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SEED EVALUATION AFTER CRYOPRESERVATION IN ONION (*ALLIUM CEPA* L.) CULTIVARS

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Onion seeds though orthodox in nature have short shelf life. Low temperature storage has been strongly recommended for the long term storage of such seeds. Cryopreservation (-180°C, vapour phase of liquid nitrogen) has proved useful in a number of orthodox seeds. These seeds could be successfully stored under cryopreservation because decline in seed germination and seedling vigour due to storage at RT can be almost completely controlled. Membrane integrity and lipid peroxidation studies were used to assess the metabolic changes accompanying seed storage. Lipid peroxidation was found to be closely associated with the decline in germination and vigour characteristics but this correlation could not be established with membrane integrity.

Key words: Onion (*Allium cepa* L.), cryopreservation, vigour index

Conservation of plant genetic resources is aimed to ensure the supply of germplasm collections to a large panel of users, from plant breeders to fundamental geneticists in crop improvement programmes. Gene banks have been established for medium and long term conservation having representatives of the genetic diversity of crop species and its wild relatives. Storability of seeds and accompanying biochemical changes is an area of interest and information generated would help in developing appropriate conservation strategies. Onion seeds exhibit desiccation as well as freezing tolerance and are therefore, orthodox in nature. However, these seeds are very poor storers under ambient conditions. Ultra low temperature storage can overcome the loss in viability and vigour. The degradative processes that occur under storage at ambient conditions and can be controlled by low temperature storage. The present investigations were aimed to study the structural and biochemical features associated with them.

MATERIALS AND METHODS

Seeds of two onion varieties viz., Pusa Red (PR) and Pusa White Flat (PWF) were procured from Division of Vegetable crops, IARI, New Delhi.

