



Biochemical investigations on vigour enhancement in aged seeds upon seed priming in onion

Umsha^{1*}, S.N. Vasudevan¹, K. Bhanuprakash², B. Manjunatha¹, G. Sarika³, N. Amruta³

¹Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur -584102 (Karnataka), INDIA

²Division of Seed Science and Technology, Indian Institution of Horticultural Research, Hesaraghatta, Bangalore-560089 (Karnataka), INDIA

³Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore 560065 (Karnataka), INDIA

*Corresponding author. E-mail: umesh3980@gmail.com

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Abstract: Loss in seed quality that occurs during all process, from maturation in the field to storage, leads to seed deterioration. Storage of onion seeds under ambient, hot and humid conditions is very problematic since these conditions deteriorate seed quality faster. Seed deterioration is associated with many metabolic defects that occur due to changes in enzymes and protein levels. This deterioration can be rectified to the extent possible by the technique of seed priming. The results clearly indicated that priming will restore the lost seed vigour in aged seeds due to reactivation of enzyme activity in old seeds. The germination percentage improved from 60% to 79.5% and 72.5% in GA₃ and hydroprimed, respectively. Priming will also restore the lost seed vigour in aged seeds due to reactivation of proteins in old seeds and expression of these proteins in priming treatments are related to priming induced proteins in contrast to their absence in the aged seeds which are necessary for germination and longevity of seeds.

Keywords: Esterase, Onion, Peroxidase, Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis SDS-PAGE, Vigour

INTRODUCTION

Onion (*Allium cepa* L.) is one of the important commercial vegetable crops of India and occupies a premier position amongst vegetables due to its high remunerative price and regular demand in the market. Seed quality is one of the key factors affecting the successful farming, but this seed trait inevitably declines during prolonged storage. Poor quality seeds generally show decline in their ability to germinate and emerge into vigorous seedlings, leading to problems for successful crop production (Powell *et al.*, 2000). The ever growing demand of this vegetable throughout the year exerts challenge for continuous production even during off seasons. Onion seeds are more sensitive to storage conditions like high temperature; high seed moisture content, light exposure and an extended storage period have all been found to adversely affect quality (Muhammad and Muhammad, 2002). Many physiological and biochemical changes are linked to the process of seed deterioration and metabolic defects that occur due to these changes. The defects can be rectified to the extent possible by the technique of seed priming. It is a pre-sowing treatment that involves controlled hydration of seeds, sufficient to allow pre-germinative metabolic events to take place and to restrict

radicle protrusion through the seed coat (Heydecker *et al.*, 1973). This technique has been used in some vegetables seeds including onion to augment the germination rate, total germination and seedling uniformity etc. The metabolic processes associated with priming are slightly different, with respect to their dynamics from those which occur during germination, where the water uptake is not controlled (Varier *et al.*, 2010). Pre-soaking the seeds in osmotic solutions has been demonstrated to improve the viability and vigour of aged seeds in various horticultural crops (Bhanuprakash *et al.*, 2010). It is a useful technique to exploit seed potential in arid and desert ecosystem. Little is known why priming enhances seed performance in terms of germination values.

The benefits of priming (physiological aspects) include increased germination rate, more uniform emergence, and germination under a broader range of environments, and improved seedling vigour and growth. The magic of priming may not be in what it does with regard to germination enhancement, but more in determining how the seed retains its ability to continue germination without harm after the germination process was initiated, and then subsequently stopped via dehydration. But, our fundamental understanding of the biochemistry of prim-

ing remains obscure. Very little is known about the basis of vigour enhancement in aged onion seeds upon seed priming through biochemical investigations. Hence, an investigation in this direction was chosen in order to understand how seed priming mitigate the ageing effects in onion.

MATERIALS AND METHODS

Laboratory experiment was conducted at the Division of Seed Science and Technology, Indian Institute of Horticultural Research, Hesaraghatta, Bangalore and Department of Seed Science and Technology, University of Agricultural Sciences, Raichur. Fresh seeds of onion Cv. Bellary Red were subjected to accelerated ageing to create low (60% germination) vigour seed lot. These seeds were subjected to priming with different chemicals to identify the best treatment. Among all treatments the seed priming with GA₃ @ 500 ppm and hydropriming was found best (Data not shown) and it is further analyzed to understand the basis of vigour enhancement in aged seeds upon seed priming through biochemical investigations. The fresh seeds were used as control for biochemical studies in relation to vigour enhancement in aged seeds upon priming. Electrophoretic analysis of isozyme and protein profiles were carried out according to the method prescribed by Glaszman *et al.* (1987) and Laemeli (1970) respectively.

