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RESEARCH ARTICLE

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ASSOCIATED WITH SEED INVIGORATION TREATMENTS IN RELATION TO GERMINABILITY, LIPID PEROXIDATION ACTIVITY ALON GWITH PROTEIN PROFILE OF STORED FIELD PEA (PISUM SATIVUM L.)

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ABSTRACT

Physiological and biochemical changes occurring in seed during ageing are the significant parameters for reviewing the quality of seed. The rate at which the process of ageing takes place depends on the ability of the seed to resist the deteriorative changes and to act with their protective mechanism. The present study was conducted to judge the efficacy of eco-friendly non-toxic dry dressing and wet treatment on the germinability and membrane integrity of stored freshly harvested field pea (cv. Rachna) seeds. The seeds treated with red chilli powder @ 1g/kg of seed and amla fruit powder @ 2 g/kg of seed showed significant improvement on germinability than the untreated control after subsequent ageing. The ageing of seed accelerates the rate of lipid per oxidation, increases leaching of free amino acid and results in decline of total soluble seed protein banding pattern. Physio-biochemical studies revealed that the seeds treated with red chilli powder @ 1g/kg of seed and amla fruit powder @ 2 gm/kg of seed showed lower leaching of free amino acid, lower lipid per oxide formation and more number of peptide bands over control reflecting higher membrane integrity of the dry treated seeds than the untreated seeds under subsequent storage which indicate seed invigoration treatments improved the seed quality by maintaining higher germinability under adverse storage conditions. However, based on the above results, pre-storage dry treatments with red chilli powder and amla fruit powder may be suggested for the maintenance of vigour and viability of field pea seeds.

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INTRODUCTION

Pulse is an important source of dietary protein specially for the predominantly vegetarian people of India. Besides protein, it is also a good source of carbohydrate, mineral and fats. Field pea being one of the important pulse crop have 22.5% protein, 62.1 % carbohydrate, 1.8 % fat. Deterioration of seed quality is an undesirable attribute of agriculture. Annual losses of revenue from seed /grain products due to deterioration can be as much as 25% of the total harvested crop. The total acreage and productivity of field pea in India is still suffering due to insufficiency of good quality seeds in comparison with the other developed countries of the world.

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The major field pea growing areas in India, experience a temperate climate with high relative humidity and temperature during seed storage which is a major cause of deterioration of the quality of the seed stored by the farmers. Mosavi Nik *et al*. (2011) observed that quality of seed worsen if they are stored at high temperature and humidity apart from the other factors which occurs in field before harvesting, during harvesting, drying and processing. Several workers have successfully used various plant product, chemicals, priming treatment for maintaining seed viability, vigour and field performances. Pati *et al*. (2011) demonstrated that pre treatment of pea seeds with leaf extract of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) 50g in 250 ml distilled water for six hours before accelerated ageing treatment (100% R.H. and 30 ± 2°C temperature) for 45 days had much better plant performance than untreated control. Mandal *et al.* (2010) suggested that pre storage dry physiological treatment with red chilli powder and aspirin improved the germination percentage

and yield potential of sunflower seeds. Our present experiment aimed at investigating the efficacy of various non-toxic dry treatments for maintaining the vigour and viability of stored field pea (cv. Rachna) seeds.

MATERIALS AND METHODS

Seed collection

The study was carried out on freshly harvested seeds collected from Agricultural Experimental Farm of University of Calcutta at Baruipur which was properly dried to a safe moisture content (9.5%) and then stored under ambient conditions in the rubbered stoppered glass bottle (2.5 lit. capacity) until the treatments were given out.

Methods of seed treatment

- **Dry treatment**

Dry dressing treatment was executed by mixing finely powdered crude plants materials like red chilli powder (active ingredient, capsaicin) @ 1g/kg of seed and amla fruit powder (active ingredient, phyllemblin) @ 2 g/kg of seed, chemicals like calcium carbonate @ 2g/kg of seed, iodinated calcium carbonate @ 2g/kg of seed and pharmaceutical products like aspirin @ 50mg/kg of seed properly with 250g of seeds separately for each treatment kept in the 500g capacity glass bottles and were shaked gently up to 7 days for uniform mixing of these products with the seeds.

