

POLLEN VITALITY AND GERMINATION CAPACITY IN THREE TAXA OF THE GENUS *BRASSICA* L. (BRASSICACEAE)

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Abstract

The present article pertains to the pollen vitality and germination capacity of 3 *Brassica* taxa viz. *B. rapa* ssp. *campestris* (L.) Clap, *B. oleracea* var. *capitata* L., and *B. oleracea* var. *botrytis* L. The study revealed that *B. oleracea* var. *botrytis* showed vitality for a longer period of 128 weeks at freeze-dried condition (-60°C), while the shortest period of 32 weeks was noted in *B. oleracea* var. *capitata*. In fresh form pollen of *B. oleracea* var. *botrytis* showed 72% of germination compared to 64% and 62% of *B. oleracea* var. *capitata* and *B. rapa* ssp. *campestris* respectively. In stored conditions the maximum percentage of germination (74.5%) was noted in *B. rapa* ssp. *campestris* after 4 weeks of storage compared to the lowest (10.7%) of *B. oleracea* var. *capitata*. The investigation showed that the freeze-dried condition seemed to be a better method for pollen storage contrary to the refrigerator (4°C) and freezer (-20°C, -30°C).

Key words: *Brassica rapa* ssp. *campestris* (L.) Clap, *Brassica oleracea* var. *capitata* L., and *B. oleracea* var. *botrytis* L., pollen, Germination, Viability.

Introduction

Brassicaceae is a large family having 350 genera and 3000 species, with worldwide distribution most commonly concentrated in temperate and cooler parts of the world, from Pakistan 92 genera and about 250 species are reported, of which 5 genera and 14 species are cultivated (Jafri, 1973). The genus *Brassica* L. has about 40 species abundant in the Mediterranean, while from Pakistan 7 species are reported, of those 2 are wild and the remaining are cultivated (Jafri, 1973). *Brassica* L. is an important genus of the family having many oil yielding plants along with vegetables.

Genetic conservation through pollen storage is desirable for plant breeders since pollen are known to transmit important heritable characters. Plant breeders have an access to pollen bank facility, from where anybody can draw pollen of its own choice in breeding a develop new cultivar. Several methods of pollen storage have been tried and applied of which the most important factors are controlled temperature and humidity. Generally, it is believed that pollen during a period of hot and dry weather reduces vitality both initially and in storage as well. Many short and long term pollen storage methods that reflect the competence of pollen to perform its function have been devised (Shivana & Rangaswamy, 1992). The most common method employed for pollen storage is the manipulation of temperature and humidity conditions, lowering both tend to increase the period of pollen viability. According to Usha & Kulkarni (1983) storage temperature for *Moringa oleifera*, *Capsicum frutescens*, *Ricinus communis*, *Abrus precatorius* and *Clitoria ternatea* ranged from 0°C to 14°C, while the humidity ranged from 0% to 56%, the pollen of *A. precatorius* and *M. oleifera* could be stored for one year. Knowlton (1922) stored *Antirrhinum* pollen at 5 different temperatures concluded that lower the storage temperature prolonged the pollen vitality period. Visser (1955) stored apple and pear pollen at -190°C in liquid oxygen and concluded that pollen germination was not affected even after several months of storage. Successful pollen storage is a very convenient tool

in the hand of plant breeders for effecting the hybridization of plants occurring in different regions and also those blooming in different seasons. Humidity plays a vital role in effective storage and pollen tube longevity. Most of the species maintain their pollen viability best at low temperature and relative humidity, however, it is difficult to predict the exact optimum relative humidity and temperature because a great number of results reported for different species are not comparable. Various methods have been adopted for successful storage of pollen of different taxa (Stanley & Linkson, 1974; Kopp *et al.*, 2000; Pansonen *et al.*, 2001; Kenta *et al.*, 2002; Towill, 2004; Alba *et al.*, 2011; Sores *et al.*, 2013, Hakan & Gokbayrak, 2019; Wani *et al.*, 2020).

