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Physiological and biochemical changes in relation to seed quality in ageing bell pepper (C*apsicum annuum*) seeds

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ABSTRACT

A study was conducted during 2006–08 to identify physiological (per cent germination, germination rate, seedling vigour index, germination index and standard germination) and biochemical changes (membrane injury, malonaldehyde content, total soluble sugars, amylase, peroxidase and dehydrogenase activity) in relation to seed vigour and viability in fresh and aged bell pepper seeds (C*apsicum annum* L.). Results showed an overall decrease in germination, vigour and enzyme activity with increase in ageing duration (data). Increased membrane injury (1.2–2.05 times) and lipid peroxidation (1.09–1.38 times) coupled with significant reductions in dehydrogenase (34.5–100%) and amylase activity (42.9–100%) were noticed in accelerated aged (3–15 days) seeds. Peroxidase activity remained unaltered up to 9 days after accelerated ageing but declined significantly at 12 and 15 days after accelerated ageing. Changes in protein and isozymes profiles in aged seeds compared to fresh seeds further indicated that the loss in seed quality might be due to adverse effect of ageing on proteins. The electrophoretic variations in proteins and enzymes noticed in the present study could be used as criteria in assessing the quality of bell pepper seed lots.

Key words: Ageing, Bell pepper, Biochemical changes, Physiological changes

Rapid loss of seed viability in storage under ambient conditions is reported to be one of the causes for poor germination. Loss of vigour precedes viability and hence its evaluation is the first step in predicting seed quality (Vijayakumar 2003). This would particularly be the case in pepper seeds, if carry over seed lots are used from previous production season as this species is considered to have a relatively short life span and loses viability in a medium range storage period (Ozcoban and Demir 2002). The process of deterioration under accelerated ageing or controlled deterioration conditions is arguably similar to those under normal condition as it is presumed to mimic natural ageing (Loýc Rajjou *et al*. 2008) and accordingly, this treatment is widely used by seed companies as a vigour assay. Thus accelerated ageing techniques have great potential for understanding the mechanism of ageing associated changes and its impact on seed vigour and viability. Research on ageing induced changes on warm climate crop seeds, such as pepper is scarce. Hence, an experiment was undertaken in this direction to identify various physiological and biochemical changes associated with loss of viability in bell pepper (*Capsicum annuum* L.) seeds.

MATERIALS AND METHODS

Fresh seeds of 'Arka mohini' bell pepper (*Capsicum annuum* L.) were obtained from Indian Institute of Horticultural Research, Bangalore. Four replicates of 100 seeds of each sample planted in moistened roll paper towels were germinated in a growth chamber at 25°C under dark. Seeds showing normal seedlings at the end of day 14 were considered germinated and per cent germination, germination rate, germination speed and standard germination were computed (Bhanuprakash *et al.* 2006). Accelerated ageing was done for 15 days at 45± 1°C and 75% relative humidity. Samples were drawn at three-day interval and dried back to original moisture content before evaluating for physiological and biochemical changes.

Seedling vigour index was calculated by multiplying germination per cent with total seedling length. Membrane injury was calculated using the formula (E1/E2) × 100 (Onwueme 1979). Lipid per oxidation in aged seeds was studied by TBA colour reaction and total soluble sugars were estimated by colorimetric assay in seed leachate. The α-amylase and peroxidase activities in developing seeds were estimated after incubating seeds prior to extraction of

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enzymes for 24 hr at 25°C. Dehydrogenase activity was estimated by colorimetric estimation of formazan at 480nm. The protein content in samples was quantified and characterization of seed proteins was carried on 12% resolving gel using SDS- PAGE and relative mobility of each band was calculated. For esterase isozyme studies, samples were run on 8% native-PAGE and stained in solution (50 mg Fast blue RR salt in 2 ml of 50 mg Napthyl acetate in 50% acetone added to 100 ml of 0.5 M sodium phosphate buffer *p*H 6.2) for 1 hr. All the above mentioned parameters were estimated as per procedures discussed (Varier and Dadlani 1992, Bhanuprakash *et al*. 2006). Data were arranged in completely randomized design and analysis of variance was used to assess the statistical significance of treatment differences (*P*=0.01).