- **Wet treatment**

Three types of wet treatment were given to equal quantity (250 g) of seeds viz.,

- ***Soaking-Drying*: -** Seeds were soaked in double volume of water for 1 hour before drying back to its original moisture content.
- ***Moist-sand conditioning drying*: -** Seeds were mixed thoroughly with the moist sand (6% moisture content) for controlled permeation of water into the seed for 24 hours and then seived out followed by drying back to its original moisture content.
- ***Moist-sand conditioning soaking drying*:-** Seeds were kept for 24 hours in moist sand (6% moisture content) for 24 hours and then sieved to remove the sand which was again placed in double volume of water for 1 hour before drying back to its original moisture content.

After drying all the seeds of three wet treatments, these seeds along with the dry treated seeds were kept in perforated paper packets separately having equal number of holes and placed in glass dessicator containing fused calcium chloride for four days for stabilizing the moisture content to an uniform level and then kept in the glass bottle for storage.

Accelerated ageing

To judge the efficacy of treatments in controlling the rate of seed deterioration during storage, treated seeds were subjected to accelerated ageing by placing the seeds at 98 % relative humidity and 40°C temperature for 24 days.

Germination test

Germination test were carried out 10 days after treatment i.e. under before ageing condition and after accelerated ageing at 98% relative humidity and 40°C temperature for 24 days following the method of Punjabi and Basu (1982) with minor modifications by taking over 400 seeds for each treatment (ISTA, 1996). The effect of various treatments were calculated statistically following the analysis of variance method (Fisher, 1948).

Boichemical analysis

- ***Leaching of free Amino Acid***

Free amino acid was measured following the method of Moore and Stein (1948). Six field pea seeds were soaked in 20 ml of distilled water for 6 hours. Then 0.1 ml of decanted seed steep water was taken in a test tube to which 0.9 ml of distilled water and 1 ml of freshly prepared ninhydrin solution was added. The solution was then boiled for 10 minutes at 100°C after covering the test tube with glass lid. After cooling the solution, 5 ml of diurent (equal volume of n-propanol and water) was added and the solution was kept for 15 minute and the absorbance of the solution was recorded in the systronic spectrophotometer at 570 nm and expressed in terms of µg leucin equivalent in per ml of leachate.

- ***Lipid peroxide Formation***

The level of lipid per oxidation was measured in terms of malondialdehyde (MDA) formation, a product of lipid per oxidation by the method of Bernheim *et al*. (1948). 200 mg of dry seed powder was dissolved in 5 ml of 1 % TBA solution in a test tube. Then 2 ml of 1 N H_2SO_4 was added to it. The mixture is heated at 100°C in water bath. After cooling, 5 ml of of 2 methoxy ethanol was added and the mixture was thoroughly shaken which was then centrifuged at 10000 rpm at 4°C for 10 minutes. The absorbance of the clear orange red coloured solution was read at 520 nm. Lipid peroxidation activity is expressed as MDA produced in terms of n mole/gm of fresh tissue.

- ***SDS-PAGE of total soluble protein***

SDS –PAGE of the total soluble protein was done following the method of Laemeli (1970) with minor modification. 100 mg tissue was crushed in pre chilled mortar pestle with 4 ml of sample buffer, The mixture was then centrifuged at 14000 rpm for 30 minutes at 4°C and the supernatant so collected was used for protein estimation. 50 µg of protein was used from each sample for loading in the wells on the stacking gel. A constant current of 20 mA was passed until the tracking dye crosses the stacking gel. Then a constant voltage of 80 V was applied until the tracking dye reaches the bottom of the separating gel. After that the gel was first stained in the solution containing commassie brilliant blue, R-250 and then destained using a mixture of 100 ml methanol, 100 ml acetic

acid and 800 ml of deionised water until the bands were clearly visible.