Electrophoretic analysis of esterase (EST) and peroxidase (POX) isozymes: Esterase (EST) and Peroxidase (POX) isozyme analysis was done as described by Glaszman *et al.* (1987) with slight modification.

Sample preparation: 0.5 gram of seed sample was ground thoroughly in a pestle and mortar with 50 µl of extraction buffer (0.1M Tris-HCl, pH-6.8) under ice condition. The extract was taken in 2 ml eppendorf tubes and centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected and 10 µl of tracking dye (1% bromophenol blue containing 0.05% glycerol) was added into each tube. 50 µl of sample extract was used for loading.

Electrophoresis: Resolving gel of 8 % and stacking gel of 5 % were used. The upper and lower reservoirs of electrophoretic apparatus were filled with electrode buffer (Tris glycine without Sodium Dodecyl Sulphate). Then 50 µl of enzyme extract was loaded into the wells of stacking gel using micropipette. A current of 1.5 mA per well was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel, which took eight to ten hours. α -naphthyl acetate protocol was used for esterase staining.

Staining procedure for peroxidase (POX): Gel was transferred to tray having 0.05 M sodium acetate buffer, pH 4.5 and kept for shaking for 1 hour. Just before staining 200 mg 3-9 AEC (3-Amino-9-Ethyl Carbazole) was dissolved in 3 ml DMF (Dimethyl For-

amide). This 3-9 AEC was added to 250 ml of 50 Mm sodium acetate buffer (pH 4.5) under stirring conditions to avoid formation of clods. The solution was prepared in the dark just few minutes before staining. This reaction mixture was added to the gel and kept for shaking and 1-2 ml of 30% H₂O₂ was added drop by drop till the appearance of the dark pink bands for about 5-20 minutes. Gel was preserved in 30% glycerol till documentation.

Electrophoretic analysis of soluble seed protein: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of total soluble seed protein was carried out by using 12.5 per cent polyacrylamide gel according to the method prescribed by Laemeli (1970) with slight modifications.

Sample preparation: The seed sample was crushed by pestle and mortar. The ground seed sample was defatted by using CMA for 3-4 times. To the defatted seed material, 1.5 ml of extraction buffer (Tris glycine 25 mM, pH 8.5) was added, agitated thoroughly and kept at 4^oC overnight for protein extraction. Then the suspension was centrifuged under refrigeration at 10,000 rpm for 10 min and the clear supernatant was collected. This protein extract was dissolved in an equal amount of working buffer and kept in boiling water at 90^o C for 10 minutes, and kept at 4^o C for cooling. Before loading the sample was centrifuged at 10,000 rpm for 5 minutes and supernatant was loaded in to the gel.

Electrophoresis: Resolving gel of 12.5 % and stacking gel of 5 % were used. Tankbuffer was prepared using 3 g of Tris+14g of Glycine+ 1g of SDS per litre of double distilled autocleaved water. The upper and lower reservoirs of electrophoretic unit were filled with electrode buffer. Then 20µl of protein extract was loaded into the wells of stacking gel by layering them under electrode buffer using micropipette. A current of 1.5 mA per well was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel, which took six to eight hours. Silver staining was done for gel to get protein profiles. Once the bands are developed, the gel was washed with double distilled water for 20 seconds and the gel was fixed using acetic acid.

Evaluation and documentation: The electrophoregrams of the gel were prepared by measuring the distance of each band from the point of loading. The Rm value was calculated as given below:

$$R_m = \frac{\text{Distance travelled by the protein or isozyme}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered on the basis of increasing Rm values; the relative intensities and mobilization of protein and isozyme bands and presence or absence of specific bands or combination of different bands were analyzed.

RESULTS AND DISCUSSION

The appearance or disappearance of particular peptides could serve as a marker for seed invigoration. Late embryogenesis abundant (LEA) proteins accumulated in developing seeds prior to maturation will protect the embryo from desiccation stress. It is evident that priming is reversing the detrimental effects of seed deterioration, but how is still poorly understood, and therefore, it is necessary to know the biochemical changes that may contribute for seed priming. In the present study, variations in fresh and aged seeds for proteins were noticed based on either presence or absence of specific bands at specific Rm value.

Significant changes in enzyme activities were noticed in primed seeds compared to unprimed seeds.

