Legume Research, 40 (2) 2017 : 313-318
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.legumeresearch.in

Effect of foliar application of growth activator, promoter and antioxidant on seed quality of soybean

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Received: 18-08-2015 Accepted: 21-01-2016 DOI:10.18805/lr.v0iOF.11302

ABSTRACT

Loss of seed quality in soybean is a serious problem in tropical and subtropical region. Foliar application of salicylic acid (SA), GA_3 and acetyl salicylic acid (50 ppm) at seedling and pod filling stage, K_2HPO_4 (2%), α-tocopherol (100 ppm) at flowering and pod filling stage was done on soybean varieties – NRC 7 and JS 335. Foliar application of SA showed positive effect on seed yield, seed health, germination and seed vigour during storage. Plant height was significantly improved by the GA_3 spray in both the varieties. Significantly higher seed yield was obtained with foliar spray of α-tocopherol. Application of SA was found effective to protect plants from various diseases to get quality seed. α-Tocopherol and K_2HPO_4 treatment significantly reduced MDA production in seeds. α-Tocopherol application increased super oxide dismutase activity as a result the lipid peroxidation of seed during storage was significantly reduced. Application of α-tocopherol, K_2HPO_4 and salicylic acid improved the storage or keeping quality of soybean seed.

Key words: Antioxidant, Foliar application, Salicylic acid, Soybean.

INTRODUCTION

Soybean is a cheaper source of quality protein and edible oil. Although most of soybean is grown in temperate region, there is tremendous potential to expand its production in the tropics. However, one of the major problems encountered in soybean production in tropical and sub tropical region is rapid deterioration of seed quality during storage, leading to poor germination, weak seedling and sub optimal plant stand. Quality of seed depends on how the seed plant grows and post harvest management. Seed longevity, an inherited property had been reported to vary in high yielding Indian varieties due to structural properties of seed coat (Kuchlan *et al*, 2010) and loss of viability is closely associated with the seed deterioration due to loss of membrane integrity (Basavarajappa *et al*., 1991), reduction in enzyme activities (Bailly *et al*., 1998) and peroxidative changes in polyunsaturated fatty acid. High levels of endogenous SA have been identified as an important factor in the acquired systemic resistance (ASR) in several species (Shah, 2003). Application of exogenous salicylic acid at non-toxic concentration to plants has been shown to be effective in the regulation on number of process such as biotic and abiotic stresses (Xu and Tian, 2008). SOD and catalase are antioxidant enzymes which protect seeds from peroxidative deterioration (Wilson and McDonald, 1986). Increase in plant growth, seed yield by α-tocopherol, ascorbic acid application in *Vicia fava* (Hala *et al*., 2005), by salicylic acid in *Vigna mungo* (Jeyakumar *et al*., 2008) and split doses of foliar applications of di-ammonium phosphate in chickpea (Singh and Singh, 2014) had been reported but effect on seed quality and germination after harvest and during storage for next crop is scanty. Therefore, present investigation was undertaken to incorporate seed protecting growth activator and antioxidant through foliar application to improve seed yield and seed quality of soybean.

MATERIALS AND METHODS

Experiment was conducted at Directorate of Soybean Research, Indore during 2010-2013. Experiment was sown in a randomized block design in three replications. Foliar application of salicylic acid (SA), GA_3 and acetyl salicylic acid (ASA) (50 ppm) was done at seedling and pod filling stage; K_2HPO_4 and α-tocopherol (100 ppm) at flowering and pod filling stage on two soybean varieties namely NRC 7 and JS 335. Observation on plant height was taken during R1 stage.

The germination of the seed was determined by 'Between Paper' method (Anonymous, 2009). Four replications of 100 seeds were placed between two layers of moist germination paper and incubated in a germinator at 25^0 C to take final count of germination was on 7^{th} day.

Electrical conductivity was measured by the methods given by Alison and Stan (1986). 5g seeds were soaked in 25 ml deionized water at 25^0 C for 17 hours. The electrical conductance of the leachate was measured at room temperature with a conductivity bridge model (PCS Testr 35, Eutech Instrument).

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Vigour Index-I ((V.I.) was calculated by using the formula,

Vigour Index-I = germination percentage x seedling length (cm).

Malondialdehyde (MDA) was estimated following Cakmak and Horst, (1991) protocol. Weighted10 seeds soaked for 17-18 hours in 25^{0}C were homogenized after removal of seed coat in 7 ml distilled water and incubated at 95^0 C for 30 minute after addition of 7 ml of 0.5% TBA-TCA reagent. The absorbance of supernatant centrifuged at 5000xg for 10 min was measured at (w) 535 nm and (s) 600 nm. Difference in OD values measured at 535 nm and 600 nm was used to compare the effect of treatments.

