

## Changes in Antioxidant Enzymes during Ageing of Onion Seeds

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### Abstract

Onion seed viability during storage and drying declines with genotype specificity. Changes in antioxidant enzymes at different viability levels were investigated in seeds of the three onion cultivars 'Akgün-12', 'Valencia' and 'TEG-502'. For this purpose, 95, 80, 60, 40 and 20% viability levels were obtained through the use of controlled deterioration treatments in seeds of each onion cultivar. Subsequently, catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activity measurements were conducted at these viability levels. POX activity was below the detection limit in aged onion seeds. The activities of CAT and SOD decreased due to seed ageing in each cultivar. The decreases observed in CAT and SOD activities were more evident when seed viability decreased below a 60% level. Moreover, a high level of correlation was found between the loss of seed viability and the decreases that occurred in CAT and SOD activities, in the seeds.

**Keywords:** *Allium cepa* L., seed, controlled deterioration, viability, catalase, superoxide dismutase

### Introduction

Seed viability is a matter of great concern and measures to maintain germination efficiency of stored seeds have significant economical implications. A range of pre-harvest, harvest and post-harvest conditions affect seed viability (Basu, 1995). On the other hand, seed ageing which is linked to loss of seed viability with time is inevitable and the best that can be done is to lower its rate (Coolbear, 1995). Many factors contribute to seed ageing. These include genetics, mechanical damage, relative humidity and temperature of the storage environment, seed water content, presence of microflora, seed maturity, etc. The rate of loss of seed viability is mainly a function of temperature and seed moisture content (McDonald, 1999; 2004). During ageing, seed viability and vigour decrease. Furthermore, the losses of viability and vigour in seeds differ with species and cultivars.

Many hypotheses have been proposed regarding causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (McDonald, 1999; Murthy *et al.*, 2003; Priestley, 1986; Smith and Berjak, 1995; Walters, 1998). Degradation and inactivation of enzymes due to changes in their macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds (Bailly, 2004; Basavarajappa *et al.*, 1991; Basra and Malik, 1994; Goel *et al.*, 2002; Kalpana and Rao, 1993; Lehner *et al.*, 2008; McDonald, 2004; Salama and Pearce, 1993). Most of these studies suggest that decreases occur in the activity of en-

zymes such as superoxide dismutase, catalase, peroxidase and glutathione reductase in aged seeds. The general decrease in enzyme activity in the seed lowers the respiratory capacity, which in turn lowers both the energy (ATP) and assimilates supply of the germinating seed. Therefore, several changes in the enzyme macromolecular structure may contribute to their lowered germination efficiency.

The objective of the present work was to determine possible changes in antioxidant enzymes occurring due to seed ageing in seeds of onion cultivars 'Akgün-12', 'Valencia' and 'TEG-502'.

### Materials and methods

Seeds of onion: cv. 'Akgün-12' (obtained from Atatürk Central Horticultural Research Institute in Yalova, Turkey) and cvs. 'Valencia' and 'TEG-502' (obtained from MayAgro Seed Corporation in Bursa, Turkey) were used in the present study. In preparation of the experimentation, it was aimed that the seed moisture contents of different onion cultivars would be as close as possible. Thus, among the various parameters that affect germination loss the seed moisture content was adjusted. Seeds of each cultivar were placed in a separate tray and spread to form a single layer. Then the trays were placed in a climate chamber running at 20°C with 50±5% relative humidity for 14 days and the seed moisture content of each cultivar was balanced to ~ 8.4%.

The seed moisture contents were determined using the Low Constant Temperature Oven Method, after drying at 103±2°C for 17 hours (ISTA, 2007). Controlled dete-

rioration treatments, at  $60 \pm 1^\circ\text{C}$ , were conducted in order to lower the seed viability. The percentage of germination efficiency ranged from ca. 95% to 80, 60, 40 and 20% for each onion cultivar. Germination tests were conducted at  $20 \pm 1^\circ\text{C}$  by placing the seeds in Petri dishes with moist filter papers, as described in ISTA Rules (ISTA, 2007) with the modification of four 50-seed replicates instead of four replicates of 100 seeds. Germination test results were recorded daily until day 21.