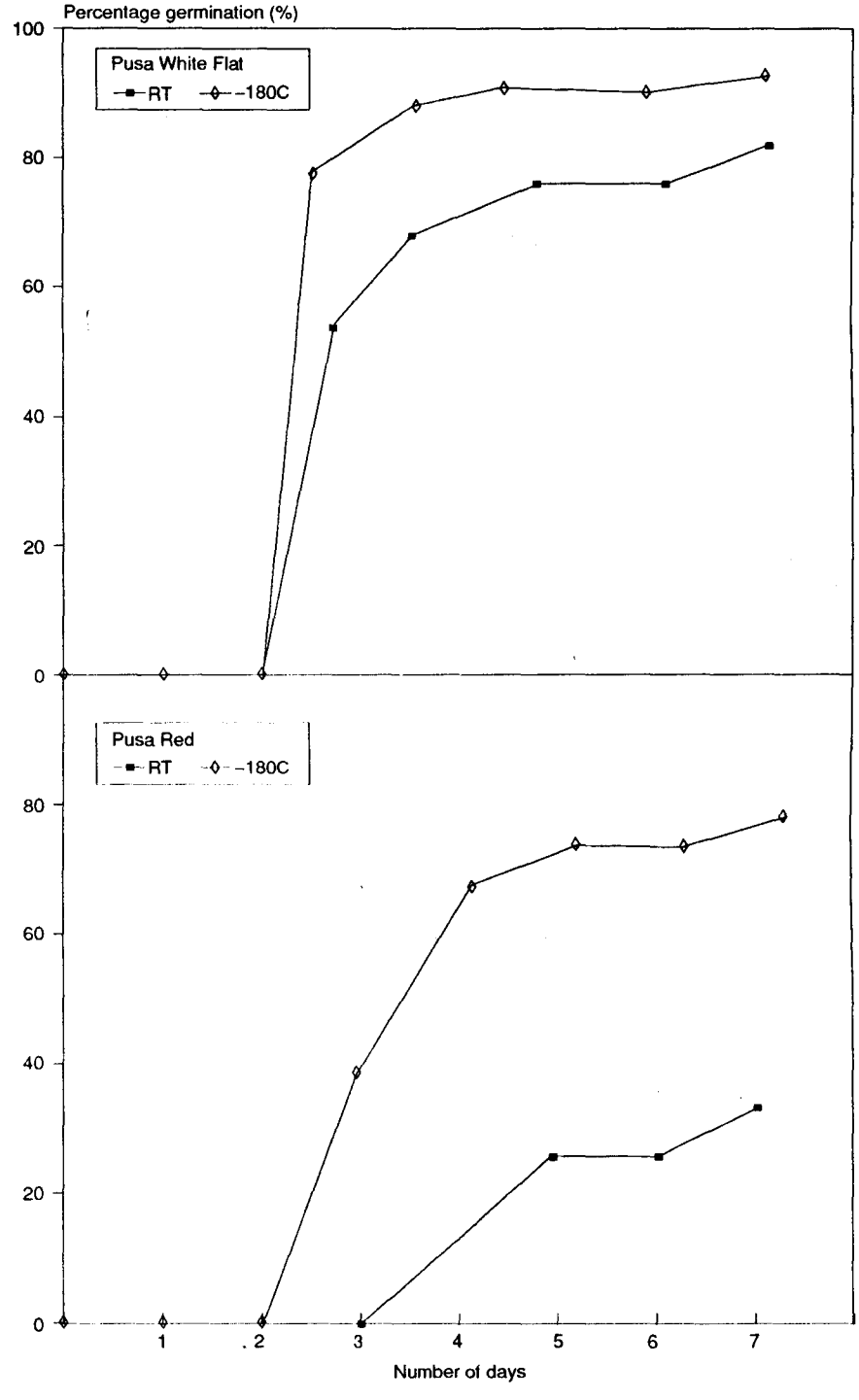

Fig. 1 : Speed of seed germination in *A. cepa* L. cultivars after storage for nine months

Initial moisture content was determined and brought down to 6-7 per cent using seed dryer (Bry-Air, India). Moisture content determinations on fresh weight basis were done by drying the seeds at 105 ± 1°C for sixteen hours (ISTA, 1976) in three replicates of 10 g each. Seeds were stored in polypropylene vial in -180°C at vapour phase of liquid nitrogen in the cryotank (CMS 328, USA) also in ambient conditions to be used as controls.

Stored seeds were retrieved after nine months. For retrieval from liquid nitrogen, seed vials were immediately immersed in warm water bath (38°C) for few seconds. Seeds were germinated over moist filter paper in Petri plates at 20 ± 1°C in three replicates of 100 seeds each and germination was recorded every day upto 7th day. The criterion of germination was normal seedling development (ISTA, 1993). For determination of seedling vigour, seeds were placed in folds of germination paper and kept in dark at 20 ± 1°C on template stands. Lengths of radicle and plumule were measured after nine days and vigour index was calculated following Abdul-Baki and Anderson (1973).

Lipid peroxide formation was studied by Thiobarbituric acid (TBA) colour reaction following Bernheim *et al.* (1948). 0.5g seeds of each treatment were homogenised in 5ml of TBA-TCA reagent (0.5% TBA in 20% TCA). Homogenate was incubated in an oven maintained at 95°C for 1 hour followed by transfer to ice bath immediately. Extracts were centrifuged at 1000 × g and absorbance of clear supernatant was measured at 535nm and 600nm using TBA-TCA reagent as blank. Maloneldialdehyde (MDA) content was estimated by sub-stracting A_{600} from A_{535}nm.

For membrane permeability studies, 0.5g seeds of each treatment were soaked in 10ml of distilled water in three replicates. Electrical conductivity was recorded on a conductivity bridge (cell constant of 1.2 x 10^{-6}mhos) after every 15 min. upto 1 hr. and then after 2 hrs., 3.5 hrs., 5 hrs., and 24 hrs.

Spectrophotometric absorbance of seed leachate was also measured in ultraviolet region for estimating organic constituents. For this, leachate obtained after soaking 0.5g of seeds in 10ml of distilled water for 24 hrs. was used. Absorbance was recorded at 240nm with distilled water as the blank.

The statistical analysis were done by using MSTATC software, Version 1.4 (R. D. Freed and S. P. Eisensmith, Crop and Soil Science Deptt. Michigan State Univ. USA). The data were subjected to ANOVA in Randomized Complete Block Design and significance was tested at the 5 per cent and 1 per cent level. The correlation coefficients were analyzed between percentage germination and conductivity, UV absorbance and lipid peroxidation.

