Cryo-preservation of Pollen for Hybrid Seed Production in Hot Pepper

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Abstract

Hot pepper is one of the important vegetable crops of Asia, and India is fast developing as hub for its seed production. In hot pepper, seed production is mainly done using cytoplasmic male sterile lines, where sterile female plants were grown in fields of seed growers. The male line is grown in a centralised facility, and only male flower buds were distributed to growers for pollen extraction and crossing of female buds. Sometimes it is difficult to supply male flower buds to growers because of distance and possibility of crop failure. Further, hot pepper seed production can be done in 9–10 months in a year in various sowing windows and in different areas. An effort to preserve pollen from these male buds was made under refrigeration and cryo-preservation which can be used to supply pollen directly instead of buds and to longer distances. The pollen from these male lines was harvested and stored at normal room temperature (control), in deep refrigeration (−20 °C) and in liquid nitrogen (−196 °C). The pollen extracted was preserved in capped vials for refrigeration, and semen straws were used for storing in liquid nitrogen. The semen straws containing pollen are kept in IBP cryo-containers which are normally used for artificial insemination. The pollen had lost viability and failed to produce seeds in female buds within 3 days when stored under room temperature. The pollen stored in deep refrigeration produced seeds up to 6 days. In case of cryo-preservation, the pollen can be stored up to 47 days and produced seeds when used for pollination up to 48 days. The pollen viability was tested using 2% acetocarmine staining, and pollen germination by hanging drop technique which showed 85–2% pollen was viable in this period. The molecular genetic purity test using protein banding pattern (to know the genetic purity) of these hot pepper hybrid seeds shows no variations for the characters of F1 as

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described by the breeder. Also there is no effect on storability and viability of seeds in modified atmospheric storage. The use of cryo-preservation for storing pollen can be effectively used for hybrid seed production which helps in reducing cost of production, genetic integrity and germ plasm security.

Keywords
Pollen cryo-preservation • Male sterility • Pollen germination

Introduction

India has the unique distinction of being a major seed production hub among the Asian countries due to its diversified agroclimatic regions. Hence, most of tropical vegetable seeds are produced in India with the exception of some *Brassica* seeds which need temperate conditions. Among the important vegetables the Asian type hot pepper is one of the important vegetables in which the commercial hybrid seed production is going on large scale. The seed production is carried out by farmers, which gives them high returns per unit area. With a land area as low as half acre, one can earn profits more than the commercial crops. The seed production of hot pepper is a skilled activity which involves emasculation and pollination. The seed production is carried out using cytoplasmic male sterile lines. The usual practice is to grow male sterile lines and fertile male plants separately, and pollen from fertile male plants is used to pollinate the male sterile lines. To grow fertile lines to supply pollen is a costly affair, and crop failure may jeopardise the entire seed production programme. In an experiment conducted in UAS, Raichur, an effort was made to store pollen of hot pepper in liquid nitrogen which can be used for pollination at various sowing windows without growing fertile lines separately, which is economically viable. Earlier studies reported that hot pepper pollen can be stored in liquid nitrogen [1, 2]. Another study reported that pollen of capsicum can be stored for more than 42 months without any effect on the pollen viability and fertility [3].

Materials and Methods

Collection of Flower Buds, Pollen Extraction and Storage

To conduct the study, the flower buds from fertile plants were collected from the crop grown in the previous season. The flower buds were plucked from the plants and stored in wet cotton cloth. Flower buds were then exposed to sunlight to induce anther dehiscence. The pollen was collected in a container made of china clay and then transferred to various containers based on the type of storage. The pollen is stored in capped vials for normal storage (under cool conditions) and for deep refrigeration. The semen straws are used for storing pollen under liquid nitrogen. These straws were normally used for artificial insemination in Department of Animal Husbandry. The IBP cryo-cans were used for cryo-preservation. The commercial grade liquid nitrogen was obtained from Karnataka Milk Federation. The cryo-cans were monitored regularly as per the earlier methods [4] and filled with fresh liquid nitrogen after every fortnight.

