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PHYSIOLOGY AND ENZYMATIC ACTIVITY OF ASIATIC CARROT SEEDS AS AFFECTED BY INVIGORATION TREATMENTS

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SUMMARY

Various seed enhancement treatments including osmo, hydro and halo priming were evaluated for their efficiency in improving vigour and uniformity of field emergence in Asiatic carrot (*Dacus carota* L.) cv. Pusa Kesar. Three seed lots of different season's harvest (1998-99, 1999-2000 & 2000-01) were used in the study. All priming treatments improved speed of germination, vigour and field emergence characters. Both osmopriming using PEG-6000 (-0.5 and -1.0 MPa) and halopriming using KNO_3 (15 and 30 mM) were found to be the best with an average improvement of 38% in speed of germination, 47% in vigour index, 5% in field emergence index and 36% in dry weight of one month old seedlings. Significant correlation between laboratory parameters (germination per cent, speed of germination and vigour indices) and field parameters (field emergence per cent, emergence index and seedling dry weight of one month old seedling) was observed. This can be used to predict the field performance of primed seeds. The interaction between seed lots and priming treatments revealed that aged seeds respond better to priming than fresh seeds with respect to seedling vigour and speed of germination. In three treatments, one each of osmo, halo and hydro priming along with untreated control, biochemical changes due to priming was evaluated. Soluble protein increased marginally by 4.7% in primed seed except in hydroprimed. Volatile aldehyde production that is an indicator of seed deterioration was negligible in osmo and halo primed seeds and reduced by 45% in hydroprimed seed compared to unprimed control. The activities of dehydrogenase (an indicator of seed viability) and peroxidase (free radical scavenging enzyme) increased by 148% and 195% respectively in osmo and halo primed seeds. Therefore, in Asiatic carrot cv. Pusa Kesar, osmopriming by PEG-6000 and halopriming by KNO_3 are useful in enhancing vigour and field emergence characteristics.

Key words: *Dacus carota* L., enzyme activity, field emergence, seed priming.

INTRODUCTION

Maintenance of desired levels of germination and vigour in seeds during storage is of great concern in tropical and sub-tropical regions, which are characterized by warm and humid climate. Hence, development of cost effective methods to enhance the vigour so as to realize the full potential of the variety is one of the major areas of research in India (Basu 1994, Thakur *et al.* 1997, Pandita and Nagarajan 2000, Pandita *et al.* 2001, Srinivasan and Saxena 2001). Seed enhancement treatments are particularly useful for ensuring uniform stand establishment in the field. Such treatments have become popular in western countries for high value vegetable seeds for better handling, improved seedling establishment, effective disease control and better crop performance (Lorenz *et al.* 1988, Parera and Cantliffe 1994, Liu *et al.* 1996). Studies are also needed to elucidate the mechanism

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of seed invigoration. In Asiatic carrot, slow and non-uniform seedling emergence is a major problem. This results in smaller plants and production of non-uniform roots of poor marketable value. Hence, various seed treatments including osmo, hydro and halo priming were evaluated for their efficiency in improving vigour and unifomity of field emergence in Asiatic carrot cv. Pusa Kesar seed lots of different ages. The biochemical mechanism of seed enhancement was also studied.

MATERIALS AND METHODS

Seed lots of Asiatic carrot (*Dacus carota* L.) variety Pusa Kesar harvested in 1998-99, 1999-2000 and 2000-01 and stored under controlled storage (15°C & 30% RH) were used as the experimental material.

Priming treatments

There were nine priming treatments including unprimed control. Osmo priming was done using PEG-6000 and the concentrations were adjusted to give two osmotic potentials, namely, -0.5 and -1.0 MPa at 25°C (Michael and Kauffman 1973). The seeds were kept on one layer of filter paper wetted with 5 ml of the osmoticum in 9 cm dia Petri-dish and replicated 10 times for each seed lot and potential. They were kept under dark in an incubator at 25°C. The priming treatment was carried out for 2 and 4 days at -0.5 MPa and 3 and 6 days at -1.0 MPa respectively. Seeds were then washed in running water and dried at room temperature. The same procedure was repeated with 15 and 30 mM solutions of KNO_3 for halo priming and with distilled water for hydro priming and incubated in dark for 24 h. For humidification treatment, seeds were kept in a muslin cloth bag and equilibrated over water kept in desiccator for 36 h. The treatment with an antioxidant, Butylated Hydroxy Toluene (BHT) was done by mixing the seeds with 1% solution of BHT in acetone at the rate of 1 ml/g seed and keeping it covered for 1h and dried at room temperature. All seed lots exposed to different pre-sowing treatments were conditioned to same moisture content (about 8%) by equilibrating over 45% Relative Humidity for 48h.