RESULTS AND DISCUSSION

Storage at ambient conditions resulted in a significant decline in germination in both the cultivars (Table 1). Seeds stored under cryopreservation

Table 1. Analysis of variance of germination and vigour characteristics in of *A. cepa* L. cultivars

Parameter	Treatment	PR	PWF		
		Mean ± SE	Mean ± SE	Treatment MS[a]	Error MSb
Germination percentage	RT	33.33 ± 6.11	77.33 ± 2.30	1640.44**	16.44
	−180°C	70.66 ± 2.30	86.66 ± 6.11		
Radicle length	RT	2.32 ± 0.37	2.30± 0.18	5.03**	0.14**
	−180°C	3.59 ± 0.35	4.63 ± 0.46		
Plumule length	RT	4.22 ± 0.23	5.30 ± 0.14	8.78**	0.06**
	−180°C	6.55 ± 0.25	7.63± 0.42		
Vigour index	RT	142.97	588.32		
	−180°C	716.49	1062.62		

[a]: df = 3, [b]: df = 6, **: Significant at p 0.01, -: Not determined

did not show such decline and in fact had slightly higher germination values in comparison to the initial values. The radicle and plumule lengths were also adversely affected in RT stored seeds in comparison to cryopreserved seeds. Therefore, ultralow temperature of liquid nitrogen could control the degradative processes occurring at ambient conditions which result in loss of germination and reduce the vigour of seedlings grown from the surviving seed population.

Table 2. Effect of natural ageing on electrolyte concentration and UV absorbance (A_{240}) of seed leachate after 24 hr. leakage

Treatment		Conductivity (μScm^{-1} g d.wt^{-1})	UV absorbance A_{240}
		Mean ± SE	Mean ± SE
PR	Dead	14.25± 1.88	0.53 ± 0.29
	RT	14.33 ± 1.15	0.53 ± 0.05
	−180°C	16.58 ± 0.62	0.85 ± 0.09
PWF	Dead	9.43 ± 0.60	0.35 ± 0.07
	RT	12.33 ± 0.28	0.44 ± 0.05
	−180°C	14.83 ± 2.75	0.55 ± 0.01
Treatment MS		16.72*	0.08**
Error MS		2.47*	0.001**

* and ** : Significant at p < 0.05 and p < 0.01 respectively.

Membrane permeability studies using conductivity measurements of electrolyte leakage from seeds after various time intervals showed lower values for dead seeds and were almost similar in RT stored and cryopreserved seeds in cv. PR. In cv. PWF also, dead seeds showed less leakage of electrolytes

in comparison to both RT and cryopreserved seeds (Fig. 2). In fact, cv. PWF seeds retrieved from cryopreservation showed the highest leakage of electrolytes.

A spectrophotometric scan between 200-400 nm showed presence of UV absorbing substances in the leachate. An λ_{max} was observed at 240 nm and, therefore, A_{240} values were recorded for all treatments (Table 3). Pattern of

Table 3. Effect of natural ageing on lipid peroxidation in seeds of *A. cepa* cultivars

Treatment		Lipid peroxidation
		Mean ± SE
PR	Dead	0.0247 ± 0.0095
	RT	0.0198 ± 0.0080
	−180°C	0.0175 ± 0.0093
PWF	Dead	0.0341 ± 0.0060
	RT	0.0227 ± 0.0030
	−180°C	0.0191 ± 0.0020

leakage of UV absorbing substances was same as observed in case of conductivity measurements, as highest leakage was observed in case of LN_2 stored seeds. Membrane integrity has been used as an index of seed viability (Bewley, 1986; Ching and Schoolcraft, 1986) and loss of integrity has been reported to be directly proportional to loss in viability. However, in the present study electrolyte leakage from the seeds retrieved from cryopreservation and thus having higher germination interestingly showed more leakage of electrolytes in comparison to seeds with lower germination and even dead seeds. These results were confirmed by analysis of UV absorbing substances spectrophotometrically. No correlation was found between percent germination and leakage of electrolytes as well as UV absorbing substances (Table 4).

Lipid peroxidation was the highest in the dead seeds followed by RT stored seeds and the lowest values were recorded for cryopreserved seeds. In both varieties, a significant negative correlation was exhibited between percent germination and lipid peroxidation (Table 4). Lipid peroxidation is considered to be a result of free radicle oxygen generation that accompanies the production of malonedialdehyde (MDA). Highly reactive free radical oxygen causes alteration in the cell structure and in turn the cellular functions (Mack *et al.*, 1991).