Morphologically pollen germination is a swelling of pollen grain followed by the protrusion of the pollen tube. Pollen has been a favorite subject for physiological studies due to its simple structure, readiness to grow in controlled conditions, and sensitivity to environmental factors. Nair (1964) correlated pollen morphology with physiological potential by studying pollen germination in different varieties of *Vitis vinifera*. *In vitro* pollen germination and short term pollen storage in *Caladium* was studied by Deng *et al.*, (2004). Conservation of the germination capacity of pollen grains in three varieties of *Zea mays* L. has been examined by Youmbi *et al.*, (2007). Zhang *et al.*, (2011) examined the effect of temperature on *In vitro* pollen germination and storage of Peony pollen. Bhat *et al.*, (2012) studied the influence of storage temperature on viability and *In vitro* germination capacity of pear pollen. Dickinson *et al.*, (2018) reported *In vitro* and semi in-vivo methods for *Arabidopsis thaliana* pollen, and tube growth. Mankad (2012) examined *In vitro* pollen germination and storage of *Crinum asiaticum* L. Polat & Pirlak (1999) investigate pollen viability, germination, and tube growth in some stone fruits. It is well known that the pollen has a direct effect on the fertilizing process in plant breeding (Androulakis & Loupassaki, 1990; Ateyyeh *et al.*, 2000; Mehri *et al.*, 2003; Jayaparksh, 2018).

The present investigation is undertaken to examine the pollen vitality and germination capacity of the three important vegetables and oil yielding taxa of the genus *Brassica* viz., *B. oleracea* var. *capitata*, *B. oleracea* var. *botrytis*, and *B. rapa* var. *campestris*. All the three taxa are cultivated in most parts of the world including Pakistan for their veggies, fodder and oil yielding value.

Materials and Methods

Polliniferous material was collected from the Center for Plant Conservation (Botanic Garden), University of Karachi, and Malir Memon Ghott during peak flowering period. Pollen viability was tested immediately according to the method of Alexander (1969) and then stored at different stored conditions including a refrigerator (4°C), freezer (-20°C, -30°C), and freeze drier (-60°C). Pollen culture medium was prepared according to the standard method of Brewbacker & Kwack (1963). The germination was scored after 3-6 hours of incubation at room temperature in humid chambers using different solutions (10%-100%). Approximately 100 pollen per slide and 10 slides per taxon were prepared to find out the germination percentage. Pollen produced pollen tubes and grew at least twice the diameter of pollen were counted as germinated, while burst pollen was counted as ungerminated. The viability of stored pollen was assessed in terms of percent germination.

Results and Discussion

The pollen germination capacity of the three *Brassica* taxa viz., *Brassica oleracea* var. *botrytis* L. *B. oleracea* var. *capitata* L. and *B. rapa* subsp *campestris* (L.) Clapham was studied and pollen were stored up to 48 weeks at different temperature and moisture conditions i.e. refrigerator (4°C), freezer (-20°C, -30°C) and freeze drier (-60°C). Among the three taxa, *B. rapa* subsp *campestris* & *B. oleracea* var. *botrytis* showed better germination percentage and maintained viability after 48 weeks of storage with variable germination percentage compared to *B. oleracea* var. *capitata* which showed poor germination percentage in early weeks and lost viability very rapidly (Figs. 1, 2 & 3). According to Fuller *et al.*, (2004) species like *Allium cepa*, *A. sativus*, *Lactuca sativa*, *Beta vulgaris* and, *Spinacia oleracea* showed a lower percentage of germination and viability in most storing conditions, supporting our findings, in case of *B. oleracea* var *capitata*. However, our other two taxa namely *B. rapa* subsp *campestris* and *B. oleracea* var. *botrytis* showed better results concerning germination percentage and vitality period. Pollen of *B. oleracea* var. *botrytis* showed a maximum 75% of germination after 4 weeks of storage at freeze drier (-60°C) compared to 74.50% and 61.10% at freezer (-20°C & -30°C), while refrigerated pollen (4°C) showed 48.40% of germination, which gradually was decreased as the period proceeded and after 48 weeks of storage freezer (-30°C) showed 48.70% of germination as compared to 25.90%, 20.30% and 5.20% at freeze drier (-60°C), freezer (-20°C) and refrigerator (4°C) respectively (Table 2). These findings were in agreement with the work of Udomedee *et al.*, (2003) who reported better germination percentage and viability at low temperature in *Cucurma* pollen.