RESULTS AND DISCUSSION

Results showed that seed ageing affected seed viability and vigour compared with the fresh seeds. Significant reductions in germination to the extent of 31, 55 and 88% were noticed in 3, 6 and 9 day accelerated aged seeds. Further increase in ageing duration to 12 and 15 days, however, resulted in complete loss of germination. Decline in vigour indices like germination rate, germination index, seedling vigour index was also noticed due to ageing in the present investigations (Table 1). Similar observations in relation to artificial ageing were noticed by Mc Donald (1999) in various crops. Studies conducted on per cent membrane injury in relation to ageing indicated that aged seeds failed to maintain stable membrane (Table 2). With increase in ageing duration from 3 to 15 days marked increase in membrane injury from 20.5–34.5% was noticed when compared to fresh (16.8%). In support of our results, Panobianco and Marcos-filho (1998) demonstrated that electrical conductivity, accelerated ageing and controlled deterioration tests were more efficient in detecting differences among physiological quality levels of the *C. annuum* seeds. Malonaldehyde content, as a measure of lipid peroxidation, was also investigated in the present experiment. Higher and progressive increase in malonaldehyde content from 1.27 to 1.60 with increasing in ageing period from 3 to 15 days was noticed against 1.16 (mM/100 g fresh weight) of fresh seeds (Table 2). These volatile aldehyde products may be a consequence of lipid peroxidation, which is mediated by free radicals and lipoxygenase enzymes. Among the enzymes studied, amylases are the ones that are affected at higher rate due to ageing. At the initial increment of ageing duration, i e 3days after accelerated ageing around 50% drop in activity was observed. Dehydrogenases followed similar trend and the per cent reductions due to ageing for 3,6,9,12 and 15 days

Table 1 Physiological changes in relation to seed quality in aged and fresh bell pepper seeds

Treatment	Per cent germination	Germination index	Germination rate	Standard germination	Total seedling length (cm)	Seedling vigour index
Fresh	100 (89.8)	115	21.4	200	26.6	2660
3 DAA	69.0 (56.0)	66.7	14.8	138	20.7	1432
6 DAA	45.0 (42.0)	30.7	5.79	63.0	17.6	788
9 DAA	12.0 (20.1)	5.67	1.71	16.0	3.40	39.6
12 DAA	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00
15 DAA	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00
SEd±	2.62 (1.73)	1.54	0.36	3.61	0.94	88.9
CD (*P*=0.01)	8.02 (5.30)	4.70	1.11	11.0	2.86	271.6

*values in parenthesis represent angular transformed values; DAA, days after accelerated ageing

Table 2 Biochemical changes in relation to seed quality in aged and fresh bell pepper seeds

Treatment	Per cent membrane injury	Total water soluble sugars (µg glucose equivalent/ml)	Malonaldehyde content (mM/100 gr.fwt)	Amylase activity (µg maltose released/ml/mt)	Dehydrogenase activity (O.D values)	Peroxidase activity (ΔO.D/min/ml)
Fresh	16.8	126	1.16	42.3	0.87	0.18
3 DAA	20.5	131	1.27	24.5	0.57	0.21
6 DAA	25.3	133	1.43	6.61	0.41	0.24
9 DAA	29.8	149	1.58	1.83	0.17	0.09
12 DAA	32.3	155	1.62	0.48	0.04	0.02
15 DAA	34.5	162	1.60	0.00	0.00	0.02
SEd±	1.00	10.95	0.05	1.57	0.029	0.030
CD (*P*=0.01)	3.10	33.48	0.15	4.81	0.088	0.093

DAA, Days after accelerated ageing

Fig 1 Schematic diagram showing alteration in protein profiles of aged and fresh bell pepper seeds (DAA, days after accelerated ageing)