Though a number of studies indicate membrane permeability to be a useful criterion for assessing the loss in germinability, in case of onion this

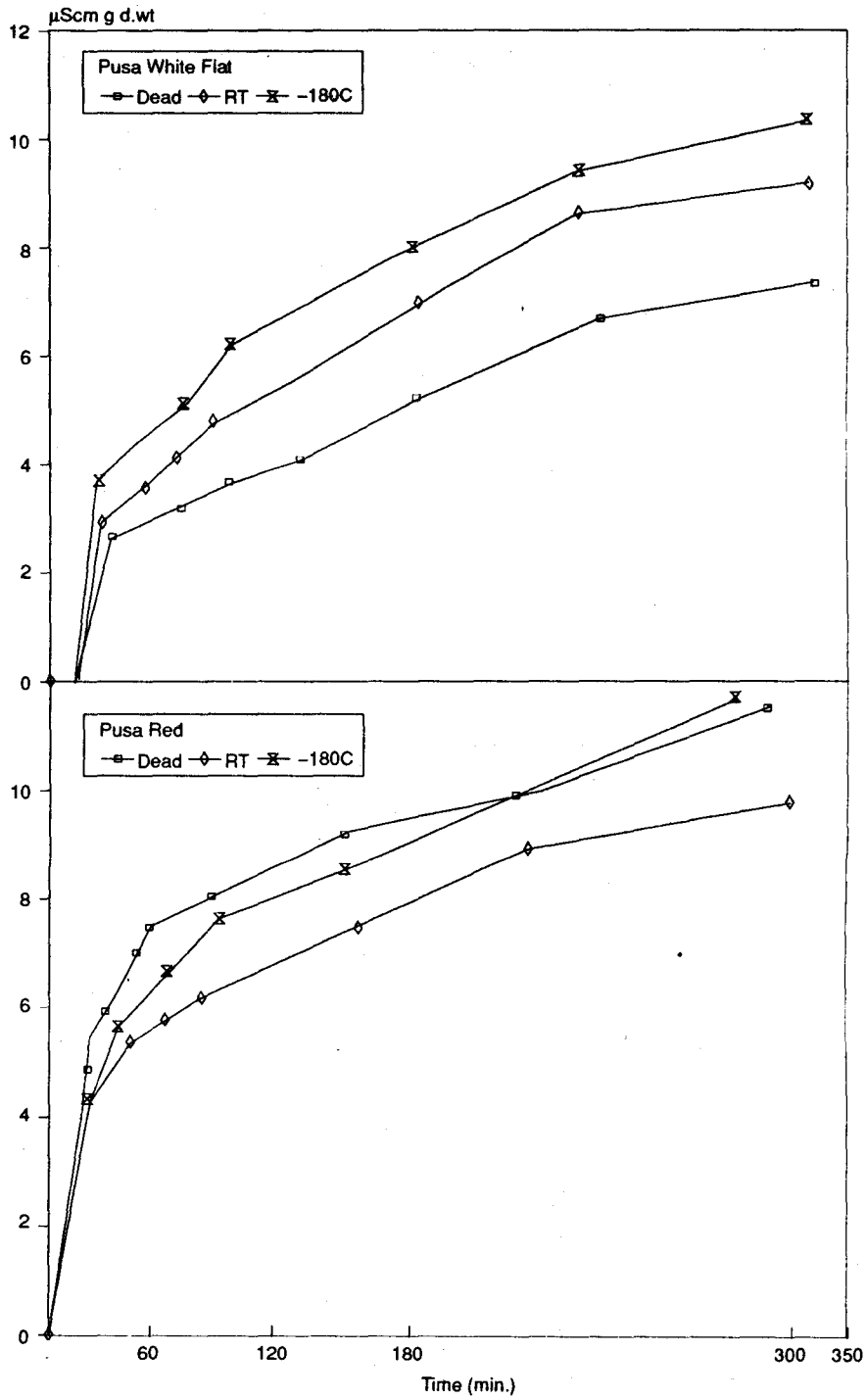

Fig. 2 : Conductivity of seed leachates measured after various time intervals in *A. cepa* L. cultivars stored for nine months

Table 4. Correlation coefficients of percentage germination with leachate conductivity, UV absorbance and lipid peroxidation in *A. cepa* L. cultivars

Parameter	Correlation coefficient with percentage germination	
	cv. PR	CV. PWF
Leachate conductivity	0.896	0.928
UV absorbance	0.882	0.886
Lipid peroxidation	–0.997*	–0.991*

*: Significant at P < 0.05 level

correlation could not be established. On the other hand, lipid peroxide formation was found to be a useful criteria to evaluate the seeds for the extent of decline in seed germination as well as the associated fall in the vigour of the seedlings under ambient conditions. This loss in germination and seedling vigour could be prevented by storing the seeds under cryopreservation where the degradative metabolic processes were also controlled as revealed by the lipid peroxidation assay.

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