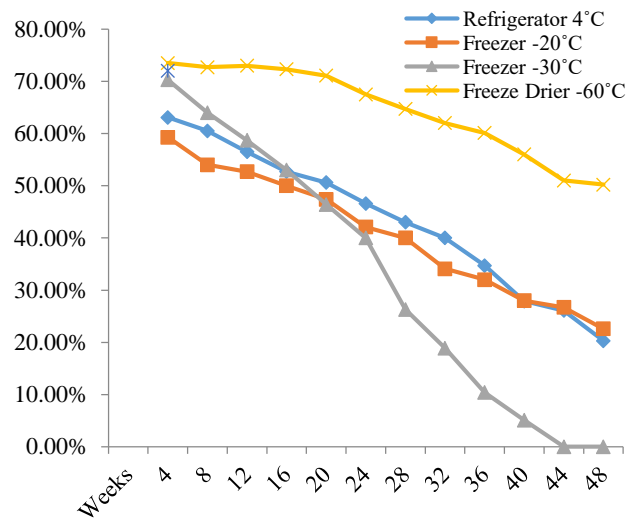


Fig. 1. Germination Capacity of stored pollen of *Brassica oleracea* var. *botrytis* up to 48 weeks.

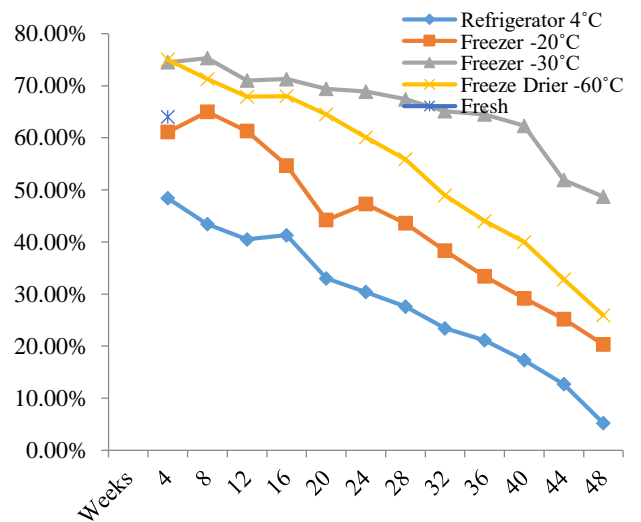


Fig. 2. Germination Capacity of stored pollen of *Brassica rapa* ssp. *campestris* up to 48 weeks.

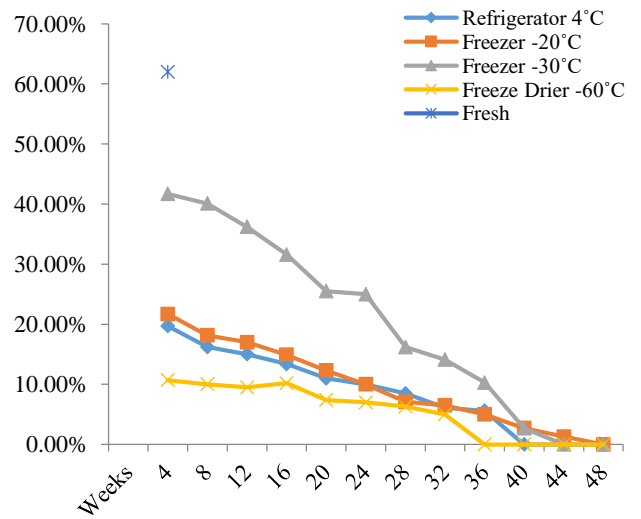


Fig. 3. Germination Capacity of stored pollen of *B. oleracea* var. *capitata* up to 48 weeks.