Laboratory germination parameters

Germination per cent was determined following ISTA (1985) procedure. One hundred seeds were placed between paper towels and kept at 22°C in a germinator for 14 days. After recording per cent normal seedlings, root and shoot lengths and dry weights of 10 randomly selected seedlings were measured. Seedling vigour was calculated following Abdul Baki and Anderson (1973). Seedling vigour I and II were calculated as germination % x root length + shoot length or dry weight of 10 seedlings respectively. There were four replicates for each lot and treatment. For determining speed of germination, twenty-five seeds of different lots and treatments were placed on moistened filter paper and kept at 22°C in an incubator with 4 replications. Daily germination count was taken till no more seed germinated. The speed of germination (x) was calculated as

$$X = \frac{\text{Number of seeds germinated}}{\text{Day of the first count}} + \text{--------} + \frac{\text{Number of seeds germinated}}{\text{Day of the final count}}$$

Field emergence parameters

The primed seeds of the three lots and untreated control were sown in well prepared seedbeds inside a shade house during September. Fifty seeds were sown in a row of 1 m length with 10 cm row-to-row distance. The experiment was laid in a split plot design with seed lots as main plots and treatments as sub-plots. There were four replications. Daily count on emergence was recorded till it became constant in all the treatments. Emergence Index was computed as per Mock and Skrdla (1978). At 30 days after sowing, plant height and dry weight were recorded on 5 plants per replication. The data were subjected to statistical analysis.

Biochemical parameters

Based on the results obtained, one each of osmo (-0.5 MPa), halo (15 mM KNO_3) and hydo priming treatments along with untreated control were chosen for biochemical studies. The pre-weighed seeds were placed on filter paper moistened with distilled water for 24 h in a Petri-dish. The hydrated seeds were used for estimation of protein and enzyme analysis. There were three replications for all the measurements. Soluble protein was estimated by Lowry's method (Lowry *et al.* 1951). Peroxidase activity was assayed as per the method described by Kar and Mishra (1976). The enzyme activity was expressed as change in absorbance units g^{-1} fresh weight min^{-1}. Volatile aldehyde assay was conducted following the method of Wilson and McDonald (1986a). The dehydrogenase

activity was determined by measuring the intensity of the colour developed due to the formation of formazan during treatment of the seeds with 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) solution (Gopandey and Basu 1981). The activity was expressed in absorbancy units.

RESULTS AND DISCUSSION

The effect of different priming treatments on laboratory germination and field emergence traits are given in Table 1. Laboratory germination improved marginally with pre-sowing treatments. However, they have improved field emergence significantly. The main effect of priming is seen in the enhancemnet of speed of germination, vigour and field emergence index. Only BHT treatment was an exception. Laboratory germination that was conducted under optimal temperature and moisture did not respond to the extra vigour provided to the seeds by priming. But this increase in vigour has helped in higher emergence per cent under field conditions. The enhancement in laboratory vigour indices is, however, reflected in the significant increase in height and dry weight of 1 month old plants. Szafirowska *et al.* (1981) showed that osmo priming with PEG-6000 has improved the emergence time, stand size, uniformity of stand in the field and increased the total root yield of carrot cvs. Nantes and Perfekcja in the cold soil. Similarly, reduced mean germination time and spread in germination time in polyethylene glycol primed carrot seeds were reported by Gray *et al.* (1991). Pill and Evans (1991) found no effect on emergence synchrony or percentage due to osmo priming, but the seedlings emerged more rapidly than those from untreated seeds in the field. Under glasshouse conditions, seeds given hydration treatments generally emerged more rapidly and gave greater seedling fresh weight than osmo-primed seeds. In our experiment, comparison of the effect of different treatments on the overall improvement of various

Table 1. Effect of different priming treatments on germination and field emergence characters of carrot cv. Pusa Kesar.

Treatments	Per cent germination	Speed of germination	Vigour index		Per cent field emergence	Field emergence index	One month old seedling	
			I	II			Height (cm)	Dry wt. (g)
PEG-6000 (-0.5 MPa) 2 days	57.2	21.9	860	1.408	59.7	71.6	16.4	1.086
PEG-6000 (-1.0 MPa) 3 days	57.0	20.7	839	1.400	66.5	71.0	16.0	0.938
PEG-6000 (-0.5 MPa) 4 days	58.1	20.5	806	1.113	54.7	69.1	14.4	0.914
PEG-6000 (-1.0 MPa) 6 days	52.8	19.1	769	1.542	57.7	69.4	13.4	0.765
KNO_3 (15 mM) 24 h	53.7	19.3	780	1.551	62.7	70.7	14.7	0.835
KNO_3 (30 mM) 24 h	53.8	19.1	732	1.565	57.8	70.2	15.1	0.856
Hydro priming- 24 h	54.9	18.7	793	1.338	57.5	69.9	14.2	0.826
BHT*	55.5	13.1	656	1.163	63.7	67.5	13.7	0.844
Humidification for 36 h	57.8	15.2	624	1.147	57.8	69.3	14.1	0.772
Dry seed control	54.0	14.4	522	1.009	52.8	67.2	11.9	0.654
CD at 5%	3.45	1.5	48.7	0.16	6.24	2.21	1.15	0.14

