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Original Research Article

Effect of Seed Priming on Antioxidant Enzyme Activity of Spinach (*Spinacia oleracea* L.)

A. K. Kulsumbi[1*], I. M. Sangeeta[1], N. M. Shakuntala[1], S. N. Vasudevan[2] and B. Kisan[3]

[1]Department of Seed Science and Technology, AC, UASR, India
[2]ADR, ZARS, VC Farm, Mandya, India
[3]Department of Biotechnology, MARS, UASR, India
**Corresponding author*

A B S T R A C T

Enzymatic antioxidants work as detoxifying mechanism which curbs the radicals generated at times of stress thus preventing seed from damage or deterioration. Antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule. This includes compounds of a non-enzymatic as well as an enzymatic nature. The present investigation was undertaken to evaluate the effect of priming on antioxidant enzyme activity of spinach. The experiment was laid down in Completely Randomized Design (CRD) with three replications. The seeds were exposed to different priming agents which include 11 treatments primed for 24 hours with seed to solution 1:2 vol/vol ratio. The results showed KNO_3 (1%) to be the best priming treatment to get the maximum antioxidants enzyme activity than compared to control and other treatments.

Keywords

spinach (*Spinacia oleracea* L.), Seed priming, Antioxidant enzymes

Introduction

Spinach (*Spinacia oleracea* L.) is an edible flowering plant in the family Amaranthaceae. Spinach is most probably a native of central and western Asia region. It was known in China as early as 647 AD. Spinach, swiss chard and garden beet has a chromosome number 2n=2x=24, indicates their close relationship. Leaves of this crop might have been first used in Bengal and hence it is known as *Beta vulgaris* var. bengalensis. Spinach is one of the most common leafy vegetables of tropical and subtropical regions. The popular spinach growing states include Uttar Pradesh, West Bengal, Maharashtra and Gujarat. However, spinach is not very popular in South India. It is primarily used as pot herb and is a rich Source of vitamin A and C and also contains appreciable amount of protein, calcium and iron. The leaves contain low oxalic acid.

Antioxidants are the substances that are present in plants or in seeds at lower concentration compared to that of oxidizable substrates, significantly delays or prevent oxidation of substrates. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules (Saisanthosh *et al.*, 2018).

Antioxidant enzymes *e.g*., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reaction. Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and other low molecular weight compounds such as glutathione and lipoic acid are involved in neutralizing free radicals.

Reactive oxygen species (ROS) occur in tissues and cells and can damage DNA, proteins, carbohydrates and lipids. The ROS comprises both free radical (O_2-, superoxide radicals; OH^- hydroxyl radical; HO_2^-, perhydroxy radical and RO^-, alloxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and $1O_2$, singlet oxygen). These deleterious reactions are controlled in part by antioxidants that eliminate ROS and scavenge free radicals. Various abiotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. Seed priming methods have been used to increase germination characteristics under stress conditions. The beneficial effects of seed priming are associated with different physiological and biochemical changes (Govindaraj *et al.,* 2017).

Priming is a pre-sowing seed treatment which permits early DNA replication, increase RNA and protein synthesis, repairs deteriorated seed parts and reduces the leakage of metabolites thus enhances the embryo growth, speed and uniformity of seedlings in field. Primed and dried seeds normally have a more rapid and uniform germination when subsequently re-hydrated, especially under adverse environmental conditions.

Hence, present studies were undertaken to determine the "Effect of seed priming on antioxidant enzyme activity of spinach (*Spinacia oleracea* L.)".

Materials and Methods

The research studies were carried out in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur. Geographically, the station is situated in the North-Eastern dry zone (Zone-2) of Karnataka State at 16° 15' North latitude and 77° 20' East longitude and at an altitude of 389 metre above mean sea level. Fresh seeds of spinach variety "Annapoorna" were obtained from University of Horticultural Sciences, Bagalkot. Spinach seeds were primed with different priming agents with seed to solution ratio of 1:2 and then seeds were dried back to their original moisture content. Later the treated seeds were used to assess the antioxidant enzyme activity *viz.*, Catalase and Peroxidase.

Catalase enzyme activity (CAT)

Catalase enzyme activity was measured following the procedure of Aebi (1984) at 25 °C with some minor modifications. The ultra violet (UV) light absorbance of hydrogen peroxide solution can be measured between 230 and 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. One gram of decoated seeds were homogenized in 15 ml of 100 mM Potassium phosphate buffer (pH 7.8) with a pinch of Poly Vinyl Pyrrolidone (PVP). The extract was centrifuged at 15000 rpm for 10 minutes at 4 °C. The supernatant was collected and used as enzyme extract. Three ml of reaction mixture contained 50 µl distilled water. The control contained enzyme extract and phosphate buffer devoid of H_2O_2. Catalase activity was estimated by the decrease in absorbance of H_2O_2 at 240 nm and expressed as µmol H_2O_2

decomposing/min/g. One unit of enzymatic activity was defined as amount that decomposes 1 µM hydrogen peroxide.

Peroxidase enzyme activity (POD)

Peroxidase enzyme activity was assayed, as increase in optical density due to oxidation of guaiacol to tetra-guaiacol following Castillo *et al.,* (1994) with minor modifications at 470 nm absorbance using a reaction mixture containing 12 mM hydrogen peroxide and 96 mM guaiacol in Potassium phosphate buffer (pH 7.0). One gram of decoated seeds were homogenized in 15 ml of 100 mM Potassium phosphate buffer (pH 7.8) with a pinch of Poly Vinyl Pyrrolidone (PVP). The extract was centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was collected and used as enzyme extract. Three ml reaction mixture contained one ml 100 mM phosphate buffer (pH 7.0); 0.5 ml each 96 mM guaiacol; 12 mM H2O2; 50 µl enzyme extract and 950 µl distilled water. Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per the extinction co-efficient of its oxidation product, tetraguaiacol E=26.6 nM/cm. enzyme activity was expressed as µmoles/cm/min/g seed fresh weight. The mean data of the laboratory experiments were statistically analyzed by adopting appropriate statistical methods as outlined by Panse and Sukhatme (1985). The critical differences were calculated at one per cent level of probability wherever 'F' test was found significant for parameters under study.