Changes in esterase profile were noticed due to seed ageing and subsequent priming (Plate 1 and Fig.1). Seed

ageing (60% seed lot) has shown loss of isomorphs of esterase isozyme at Rm values (0.07, 0.50, 0.57 and 0.63) when compared to control. Decrease in intensity of esterase isozyme was also noticed at band number 1 (in 60% seed lot). However, synthesis of new isomorphs of isozymes were noticed (band number 2 and 7) at Rm values 0.05 and 0.74 which might be due to seed ageing. Seed priming restored almost entire esterase profile that was lost due to seed ageing.

All the five isomorphs of isozymes (band 1, 2, 3, 4, 5) corresponding to Rm values 0.02, 0.07, 0.50, 0.56 and 0.63 were restored due to GA₃ and hydropriming. However, some newly synthesized proteins were noticed which may be related to priming induced vigour proteins i.e. band number 8 at Rm value 0.85. Thus our results clearly supported that priming will restore the lost seed vigour in aged seeds due to reactivation of

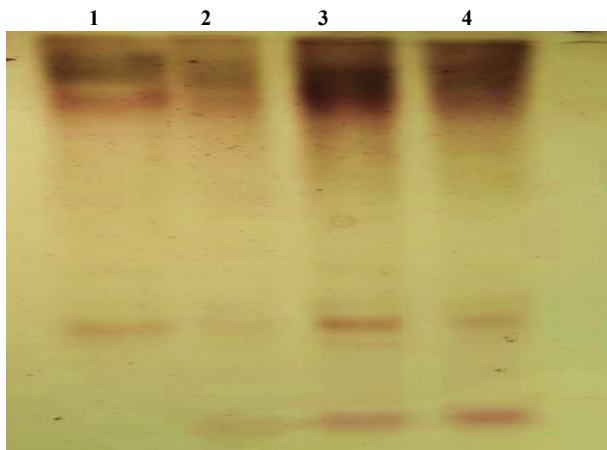


Plate 1. Esterase profiles of Primed seeds in onion Cv. Bellary red in native PAGE.
1. Control, 2. 60% aged seed lot, 3. GA₃ primed seed, 4. Hydroprimed

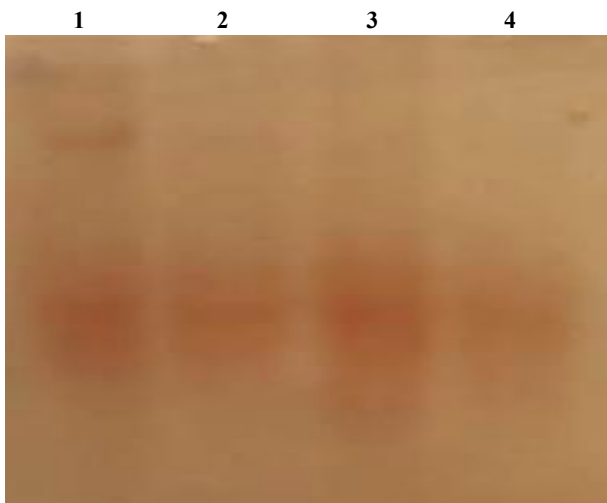


Plate 2. Peroxidase profiles of primed seeds in onion Cv. Bellary red in native PAGE.
1. Control, 2. 60% aged seed lot, 3. GA₃ primed seed, 4. Hydroprimed

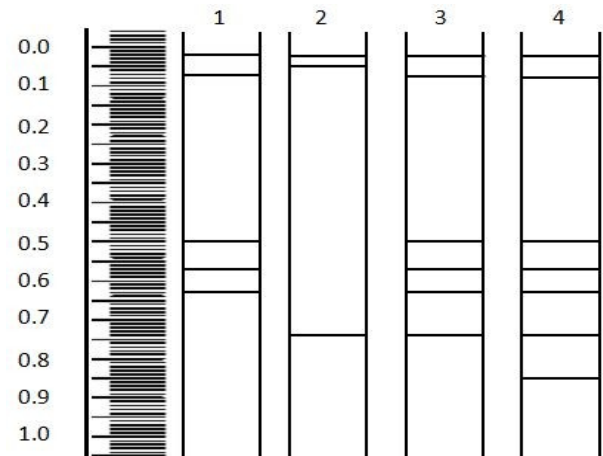


Fig. 1. Zymogram of Esterase (EST) isozyme due to seed priming in onion Cv. Bellary red .