Study of Pollen Viability and Germination

For pollen viability study, 2% acetocarmine was prepared by dissolving 2 g carmine powder in 55 ml of distilled water and 45 ml of acetic acid. This mixture was boiled gently for 5 min and filtered with No. 75 Whatman filter paper after

cooling. For studying pollen viability, the pollens were placed on a cavity slide containing 2 drops of 2% acetocarmine. These were mixed using a clean needle and examined under microscope for staining pattern. The staining patterns were classified as dark, light and unstained. The pollen grains which were stained dark and light were taken as viable. Pollen grains were observed randomly from average of five microscopic fields, and numbers of viable and nonviable pollens were recorded. The pollen germination was studied using hanging drop technique [5].

Study of Fruit and Seed Quality Parameters

The pollen after storage in various parameters was used to pollinate the flower buds of male sterile plants. Each day, ten previously tagged flower buds were pollinated. For ease in identification, various colour tags were used. The seed quality parameters such as fruit set (%), average seed set per fruit (No.) and seed germination (%) were recorded.

Collection of Data and Analysis

The data was collected every day for the first 7 days, later once in 5 days up to 47th day. The data recorded for three replicates and the experimental results were analysed statistically with randomised block design with factorial concept adopting analysis of variance technique, and critical differences were calculated whenever 'F' test was significant. Wherever necessary, the percentage data was transformed into arcsin root transformation [6].

Results and Discussion

The results of the experiment were summarised in Table 32.1. The results indicate that the pollen germination was better when pollen was stored for 3 days under ambient room temperature, for 6 days when stored under deep refrigeration and for 47 days when stored in liquid nitrogen. Though the pollen germination was observed in all storage methods, a decreasing trend was noticed. The pollen germination proves the earlier findings of pollen that can tolerate extreme ultra low temperature and effect normal fertilisation due to very low biological activity at these temperatures [7].

The fruit set % was better when pollen is stored for 3 days (99, 88 and 56%) under ambient room temperature. In case of deep refrigeration, fruit set can be obtained up to 7 days (99–24%) and better fruit set % was obtained up to 47 days, when pollen is used which was stored in liquid nitrogen (99–4%) with decreasing trend. This finding proves earlier finding of higher fruit and seed set % by using fresh pollen [8]. This also proves the fact that the normal fertilisation and fruit set is possible when pollen survival in liquid nitrogen is at least 20% [9].

The results also showed that higher seed setting per fruit was obtained when pollen is used which was stored in liquid nitrogen up to 47 days (5–168 days). Very low seed setting was observed for 3–6 days when pollen is stored under ambient room temperature and deep refrigeration. The seed germination showed no significant reduction with respect to storage methods. The seed germination ranged between 64 and 84%. This finding showed us the fact that there will not be any effect of storage method on germination which will help in storability studies as future line of work.

Conclusions

The current study on pollen cryo-preservation showed that pollen can be stored for very long period of time in liquid nitrogen and can be used for hybrid seed production. Also the pollen can tolerate extreme ultra low temperatures and can affect normal fertilisation. The pollen expresses some kind of dormant behaviour under these low temperatures and biological activity. This method can be used for short-term pollen storage