The procedures of the enzyme analyses were followed as prescribed: by Janda *et al.* (1999) for catalase (CAT), by Al *et al.* (1995) for peroxidase (POX) and by Beyer and Fridovich (1987) for superoxide dismutase (SOD). CAT and POX activities were expressed as  $\text{mmol min}^{-1} \text{g}^{-1}$  while one unit of SOD was defined as that being present in the volume of extract that caused inhibition of the photo-reduction of nitroblue tetrazolium (NBT) by 50%. The protein content of each extract was determined using the "Bio-Rad Laboratories Method 1994" and the enzyme activity was based on the protein amount. Measurements were performed three times with each extract.

The data were statistically evaluated by ANOVA and correlation analyses were conducted through the use of "SPSS 13.0 for Windows" statistics programme. Moreover, LSD tests were also used for mean separation.

## Results and discussion

In order to relate antioxidant capacity to germination efficiency, catalase activity was determined in seed extracts from different viability levels. CAT activity of onion cultivars 'Akgün-12', 'Valencia' and 'TEG-502' changed with

ageing significantly ( $P < 0.05$ ). CAT activity decrease, correlated with the decrease in seed viability (Tab. 1A). This decrease was particularly pronounced below the 60% viability for the seeds of each onion cultivar. The general trend of the results is in agreement with the studies of Saxena *et al.* (1985) on sesame seeds, Basra and Malik (1994) on onion seeds, Bailly *et al.* (1998, 2002) on sunflower seeds, Goel *et al.* (2002) on cotton seeds, and Zeng *et al.* (2004) on cucumber seeds. However, the activity differences between the cultivars are highly interesting (Tab. 1B) and provide novel information. The most significant decline was observed for the cultivar 'Valencia', while the initial decrease was similar for 'Akgün-12' and 'TEG-502' until 60% viability and more rapid in 'TEG-502' during further ageing.

The effects of different seed viability on superoxide dismutase (SOD) activity of onion cultivars 'Akgün-12', 'Valencia' and 'TEG-502' were also significant ( $P < 0.05$ ). SOD activity also decreased along with the decrease in seed viability level. Similar to that of catalase, the decrease was more pronounced below the 60% viability for seeds of each onion cultivar (Tab. 2A). These results are in line with previous findings of Goel *et al.* (2002) working with cotton seeds and Zeng *et al.* (2004) working with cucumber seeds. The relative decrease was the strongest for cv. 'Valencia' and similar for 'Akgün-12' and 'TEG-502'. At 20% seed viability residual catalase activity has dropped to 29.3% in cv. 'Valencia', but is still placed at almost twice the activity in 'TEG-502' and 'Akgün-12' (Tab. 2B).

Peroxidase activity in onion seeds was found to be very low thus close to the detection limit. Embryos of the onion seeds were detached in order to control if they contain

Tab. 1. Changes in catalase (CAT) activity ( $\text{mmol min}^{-1} \text{g}^{-1}$ ) due to seed ageing in onion cvs. 'Akgün-12', 'Valencia' and 'TEG-502'

A				B			
Germination (%)	CAT activity in onion cultivar extracts			Germination (%)	CAT activity in onion cultivar extracts (%)		
	'Akgün-12'	'Valencia'	'TEG-502'		'Akgün-12'	'Valencia'	'TEG-502'
95	1.54 <sup>a</sup>	0.98 <sup>a</sup>	1.23 <sup>a</sup>	95	100.0	100.0	100.0
80	1.32 <sup>b</sup>	0.47 <sup>b</sup>	1.13 <sup>a</sup>	80	85.5	48.4	91.9
60	1.06 <sup>c</sup>	0.38 <sup>b</sup>	0.86 <sup>b</sup>	60	68.8	38.5	69.3
40	1.07 <sup>c</sup>	0.16 <sup>c</sup>	0.66 <sup>bc</sup>	40	69.3	16.8	53.3
20	1.03 <sup>c</sup>	0.18 <sup>c</sup>	0.43 <sup>c</sup>	20	66.8	17.9	35.0

Values not associated with the same letter are significantly different at  $p < 0.05$  (A); in (B), the data were normalized to the 95% value and expressed in percent

