetocarmine dye (mixed with one drop of glycerine and kept for some time), and observed under compound microscope. During this process, viable pollen grains showed pink colour with normal size, whereas non-viable ones were colourless with shrunken size (Plate 1). For each sample, three microscopic views were observed and their mean values were calculated. Viability observations on 5th, 10th, 15th, 20th, 25th, and 30th day of storage were recorded and percent viability was calculated for each stage as mentioned below :

$$\text{Pollen viability (\%)} \quad \frac{\text{No. of viable pollen grains observed} \times 100}{\text{Total no. of pollen grains observed}}$$

Observationson pollen viability (%) recorded periodically are presented in Table 2. Treatments varied significantly during all the stages throughout the study. After one month of storage, viability of pollen grains in normal situation under ambient condition was 10.32% whereas, those in polythene bag with raw rice and rice flour pollen showed 13.10 and 9.44% viability. In Aluminium foil, viability was 20.56% and 27.78% for raw rice and rice flour respectively. In polythene bag, pollen grains stored under silica and without silica showed 26.19 and 5.56%. In Aluminium foil, pollen showed 37.37% viability with silica whereas it was 13.69% without silica.

In Refrigerator, pollen grains showed less viability (16.56%) in normal situation. Pollen grains with raw rice and rice flour in polythene bag showed 21.6 and 22.34%

viability and in aluminium foil pollen showed 20.70 and 45.24% viability. In polythene bag, pollen showed 26.58% without silica and 18.90 % with silica and in case of aluminium foil pollen showed 72.21% without silica and 83.02% with silica. In Freezer, pollen grains showed the lowest viability in normal situation (20.83%). Pollen grains with raw rice and rice flour in polythene bag showed 27.48 and 33.93% and in aluminium foil showed 33.15 and 36.11%. In polythene bag, pollen grains showed 60.10% viability without silica and 68.95% with silica and these were on par with each other. In aluminium foil, pollen showed 73.44% (without silica) and 87.24% (with silica) viability, being on par with each other.

Aluminium foil with silica helped to retain the pollen

Conditions	Normal Condition	Polythene bag with Raw rice	Polythene bag with Rice flour	Aluminum foil with raw rice	Aluminium foil with Rice flour	Polythene bag without silica	Polythene bag with silica	Aluminium foil without silica	Aluminium foil with silica
Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9
				5th day					
Ambient	43.84	36.21	67.75	31.67	65.71	36.83	61.11	63.33	62.96
Refrigerator	55.30	65	86.90	51.88	61.85	68.72	88.89	85.71	91.67
Freezer	66.30	84.87	55.91	66.02	100	92.92	93.89	96.67	100
CD	29.09								
				10th day					
Ambient	41.1	29.5	43.3	39.5	55	40.8	50.6	49.7	61.4
Refrigerator	23.7	25	31.1	35.6	34.9	57.4	86.8	96.7	100
Freezer	37.9	45	43.6	44.4	51.5	49.4	74.4	77.8	100
CD	26.33								
				15th day					
Ambient	20.56	33.94	37.50	19.17	58.89	26.33	48.57	51.48	61.67
Refrigerator	32.06	46.30	50.20	59.12	64.12	64.41	72.78	69.49	83.33
Freezer	32.78	39.52	53.21	54.60	46.67	69.96	68.24	97.91	100
CD	20.77								
				20th day					
Ambient	12.22	31.67	29.49	18.92	14.44	40.80	23.89	43.33	46.83
Refrigerator	11.43	35.89	22.42	42.22	43.61	67.60	31.83	73.75	82.06
Freezer	15.87	25.93	24.03	27.58	44.11	72.16	80.56	83.21	84.92
CD	27.73								
				25th day					
Ambient	7.14	14.21	15.74	11.11	24.44	24.85	13.43	12.04	24.97
Refrigerator	10.83	17.86	21.67	34.17	43.33	36.51	29.52	58.26	94.60
Freezer	17.22	23.42	36.94	43.61	29.80	68.27	65.08	74.20	94.66
CD	22.23								
				30th day					
Ambient	10.32^{ghi}	13.10^{ghi}	9.44^{hi}	20.56^{fghi}	27.78^{efgh}	5.56^{i}	26.19^{efgh}	13.69^{ghi}	37.37^{ef}
Refrigerator	16.56^{ghi}	21.16^{fghi}	22.34^{fghi}	20.70^{fghi}	45.24^{de}	26.58^{efgh}	18.90^{fghi}	72.21^{ab}	83.02^{a}
Freezer	20.83^{fghi}	27.48^{efgh}	33.93^{efg}	33.15^{efg}	36.11^{ef}	60.10^{abcd}	68.95^{abc}	73.44^{ab}	87.20^{a}
CD	19.08								

viablility to the highest extent (> 80%) in both freezer and refreigerator. Even without silica also, pollen grains stored in Aluminium foil retained >70% viablity after month of storage, in both freezer an refrigerator. Pollen stored at low temperature (-120°C and -20°C) showed a better viability and germination percentage as compared to pollen stored at room temperature and 4°C (Bhat*et al.*, 2012). Similar to this report, in the present study also, better pollen viability was observed for the pollen stored at lower temperature (Freezer) as compared to pollen stored at Refrigerator or Ambient condition.

Pollen grains could be well preserved for a period of 20 days at high relative humidity (RH) and low temperature (Saoji and Rewatkar, 2015). Deshpande and Priya (2016) reported lower values of seed setting percentage and 100 seed weight for pollen stored at ambient condition than pollen stored in refrigerator and earthen pot.

In the present study, after one month of storage, pollen grains kept under ambient condition showed the lowest viability whereas those kept under refrigerator showed moderate viability. Pollen grains stored in freezer condition showed the highest viability throughout the experiment. So it was revealed that freezer was the better condition for pollen storage.

The viability of pollen grains was the lowest in normal storage in all conditions like ambient, refrigerator and freezer, except in 30th day under ambient condition, wherein the lowest viability percentage was observed in polythene bag without silica. Compared to polythene bag, better viability was observed for pollen grains in Aluminium foil, but a slight variation was observed in the presence of desiccators (silica gel, raw rice and rice flour). However, pollen grains in polythene bag (with and without silica) showed more viability than those in aluminium foil with rice flour and raw rice. This indicates that the viability observed under a particular storage material depended on the desiccator present in it. Among the dessicators, pollen grains stored along with silica showed the most superior viability. In ambient condition, pollen showed 37.37% viability in aluminium foil with silica while in refrigerator, pollen showed 83.02% and those in freezer, pollen showed 87.24% under aluminium foil with silica respectively.

So, based on the current study, it can be concluded that the most suitable storage condition for rice pollen grains to retain their high viability for longer time is to keep them in Aluminium foil packets along with silica and store in freezer condition.

Much studies could not be located in the previous literature related to the present study. Hence this study finds unique place in this field of research.

REFERENCES

Bhat, Z.A., Dhillon, W.S., Shafi, R.H and Rather, J.A. 2012. Influence of storage temperature on viability and *in vitro* germination capacity of pear pollen. *Journal of Agricultural Science*. 4(11): 128-135. [Cross Ref]

Deshpande, V.and Priya, K. 2016. Effect of Pollen viability and method of storage on seed set, yield and seed quality under condition of sunflower.*International Journal of Plant Sciences.* 11(2): 318-321. [Cross Ref]

Saoji, A.A. and Rewatkar, K.G. 2015. Studies on pollen storage at different temperatures and humidity conditions in *Oryza sativa* L. *International journal of researches in Biosciences, Agriculture and Technology*. 11(3): 304-305