* BHT = Butylated Hydroxy Toluene
Details of treatments given in text

parameters showed the superiority of halo priming followed by osmo and hydro priming. Humidification and BHT treatment did not improve all traits uniformly.

The relative performance of the different seed lots showed that the lot 3 seed (3 year old) performed poorly compared to lot 2 (2 year old) and lot 1 (1 year old) (Table 2). Though laboratory germination characters of lot 3 showed significant reduction, the field emergence index, seedling height and dry weight in the field were comparable with that of lot 1 and lot 2 seeds. This signifies the improvement in field performance of aged seeds by pre-sowing treatments. The interaction effect (data not given) also showed the higher response of aged seeds to priming treatments compared to fresh seeds in improving speed of germination, vigour and field emergence. Slow germinating carrot seed lots were found to benefit more from osmo priming than faster ones (Drew *et al.* 1997).

Table 2. Laboratory germination and field emergence characters of three different seed lots of carrot cv. Pusa Kesar.

Character	Lot 1	Lot 2	Lot 3	CD at 5%
Germination (%)	59.0	59.6	47.8	1.89
Speed of germination	20.6	22.4	11.6	0.82
Vigour index I	797	743	675	32.5
Vigour index II	1.489	1.443	1.039	0.16
Field emergence (%)	62.4	62.9	52.1	3.41
Field emergence index	70.0	71.0	67.7	1.21
Seedling height (cm)	14.8	14.3	14.1	NS
Seedling dry wt. (g)	0.883	0.827	0.837	NS

The high correlation between laboratory measured parameters and field observed traits like field emergence, speed of emergence and seedling height was observed (Table 3). Seedling dry weight in the field correlated only with speed of germination and vigour index I. These relations may give an opportunity to predict the field performance of primed seeds from the laboratory observations.

Soluble protein content on fresh weight basis did not vary much among the four treatments. An increase in soluble protein content in primed seeds of groundnut (Jeng and Sung 1994) and in radish (Srinivasan and Saxena 2001) has been reported. But we did not observe any increase in soluble protein due to priming and rather a small reduction was found in hydro primed seeds. The volatile aldehyde production dramatically reduced in halo primed seeds as compared to untreated control. Both osmo and hydro primed seeds also reduced their activity by more than 50%. According to the lipid peroxidation model of seed deterioration (Wilson and McDonald 1986b), many deleterious reactions may occur in the seed as a result of reactive oxygen species (ROS) attack on seed lipids. While lipid peroxidation could proceed via autooxidation to the hydroperoxide stage in the dry seed, further break down to volatile products appears to result from enzymatic activity (Sekiya *et al.* 1979). Based on passive trapping of aldehyde evolution from germinating soybean seeds, a rapid vigour test has been developed and correlated with field emergence. In our study also a clear

Table 3. Correlation matrix between laboratory parameters and field emergence characters in primed seeds of carrot cv. Pusa Kesar.

Laboratory parameters	Field parameters			
	Field emergence	Emergence index	Seedling height	Seedling dry weight
Germination (%)	0.499***	0.355***	0.268**	0.087 NS
Speed of germination	0.430***	0.499***	0.344***	0.183*
Vigour index I	0.300***	0.279**	0.568***	0.386***
Vigour index II	0.399***	0.272**	0.227*	0.096 NS

*, **, ***- significant at P = 0.05, 0.01 and 0.001 levels respectively.

negative relation between aldehyde released from the treated seeds and their field performance was observed.