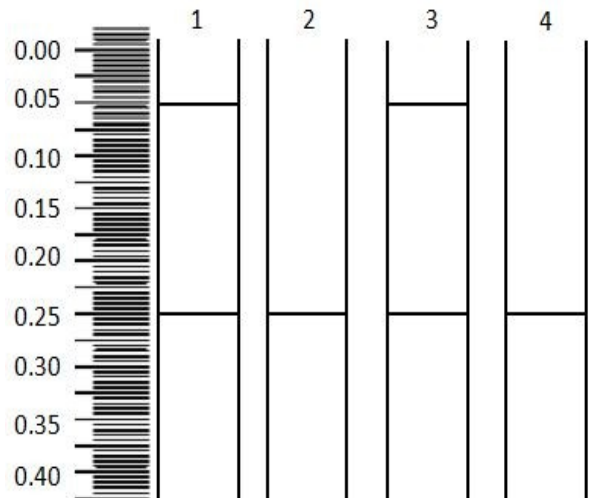


Fig. 2. Zymogram of peroxidase (POX) isozyme due to seed priming in onion Cv. Bellary red.

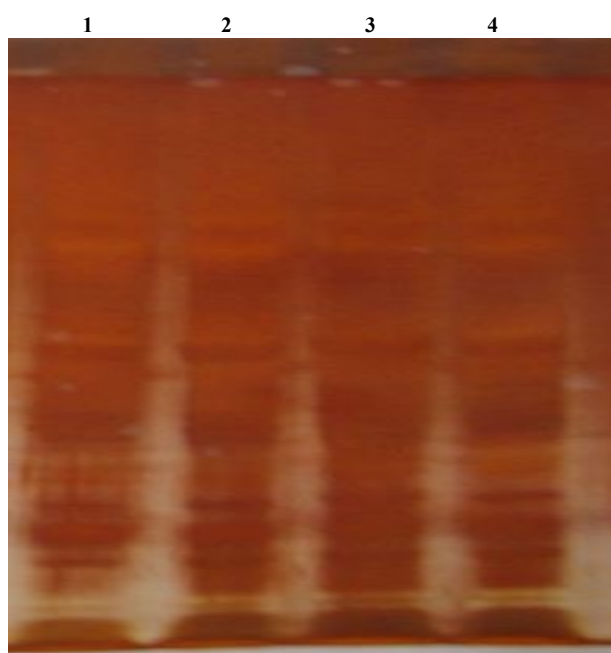


Plate 3. Protein profiles of primed seeds in onion Cv. Bellary Red in SDS PAGE.

1. Control, 2. 60% aged seed lot, 3. GA₃ primed seed, 4. Hydroprimed

enzyme activity in old seeds. The germination percentage has improved from 60% to 79.5% and 72.5% in GA₃ and hydroprimed, respectively. The increase in the germination of primed seeds might be due to increase in the synthesis of these enzymes. Similar results also have been reported in various crops (McDonald, 1999, Soumya, 2011, Sathish *et al.* 2012,).

Several enzymes have been investigated in relation to seed priming which are associated with early hours of germination by various scientists on a variety of crops. An attempt was made to examine the role of peroxidase upon priming in onion.

The detailed electrophorogram of peroxidase isozyme of primed seeds is depicted in plate 2 and fig. 2. The banding profile of peroxidase isozyme consists of 2 bands having Rm values ranging from 0.05 to 0.25. Band number 2 expressed its presence with low intensity at Rm value 0.25 in all the treatments. While band 1 (Rm: 0.05) was noticed in aged seeds primed with GA₃ and control. Further, it was absent in aged seeds (60% seed lot) and aged hydroprimed seeds. Thus our results clearly supported that priming will restore the lost seed vigour in aged seeds due to reactivation of enzyme activity in old seeds. It is also recognized that peroxidase isozyme is mostly contained to root system, but in the present study since fully imbibed seeds were used as source enzyme, complete expression of the enzyme did not perceived as such enough polymorphism could not be established in peroxidase isozyme. In onion seeds (Basu, 1976) it has been demonstrated that hydro priming treatments effectively slow down the physiological deterioration under natural and accelerated ageing con-

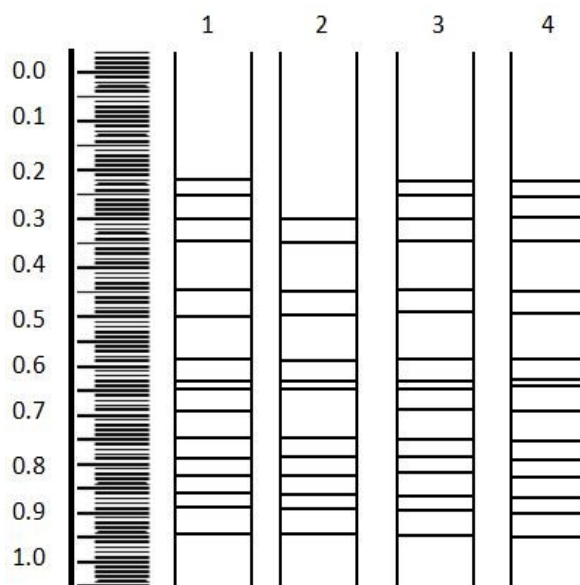


Fig. 3. Zymogram of protein profile due to seed priming in onion Cv. Bellary Red.