Tab. 2. Changes in superoxide dismutase (SOD) activity (unit) due to seed ageing in onion cvs. 'Akgün-12', 'Valencia' and 'TEG-502'

A				B			
Germination (%)	SOD activity in onion cultivars			Germination (%)	SOD activity in onion cultivars (%)		
	'Akgün-12'	'Valencia'	'TEG-502'		'Akgün-12'	'Valencia'	'TEG-502'
95	1.39 <sup>a</sup>	0.75 <sup>a</sup>	1.64 <sup>a</sup>	95	100.0	100.0	100.0
80	1.37 <sup>a</sup>	0.65 <sup>ab</sup>	1.37 <sup>b</sup>	80	98.6	86.7	83.5
60	1.02 <sup>ab</sup>	0.49 <sup>bc</sup>	1.31 <sup>b</sup>	60	73.4	65.3	79.9
40	0.95 <sup>b</sup>	0.31 <sup>cd</sup>	1.06 <sup>c</sup>	40	68.3	41.3	64.6
20	0.67 <sup>b</sup>	0.22 <sup>d</sup>	0.93 <sup>d</sup>	20	48.2	29.3	56.7

Values not associated with the same letter are significantly different ( $P < 0.05$ ) (A); in (B) the data were normalized to the 95% value and expressed in percent

Tab. 3. Correlation matrices of germination percentage (GP), catalase (CAT) and superoxide dismutase (SOD) activities in seeds of onion cvs. 'Akgün-12', 'Valencia' and 'TEG-502'

'Akgün-12'			'Valencia'				'TEG-502'			
	GP	CAT	SOD	GP	GP	CAT	SOD	GP	CAT	SOD
GP	1.00			GP	1.00			GP	1.00	
CAT	0.810*	1.00		CAT	0.879*	1.00		CAT	0.933*	1.00
SOD	0.829*	0.662*	1.00	SOD	0.866*	0.732*	1.00	SOD	0.914*	0.790*

\* Significant at P<0.01 level.

measurable peroxidase activity. Peroxidase activity was found to be close to detection limit in extracts prepared from isolated embryos. Consequently, correlation matrices of peroxidase activity could not be accomplished.

Tab. 3 shows a high level of positive correlation between germination percentage and CAT and SOD activities in seeds of onion cultivars 'Akgün-12', 'Valencia' and 'TEG-502'. In other words, an eminent relationship was found between viability loss and CAT and SOD disintegration. These results are parallel to those of the Goel *et al.* (2002) in cotton seeds.

Results obtained from this study show similarities with the results of the ageing model of onion seeds developed by Blackman and Leopold (1993). According to this model, ageing coincides with protein denaturation and degradation, inactivation of enzymes, breakdown of phospholipids and depository lipids, lipid peroxidation and alteration of membrane permeability. SOD and CAT activities in onion seeds are observed to decrease with ageing in the present study. These results support the hypothesis of Bailly *et al.* (1996) that a decrease in antioxidant enzymes is linked to an increased lipid peroxidation and accelerated ageing. Subsequently, Bailly *et al.* (2000, 2002) proposed a positive relationship between antioxidant enzyme capacity and the vigour of the seed.

### Conclusions

Consequently, our results support the hypothesis of inactivation of free radical scavenging enzymes (*i.e.*, SOD and CAT) during ageing and showed a direct relationship with the germination efficiency of ageing onion seeds. Moreover, our results provide convincing evidence that inactivation of these enzymes strongly increases below the 60% viability level in onion seeds. During storage, free radicals may be formed in the presence of even traces of oxygen. Initially, unsaturated fatty acid moieties get decomposed to form fatty acid hydroperoxide followed by hemolytic breakdown into alkoxyl radicals (Hopin *et al.*, 1996). In the absence of active enzymes scavenging free radicals, degradation products of thermo-labile lipid peroxidation accumulate in the ageing seeds, finally resulting in complete loss of seed viability (Rao *et al.*, 2006).

Our studies are in progress regarding the effects of post-storage priming and humidification treatments on

lipid peroxidation and antioxidant enzyme activity during germination and stand establishment of onion seeds.

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