Dehydrogenase activity increased significantly in osmo and halo primed seeds while it marginally increased due to hydro priming. Increase in amylase and dehydrogenase activity in primed soybean seeds compared to unprimed seeds (Saha *et al.* 1990) and greater glucose-6-phosphate dehydrogenase activity in primed sweet corn seeds (Smith and Cobb 1991) have been reported. In carrot, priming was found to increase embryo volume by 43% and doubled cell number per embryo thereby enhancing embryo development (Gray *et al.* 1990). Also, priming of carrot seeds has been reported to loosen the endosperm-testa region that permits germination at sub-optimal temperatures (Davidowicz-Grzegorzewska 1997). These observations explain the higher intensity of formazan in primed seeds compared to unprimed seeds. The increase in dehydrogenase activity is due to priming induced depletion of some toxic products generated during ageing and thus preventing the loss of viability of the seeds (Gopandey and Basu 1981). A similar increase in peroxidase activity was obtained in primed seeds. Higher peroxidase activities have been reported in primed seeds of tomato and spinach than unprimed seeds (Parera and Cantliffe 1994). Since, this is a known free radical scavenging enzyme, the improved germination of primed seeds may be attributed to the counteraction of free radicals and re-synthesis of membrane bound enzymes (Basu and Dasgupta 1978, Saha *et al.* 1990). Overall biochemical changes indicated that halo priming has improved seed quality to the maximum followed by osmo and hyro priming. The performance of these seeds in the laboratory and field experiments was also in the same order that explains the biochemical basis of the seed enhancement treatments.

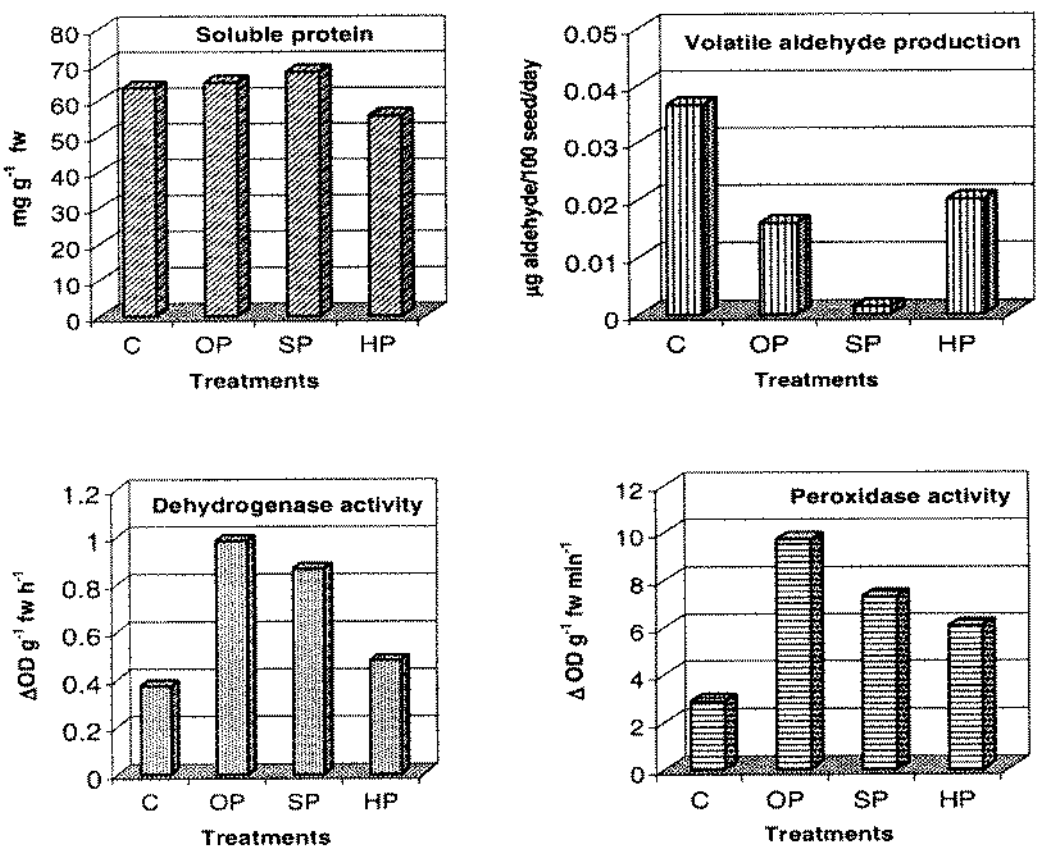

Fig. 1. Effect of seed priming treatments on soluble protein content, volatile aldehyde production, dehydrogenase and peroxidase activity in carrot cv. Pusa Kesar. C-Control, OP-PEG-6000 (-0.5 MPa), SP-KNO_3 (15 mM), HP-hydropriming. LSD at 1% is 8.64 for soluble protein, 0.021 for aldehyde, 0.023 for dehydrogenase and 1.07 for peroxidase.

The study compared the effect of different pre-sowing treatments on the enhancement of germination and field emergence characters of different carrot seed lots. Halo and osmo priming were superior to others and the aged seeds responded more than the fresh seed lots to the treatments. These seed enhancements were explained in terms of changes in some biochemical parameters.

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