RESULTS

Germination test conducted immediately after treatment (after 10 days) did not show any conspicuous difference on germination percentage and seedling length over untreated control except the soaking-drying treated seeds which had adverse effect on the germinability that might be due to rapid imbibition of water causing soaking injury to the seed (Table 1). But after accelerated ageing at 98 % relative humidity and 40°C temperature for 24 days, all the dry and wet treatments except soaking-drying treatment showed beneficial effect on germination percentage and vigour of the seedling over untreated control (Table 1). Pre-storage dry seed treatments performed better results in slowing down seed deterioration than the pre-storage wet treatments. Among the pre-storage dry treatments, red chilli powder and amla fruit powder proved better results in improving germinability and has the ability to withstand the stress conditions under subsequent storage (Table 1). Biochemical tests of treated and untreated seeds carried out immediately after pre-storage treatments also did not show any noticeable difference on membrane integrity (Table 2).

But after accelerated ageing at 98% relative humidity and 40°C temperature for 24 days, the membrane permeability of pre-storage treated seeds showed significantly lower leakage of free amino acid and less production of malondialdehyde than the untreated control. Among the pre-storage treatments, red chilli powder and amla fruit powder showed significantly less leakage of free amino acids and production of malondialdehyde, an end product of lipid peroxidation than the control thus indicating greater membrane integrity and better seed quality as compared to the other treatments and control (Table 2). In our present study, effort was made to compare the protein profile of treated and untreated seeds at before and after accelerated ageing of seeds at 98% relative humidity and 40°C temperature for 24 days.

Table 1. Effect of pre-storage seed invigoration treatments on germinability of pea (cv. Rachna) before (immediately after the treatment) and after accelerated ageing at 98% relative humidity 40°C temperature for 24 days

Treatments	Before Ageing				After Ageing			
	Germination		Seedling Length	Vigour Index	Germination		Seedling Length	Vigour Index
	(%)	Arc Sin Value	(mm)		(%)	Arc Sin Value	(mm)	
Dry treatments								
Control	100	90	76.50	7650	75.0	60.0	40.13	3010
Red Chilli Powder	100	90	78.62	7862	91.6	73.2	56.39	5165
Bleaching Powder	100	90	77.45	7745	83.3	65.9	51.97	4329
Aspirin	100	90	77.40	7740	83.3	65.9	51.72	4302
Calcium Carbonate	100	90	75.64	7564	75.0	60.0	53.99	4049
Iodinated Calcium Carbonate	100	90	77.10	7710	83.3	65.9	57.56	4795
Amla Fruit Powder	100	90	78.82	7882	91.6	73.2	57.25	5244
Wet treatments								
Soaking Drying	100	90	70.59	7059	50.0	45.0	44.52	2226
Moist Sand conditioning drying	100	90	75.80	7580	83.3	65.9	51.74	4310
Moist Sand Condition Soaking Drying	100	90	76.32	7632	83.3	65.9	53.72	4475
LSD at 0.05 P	—	NS	NS	NS	—	8.3	9.3	234.48

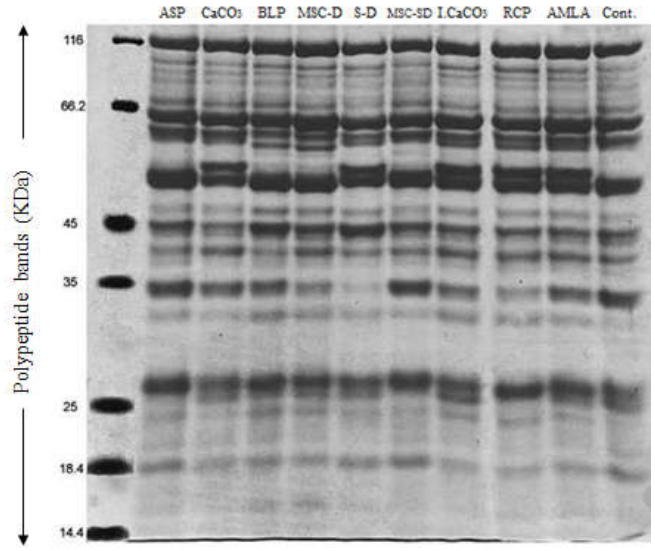
Vigour Index = G (%) × Seedling length (cm)
NS = Non significant