Table 32.1 Influence of cryo-preservation on pollen and seed quality parameters

	Pollen germination %				Fruit set %				Average seed set no.				Seed germination %			
Storage days (T)	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
T1–1 DAS	85	85	85	85	99	99	98	99	161	166	168	165	84	84	83	84
T2–2 DAS	63	78	86	76	88	92	94	91	132	146	155	144	83	84	83	83
T3–3 DAS	17	77	75	56	56	86	91	78	32	125	153	103	76	82	82	80
T4–4 DAS	0	51	72	41	20	76	90	62	0	117	145	87	76	81	82	80
T5–5 DAS	0	28	68	32	3	66	89	53	0	73	139	71	74	77	82	78
T6–6 DAS	0	11	63	25	0	54	88	47	0	23	133	52	0	77	82	53
T7–7 DAS	0	1	62	21	0	24	86	37	0	0	128	43	0	76	80	52
T8–12 DAS	0	0	53	18	0	0	68	23	0	0	107	36	0	0	75	25
T9–17 DAS	0	0	51	17	0	0	62	21	0	0	92	31	0	0	73	24
T10–22 DAS	0	0	46	15	0	0	53	18	0	0	72	24	0	0	69	23
T11–27 DAS	0	0	38	13	0	0	48	16	0	0	62	21	0	0	69	23
T12–32 DAS	0	0	34	11	0	0	42	14	0	0	47	16	0	0	66	22
T13–37 DAS	0	0	31	10	0	0	43	14	0	0	21	7	0	0	68	23
T14–42 DAS	0	0	23	8	0	0	33	11	0	0	10	3	0	0	65	22
T15–47 DAS	0	0	7	2	0	0	13	4	0	0	5	2	0	0	63	21
Mean	11	22	53	29	18	33	67	39	22	43	96	54	26	37	75	46
	S.Em ±		CD at 5%		S.Em ±		CD at 5%		S.Em ±		CD at 5%		S.Em ±		CD at 5%	
T	0.74		1.81		0.56		1.81		0.65		1.81		0.33		1.81	
C	1.65		3.10		1.25		3.10		1.46		3.10		0.73		3.10	
T×C	2.86		1.60		2.17		1.60		2.53		1.60		1.27		1.60	

Legends
C1 Pollen stored in ambient room temperature
C2 Pollen stored in deep freezer (−20° C)
C3 Pollen stored in liquid nitrogen (−196° C)
DAS Days after storage

(less than 3 months) and can be used for hybrid seed production which also helps in reducing cost of production, to ensure genetic integrity and germ plasm security. The cryo-preservation also has application in breeding programmes, distributing and exchanging germ plasm among locations as well as for studies in physiology, biotechnology and *in vitro* fertilisation [10].

References

1. Kristof EB, Barnabes B. Deep freezing storage of paprika pollen. Capsicum Newsl. 1986;5:27–8.
2. Benzoickova A. Viability of sweet pepper pollen stored at cryogenic temperature. Capsicum Newsl. 1988;7:30–1.
3. Alexander MP, Ganeshan S, Rajasekharan PE. Freeze preservation of capsicum pollen in liquid nitrogen (−196 °C) for 42 months-effect on pollen viability and fertility. Plant Cell Incompat Newsl. 1991;23:1–4.
4. Rajasekharan PE. Seed cryopreservation: problems and prospects. Paper presented at the ICAR Short Course on In Vitro Conservation and Cryopreservation-New Options to Conserve Horticultural Genetic Resources, Bangalore, India, 21–30 Sept 2006; 2006. pp. 51–65.
5. Van Tiegham PM. Ann. Sci. Natbotan, S., Ser, T. Cited in Stanley, R. G. and H. F. Linskens. Pollen Biol Biochem Manag. 1869;XII:314 (69, 195, 2.1).
6. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi, India; 1978. pp. 112–119.
7. Sacks EJ, St. Clair DA. Cryo-preservation of tomato pollen. Hort Sci. 1995;30(4):797.
8. Rajashekhar PE. Studies on seed production and pollen viability on KBSH-1 hybrid sunflower. MSc-Agri thesis, University of Agricultural Sciences, Bangalore (India); 2000.
9. Reed BM. Implementing cryogenic storage of clonally propagated plants. Cryo Letters. 2001;22:97–104.
10. Towill LE, Walters C. Cryopreservation of pollen. In: Engelmann F, Takagi H, editors. Cryopreservation of tropical plant germ plasm. Current Research Progress and Application, Barak, Japan, JIRCAS/IPGRI; 2000. pp. 115–129.