ditions, with the effect being dependent on seed vigour, this improved stability was associated with greater dehydrogenase activity and appreciably lowered the catalase activity, increased hydration enhanced membrane repair in seeds attributed this to the stimulation of free radical scavenging enzymes such as superoxide dismutase, catalase, peroxidase and glyoxysomes enzymes, these enzymes control ageing process by counteracting with lipid peroxidation.

Loss of proteins during drying of primed seeds leads to lack of desiccation tolerance in primed seeds. The entire electrophoretic protein banding profile consists of 16 bands with Rm values ranging from 0.22 to 0.94 (Plate 3 and Fig. 3). The band 1, 2 and 8 at Rm value 0.22, 0.25 and 0.63 were absent in aged seeds (60% seed lot). Seed priming restored almost entire protein profile that was lost due to seed ageing. This may be due to the reason that the protein disappeared after subjecting the seeds for ageing treatment, and reappeared after priming. All the bands (band 1, 2 and 8) corresponding to Rm values (0.22, 0.25 and 0.63) was restored due to GA₃ and hydropriming. Thus our results clearly supported that priming will restore the lost seed vigour in aged seeds due to reactivation of proteins in old seeds and expression of these proteins in priming treatments are related to priming induced proteins in contrast to their absence in the aged seeds which are necessary for germination and longevity of seeds. Haroun and Hussien (2003) reported that seed priming increased the intensities of protein bands from 20-32 kDa in *Lupinus*. The quantitative increase in protein profile might be due to the presence of all the components necessary for resumption of protein synthesis except polysomes within the cells of mature dry embryos. However, polysomes might have been formed immediately after imbibition by combination of single ribosomes which might have initiated the process of protein synthe-

sis as suggested by Bewley (1997). Dure *et al.* (1989) also suggested that the LEA proteins may be related to maintenance of seed desiccation tolerance. As the radical grows, LEA protein levels decreases and the loss of desiccation tolerance in germinating seeds is associated with degradation of LEA proteins. Gurusinghe *et al.* (2002) also opined that the induction of heat-shock protein (hsp70) in tomato seeds, the abundance of Bip (78kD binding protein) and 'class I small hsp' in primed seeds subjected to post-priming treatment that could involve in the extension of seed longevity. Expression of vigour related proteins of cauliflower seeds have been found during early germination. They are expressed more in primed seeds than in control seeds. (Fujikora and Karssen, 1992). The variations in the appearance of bands may be due to activation of membrane bound enzymes which actively participate in synthesizing proteins necessary to carry out complete emergence of seedlings (Job *et al.*, 1997). Thus the protein analysis study clearly revealed the appearance and disappearance of peptides at specific Rm values that can be employed as a marker for priming.

Conclusion

The total soluble seed protein profile had revealed polymorphism (16 bands with Rm value ranging from 0.22 to 0.94) with respect to appearance and disappearance of peptides at specific Rm values. It can be employed as a marker for priming. The band 1, 2 and 8 at Rm value 0.22, 0.25 and 0.63 were absent in aged seeds (60% seed lot) and present in primed aged seeds. Esterase isozymes expression in relation to priming clearly revealed polymorphism. All the five isomorphs of isozymes (band 1, 2, 3, 4, 5) corresponding to Rm values 0.02, 0.07, 0.50 0.56 and 0.63 were restored due to priming. Synthesis of new isomorphs of isozymes were noticed (band number 2 and 7) at Rm values 0.05 and 0.74 which might be due to seed ageing. The present study concluded that priming in aged seeds of onion showed increased enzyme activity, restored almost entire protein profile and esterase isozyme profile as it allowed repair system to combat subcellular damage, activated enzyme synthesis and thus restored deterioration process to certain extent started due to ageing. The changes in the activities of the enzyme, upon priming, suggest that mobilization of storage material may be responsible for increased germination and vigour in primed seeds when compared to unprimed aged seeds. Therefore priming can be used as a good technique for enhancing vigour of low vigour seeds lots of onion.

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