Table 2. Effect of pre-storage seed invigoration treatments on membrane integrity and lipid peroxidation activity of pea (cv. Rachna) before (immediately after the treatment) and after accelerated ageing at 98% relative humidity 40°C temperature for 24 days

Treatments	Before Ageing		After Ageing	
	Free amino acid (µg leucin/ml of leachate)	Lipid peroxidation activity (n mole/g fresh tissue)	Free amino acid (µg leucin/ml of leachate)	Lipid peroxidation activity (n mole/g fresh tissue)
Dry treatments				
Control	22.9	96.5	204.4	354.3
Red Chilli Powder	21.4	100.9	127.7	253.4
Bleaching Powder	24.3	98.7	137.2	272.0
Aspirin	23.6	99.8	146.7	291.7
Calcium Carbonate	27.1	104.1	179.5	306.1
Iodinated Calcium Carbonate	20.1	97.6	138.4	274.2
Amla Fruit Powder	26.4	102.0	129.4	258.8
Wet treatments				
Soaking Drying	28.6	111.9	190.6	376.2
Moist Sand conditioning drying	25.7	93.2	160.8	303.8
Moist Sand Condition Soaking Drying	20.7	103.9	182.2	330.1
LSD at 0.05 P	NS	NS	11.2	19.8

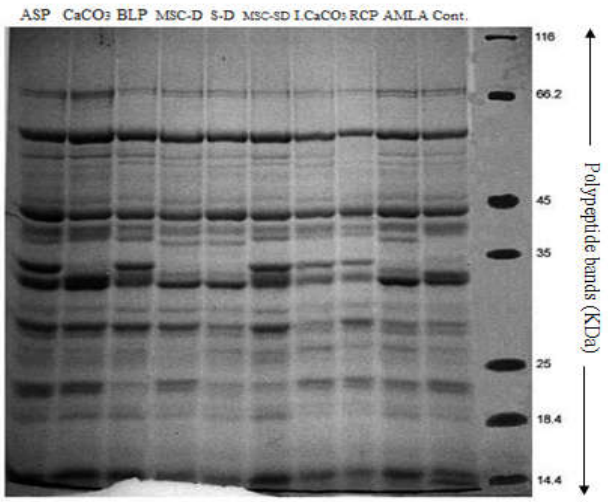
Other details are same as in Table 1.

Protein fractions of treated and untreated seeds at before ageing condition were analysed by SDS-PAGE indicating that there was a total of 17 polypeptide bands of diverse molecular weight ranging from 18 KDa to 116 KDa (Fig. 1). Seeds treated with red chilli powder showed maximum 17 number of peptide bands and minimum of 11 peptide bands were seen in the protein sample of the seeds treated with soaking- drying treatment whereas untreated control showed 12 peptide bands.



Treatment : ASP – Aspirin, $CaCO_3$ – Calcium Carbonate, BLP – Bleaching Powder, MSC-D – Moist sand conditioning drying, S-D – Soaking drying, MSC-SD – Moist sand conditioning soaking drying, I. $CaCO_3$ – Iodinated Calcium Carbonate, RCP – Red Chilli Powder, AMLA – Amla fruit powder, Cont. – Control.

Fig. 1. Peptide profile of treated and untreated field pea seeds at before ageing condition



Other details are same as in Fig. 1.

Fig. 2. Peptide profile of treated and untreated field pea seeds at after ageing condition

However, after accelerated ageing at 98% relative humidity and 40°C temperature for 24 days, seed samples had a maximum of 14 polypeptide bands of diverse molecular weight ranging from 14.4 to 66 KDa reflecting a decrease in the total number of protein bands (Fig. 2). Seeds treated with red chilli powder showed highest 14 number of peptide bands and a minimum of 8 number of polypeptide bands was found in the protein sample of the seeds treated with soaking-drying indicating that seeds treated with red chilli powder have better ability to resist disintegration of high molecular weight peptide band as compared to other treated seeds and control though the possible reason behind it is still needed to be elucidated.