Results and Discussion

Significantly highest catalase and peroxidase enzyme activity were observed in seeds treated with KNO_3 (1%) (481 and 630 nmol/g, respectively) (Fig. 1, Table 1 and 2) followed by GA_3 (100 ppm) (381 and 613 nmol/g, respectively). However significantly lowest was observed in control (169 and 267 nmol/g, respectively. In response to abiotic stresses, antioxidant enzymes like CAT and POD content increased their activity.

Furthermore, many reports demonstrated that the effect of abiotic stress is genotype specific where different genotypes showed different responses in the same stress condition. The antioxidant enzymes increased but considerable differences were noticed among the treatments with respect to different seed quality parameters, however it may depend on the tissue type, length and intensity of the stress as well as on developmental stage proving the complexity of the mechanisms of production and detoxification of ROS and the effect of ROS (oxidative stress) on antioxidant systems as it has also noticed in wheat as suggested by Slesak *et al.,* (2007). Peroxidases: Peroxidase catalyses the oxidation of a wide variety of electron donors with the help of H_2 and helps in scavenging the endogenous H_2O_2 (Mazumdar *et al.,* 1997). The peroxidase activity was positively associated with the germination percentage in maize (Oliver *et al.,* 1990). Lipid peroxidation produces highly reactive free radical intermediates that can damage membranes, proteins and nucleic acids and was observed to precede the loss of viability in maize (Leprince *et al.,* 1990). A decrease in peroxidase activity was probably associated with lipid peroxidation (Li and Sun, 1999 in cocoa).

Catalase

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals) which catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS).

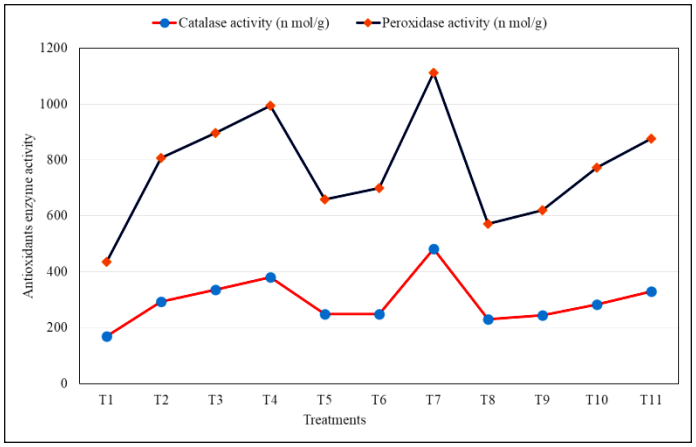
Table.1 Different priming treatments

Treatment No.	Treatment type
T_1	Control
T_2	Hydropriming
T_3	GA_3 50ppm
T_4	GA_3 100ppm
T_5	Ethrel 100ppm
T_6	Ethrel 150ppm
T_7	KNO_3 1%
T_8	Coconut water 50%
T_9	Coconut water 100%
T_{10}	Custard apple 3%
T_{11}	Custard apple 6%

Table.2 Effect of seed priming on antioxidant enzyme activity

Treatments	Catalase activity (n mol/g)	Peroxidase activity (n mol/g)
T_1- Control	169	267
T_2- Hydropriming	292	516
T_3-GA_3 50ppm	335	561
T_4-GA_3 100ppm	381	613
T_5- Ethrel 100ppm	248	410
T_6- Ethrel 150ppm	249	451
T_7-KNO_3 1%	481	630
T_8- Coconut water 50%	229	343
T_9- Coconut water 100%	245	375
T_{10}- Custard apple 3%	283	490
T_{11}- Custard apple 6%	329	547
MEAN	295	473

Fig.1 Effect of seed priming treatments on catalase and peroxidase enzyme activity (nmol/g)



Catalase, one of the most efficient protein catalysts that promote the redox reaction, is localized in the peroxisomes or the microperoxisomes. It catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and OH^- (Bandopadhyay *et al.*, 1999). Catalases are good scavenging enzymes involved in free radical mechanism on lipid peroxidation and protects the mitochondrial components from oxidative damage (Chander and Kapoor, 1990). Decreased catalase activity was associated with ageing, accompanied by an increase in lipid peroxidation and loss of vigour and viability in maize (Oliver *et al.*, 1990), sunflower (Bailly *et al.*, 2002). Stimulation of catalase activity during germination has been reported in seeds of soybean (Gidrol *et al.*, 1994) and maize (Guan and Scandalios, 2002). Hence catalase deficit serves as one of the important markers of oxidative damage.

Significantly highest catalase and peroxidase antioxidant enzyme activity was observed in the seeds primed with KNO_3 at 1 per cent (481 and 630 n mol/g, respectively) followed by the treatments GA_3 at 100 ppm (T4) (381 and 613 n mol/g, respectively) and GA_3 at 50 ppm (T3) (335 and 561 n mol/g, respectively). Whereas, significantly lowest was recorded in control (169 and 267 n mol/g, respectively). Hence, seed priming with KNO_3 1 per cent proved to be the best for improving the activity of antioxidants *viz*., catalase and peroxidase, which in turn strengthens the seed defense against cell membrane damage and seed ageing.

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