Table 1. Germination capacity of stored pollen of *Brassica oleracea* var. *botrytis* upto 48 weeks.

weeks	Fresh: 72%			
	Refrigerator 4°C	Freezer -20°C	Freezer -30°C	Freeze drier -60°C
4	63.10%	59.30%	70.30%	73.50%
8	60.50%	54%	64%	72.70%
12	56.50%	52.70%	58.70%	73%
16	52.70%	50%	53%	72.30%
20	50.60%	47.40%	46.40%	71.10%
24	46.60%	42.10%	40%	67.50%
28	43%	40%	26.30%	64.70%
32	40%	34.10%	18.90%	62%
36	34.70%	32%	10.40%	60.10%
40	27.90%	28%	5.10%	56%
44	26.10%	26.70%	00%	51%
48	20.30%	22.60%	00%	50.20%

Table 2. Germination capacity of stored pollen of *Brassica rapa* ssp. *campestris* upto 48 weeks.

weeks	Fresh: 64%			
	Refrigerator 4°C	Freezer -20°C	Freezer -30°C	Freeze drier -60°C
4	48.40%	61.10%	74.50%	75%
8	43.40%	65%	75.30%	71.30%
12	40.50%	61.30%	71.00%	67.90%
16	41.30%	54.70%	71.30%	68%
20	33%	44.20%	69.40%	64.50%
24	30.40%	47.30%	68.90%	60.10%
28	27.60%	43.60%	67.40%	55.90%
32	23.40%	38.30%	65.10%	48.90%
36	21.10%	33.40%	64.50%	44%
40	17.30%	29.20%	62.30%	40%
44	12.70%	25.20%	51.90%	32.80%
48	5.20%	20.30%	48.70%	25.90%

Table 3. Germination capacity of stored pollen of *Brassica oleracea* var. *capitata* upto 48 weeks.

weeks	Fresh: 62%			
	Refrigerator 4°C	Freezer -20°C	Freezer -30°C	Freeze drier -60°C
4	19.70%	21.70%	41.70%	10.70%
8	16.20%	18.15%	40.10%	10%
12	15%	17%	36.20%	9.50%
16	13.40%	14.90%	31.60%	10.20%
20	10%	12.30%	25.50%	7.40%
24	10%	10%	25%	7%
28	8.50%	7.10%	16.20%	6.30%
32	6.10%	6.50%	14.10%	5%
36	5.60%	5.00%	10.30%	00%
40	00%	2.70%	2.70%	00%
44	00%	1.30%	00%	00%
48	00%	00%	00%	00%

The germination percentage at fresh and pollen stored at 4°C showed slight variation in *B. oleracea* var. *botrytis* and *B. rapa* ssp. *campestris* after 4 weeks of storage, while *B. oleracea* var. *capitata* pollen showed significantly better germination as 62% at fresh compared to 19.70%, 21.30%, 41.50 and 10.70% at 4°C, -20°C, -30°C and -60°C respectively, Gawta *et al.*, (2003) observed that variation in *In vitro* germination was due to the complex interaction between morphology and physiology of pollen. The view of Kakani *et al.*, (2005) supports our findings that the variations in *In vitro* germination and pollen tube growth is due to the variations in varieties of the plant species. Souza-Lang & Pinto Junier (1997) observed the highest percentage of germination in *Araucaria angustifolia* in a sugar-free medium, but our findings showed better germination percentage and vitality in a medium having 10% to 20% sucrose, while high concentration showed poor results, which confirm the Premachandra *et al.*, (1992) hypothesis i.e. when the concentration of sucrose increase in the medium result in the increase of carbon concentration which inhabits pollen germination. Lora *et al.*, (2006) reported a progressive decline in germination percentage of *Annona charimola* pollen stored at low temperature and observed minimum vitality after 3 months of storage, our findings support the view of Lora *et al.*, (2006) in the case of *B. oleracea* var. *botrytis* and *B. rapa* ssp. *campestris* in which gradual vitality and germination were gradually decline as the storage time progresses, while *B. oleracea* var. *capitata* lost viability after 40 weeks at refrigerator (4°C) and freeze drier (-60°C), (Tables 1, 2 and 3).

Conclusion

Conclusively low temperature and relative humidity not only showed better germination percentage after 48 weeks of storage in 10% and 20% sucrose solutions in the case of *B. rapa* subsp *campestris* & *B. oleracea* var. *botrytis*, but also maintained viability for a longer period.

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