DISCUSSION

Seed deterioration is an irreversible degenerative process that occurs during storage ambience. The rate of deterioration is however influenced by the seed moisture content and temperature of the storage, an increase in either leads to more rapid seed deterioration (Ellis *et al*., 1992). Yang-ya ping *et al*., 2008 observed that artificially ageing treatment of rice (cultivar 99- zao-677 and Xiang za oxian 24) reduced the germination percentage due to injury in cell membrane resulting in higher relative electro-conductivity and free amino acid content and decrease in total seed protein. Several workers have observed that seeds if treated with crude plants materials, chemicals etc. before storing could successfully maintain their germinability and productivity. De *et al*. (2004) reported that dry physiological treatment with bleaching powder and red chilli powder resulted in improvement in germination percentage and field emergence of stored soybean seed with reduced lipid peroxidation and increased dehydrogenase enzyme activity. Our study are in agreement with the earlier findings of workers in this field.

Bailly *et al*. (1996) concluded that sunflower seed deterioration during accelerated ageing is closely related to decrease in the activities of detoxifying enzymes like superoxide dismutase, catalase, glutathione reductase and to an increase in lipid peroxidation. Chakraborty *et al*. (1991) revealed that during storage of ground nut seed under ambient conditions for four months (Kharif crops) or 8 months (summer crop)there was an increase in moisture content, lipase activity, free fatty acids, malondialdehyde concentration and electrical conductivity and a decrease in phospholipid concentration. Kaewnaree *et al*. (2011) reported that low vigour seed have higher lipid per oxidation activity reflecting a reduced germination or vigour index which is one of the prime cause of poor quality seed. Crude plant materials might play a role in reducing the lipid peroxidation and maintaining the membrane permeability of seed. Our results are in conformity with the earlier findings of Mandal *et al*. (2000) and Guha and Mandal (2013). In this experiment it was noticed that due to accelerated ageing highest molecular weight sub units were disintegrated into low molecular sub units. Machado *et al*. (2001) reported that when seeds were placed at high relative humidity and temperature, there was a change in protein electrophoretic pattern as increased moisture is enough to damage the seed on its structure. Many researchers found that as seed ageing progresses there is reduction in protein quantity, number of peptide bands and its intensity. Our results are in accordance with those of several others workers like Kehinde *et al*., 2013 in amaranthus, Vasudevan *et al*., 2012 in pea nut and Das *et al*., 2010 in rice who reported similar changes in number and intensity in protein banding of low vigour seeds. The mode of action of these dry dressing treatments is not still completely clear. Capsaicin an active ingredient of red chilli powder is an

inhibitor of lipid per oxidation (Brand *et al*., 1990; Dey and Ghosh,1993). Protein protective role of aspirin (*ortho* acetyl salicylic acid) have been reported by several workers which helps in maintaining the seed health (Pal and Basu, 1994, De *et al*., 1998).

Basu and Rudrapal (1980) suggested the role of iodine in the stabilizing the double bond of unsaturated fatty acid moieties and lipoprotein membranes which is a possible reason for maintain vigou and viability of seed. In legume seed soaking injury due to rapid water uptake is a known phenomenon. Soaking injury could be reduced by use of osmoticum polyethelene glycol very as it helps in controlled permeation of water into the seed (Woodstock and Tao, 1981; Saha and Basu, 1984). Number of research worker have found amla as an effective broadspectrum antioxidants and free radical scavengers helping to reduce disease and slow ageing process (Kumaran *et al*., 2006; Rao *et al*., 2005). Whatever may be the mode of action of these treatments, red chilli powder @ 1g/kg of seed and amla fruit powder @ 2g/kg of seed may be suggested for the maintenance of vigour and viability of stored field pea (cv. rachna) seed which would definitely increase the planting value and yield potential as well.

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