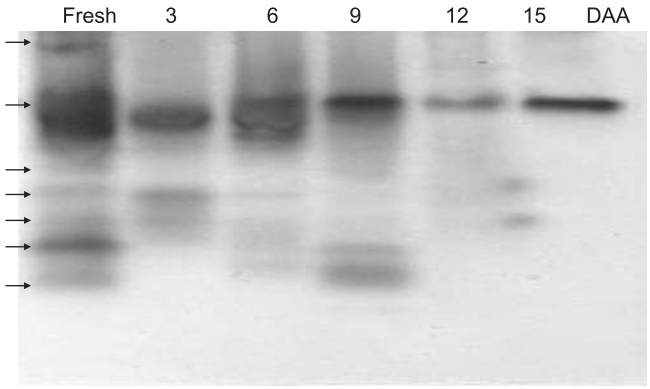

Fig 2 Zymogram showing variation in esterase isozymes in aged and fresh bell pepper seeds; (DAA, days after accelerated ageing)

was 34.5,52.8,80.5,95.4 and 100, respectively, over fresh. The activity of peroxidase unaltered till 9 days after accelerated ageing but declined at 12 and 15 days after accelerated ageing (Table 2). Ageing induced deterioration increases the extent of protein oxidation thus inducing loss of functional properties of proteins and enzymes (Loýc Rajjou *et al*. 2008).

Alteration in protein profiles due to ageing was noticed (Fig 1). Aged seeds exhibited lesser number of protein bands (3–7) as compared to fresh seeds (9). Proteins with low mobility, i e high molecular weight proteins (band 1 and 2) were present only in fresh seeds and disappeared upon ageing. Similarly proteins of intermediate mobility (band 3 and 7) were seen up to 9 days of ageing but disappeared later. Band 6 was absent only in 15 days after accelerated ageing aged seeds. Further, in the present investigation, appearance of one more protein (band 8) was noticed from 6 days after accelerated ageing period (Fig 1). These lower molecular weight protein bands might have been produced as a result of of the breakdown of the higher molecular weight band or may be synthesized in response to heat shock during ageing as reported by Varier and Dadlani (1992) in cotton. Since, specific bands are characteristic of particular ageing treatment having different seed viability levels, these profiles can be used as criteria in assessing the seed quality. Electrophoretic variations in proteins and enzymes can be used to assay the amount of deterioration of the seed during ageing.

Esterases are necessary for the breakdown of storage lipids into fatty acid moieties, which provide the biosynthetic energy necessary for seedling growth. Alteration in isozyme profiles noticed in aged seeds when compared to fresh indicate that the pattern of enzyme polymorphism changes during seed ageing (Fig 2). Seeds aged for 15 days exhibited disappearance of entire isozyme profile except retaining only one band. These amply demonstrate that there is qualitative change in enzymes of aged seeds and these changes lead to seed deterioration. The hypothesis that low quality seeds could not synthesize more denovo esterases than high quality seeds appear tenable. However, appearance of extra bands was noticed in 6 and 9 days aged seeds in contrast to fresh seeds. This might be due to breakdown of high molecular weight proteins or new isoforms appeared during ageing (Fig 2). In similarity to our findings, Varier and Dadlani (1992) observed disappearance of a prominent band and appearance of two new bands in aged cotton seeds. Precise identification of these isoforms has been unclear as some may result from expression of different genes and others from the post translation modification of products of single genes as reported by Bing Mo and Koster (2006) in their studies on changes in isoforms during pea seed germination.

In conclusion, seed ageing affected various physiological and biochemical events essential to maintain seed viability and vigour. Accelerated ageing test can be used as a successful technique to obtain seed lots of different quality levels thereby the reasons associated with loss in seed quality could be explored. Studies on protein profiles and isozymes in aged seeds further substantiated the fact that the loss in seed quality might be due to adverse effect of ageing on proteins. The electrophoretic variations in proteins and isozymes noticed in the present study could be used as criteria in assessing the quality of bell pepper seeds.

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