Super oxide dismutase activity was assayed according to Bailly *et al*. (1996). 1 g defatted seed flour was homogenized in 1.5 ml of 50 mM potassium phosphate buffer (pH 7.5) and centrifuged at 15000 x g for 10 min at 4^{0}C. 12 µl of supernatant which acted as enzyme extract was added to a mixture of 1 ml of 50 mM potassium phosphate buffer (pH 7.5); 100 µl of 2.25 mM NBT, 100µl of 3 mM EDTA, 200 µl of 200mM L-methionine and 1.938 ml distilled water, and 150 µl of 0.075 mM riboflavin. The test tubes were placed at a distance of 30 cm away from 15 W fluorescent lamps for 6 min. Reaction mixture without enzyme developed maximum color and was used as control. The absorbance was recorded at 560 nm. A 50% inhibition in enzyme activity was calculated as unit of SOD. The activity of enzymes was calculated using the following formula

$$\frac{\text{(OD value of control-OD value of sample) x dilution factor/time}}{\text{(OD value of sample – OD value of blank)}}$$

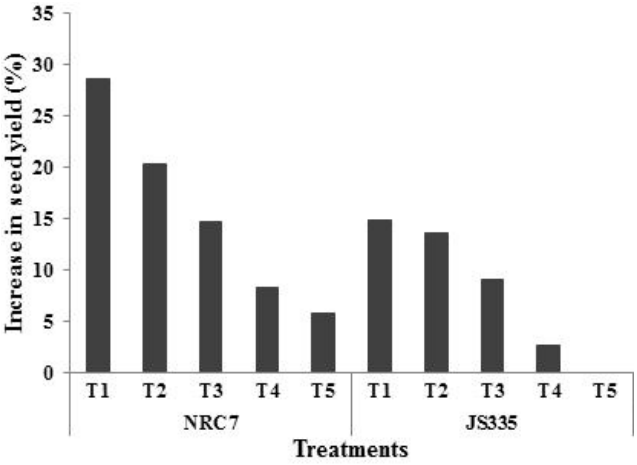
Seed heath of harvested seed were tested by agar plate method to find out seed borne pathogens (infection/ inoculums) present in the seed.100 seeds were used for seed health testing as 10 surface sterilized seeds were placed in each Petri plates of 10 cm diameter containing potato dextrose agar. After 7 days of incubation at 25^{0}C the plates were observed under stereo binocular microscope.

RESULTS AND DISCUSSION

The application of growth promoter had influence on the plant height. Exogenous application of GA_3 significantly improved plant height in both NRC 7 and JS 335 as compared to unsprayed plants (Fig1) (Table 1). Highest plant height (56.8cm) was noted with 50ppm GA_3 application and in control it was 44.4cm. Verma and Sen (2008) found enhanced growth with foliar application of GA_3 and NAA in coriander. Reuveni *et al.* (1993) reported that a single spray of K_2HP0_4 on leaf stage applied 2 or 4 days before disease inoculation, stimulated plant growth. α-Tocopherol and salicylic acid treatment significantly ($P \leq 0.05$) increased mean seed yield (Table 1). Maximum increase in seed yield was obtained by α-tocopherol (100ppm) which was 28% and 14.8% over the control in NRC 7 and JS 335 respectively (Fig 1). Hasnaa *et al*., (2009) reported similar effect of α-Tocopherol with increase in yield in geranium. SA increased plant height, seed yield and seed protein content in black gram (*Vigna mungo*) (Jeyakumar *et al*. 2008; Ali and Mahmoud, 2012).

Table 1: Effect of foliar application of growth activator and promoter on plant height and seed yield in NRC 7 and JS 335

Treatments	Plant height (cm)		Seed yield /plot (kg)	
	NRC 7	JS 335	NRC 7	JS 335
SA	42.04	49.42	1.89	1.76
GA_3	56.21	56.77	1.66	1.55
K_2HPO_4	44.01	53.82	1.80	1.69
ASA	49.47	46.48	1.70	1.59
α-tocopherol	47.23	49.02	2.02	1.78
Control	43.71	44.41	1.57	1.55
CD(.05)	2.96	3.99	0.18	0.16

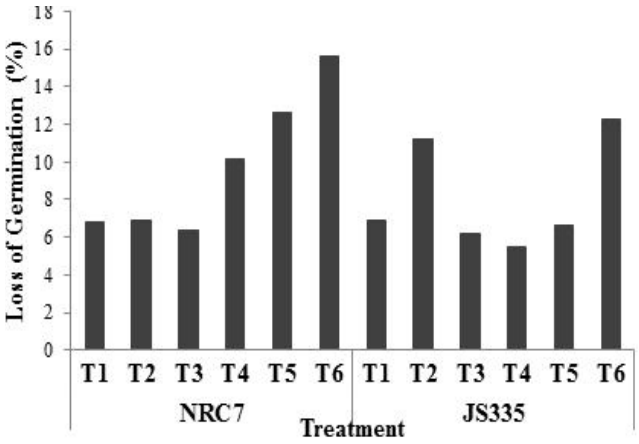


T1- α-Tocopherol, **T2**- SA, **T3**- K_2HPO_4, **T4**-ASA, **T5**-GA_3, **T6**- Control

Fig 1: Percent increase in seed yield over control as influenced by foliar application of growth activators and antioxidant

Reduction in seed germination during storage is a natural process. Effect of salicylic acid and antioxidant (α-Tocopherol) to minimize seed quality deterioration was very significant. Maximum loss of germination (12-15%) was observed in control after six month of storage. Reduction in seed germination level was minimum was with α-Tocopherol (6%) and K_2HPO_4 (7%) during storage (Fig 2). Alonso-Ramirez *et al*., (2009) reported exogenous application of 50µM SA was able to both revert the inhibitory effect of PCB on seed germination and improve germination of GA-deficient mutant *gal*-3. They also reported that addition of SA in the medium increased germination percentage up to 9%.

Seedling vigour is an important quality parameter which needs to be assessed to supplement germination and viability test to gain an insight in to the performance of a seed lot in the field or in storage. Effect of foliar spray of different growth activator and antioxidant were found with



T1- α-Tocopherol, **T2**- SA, **T3**- K_2HPO_4, **T4**-ASA ,**T5**-GA_3, **T6**- Control

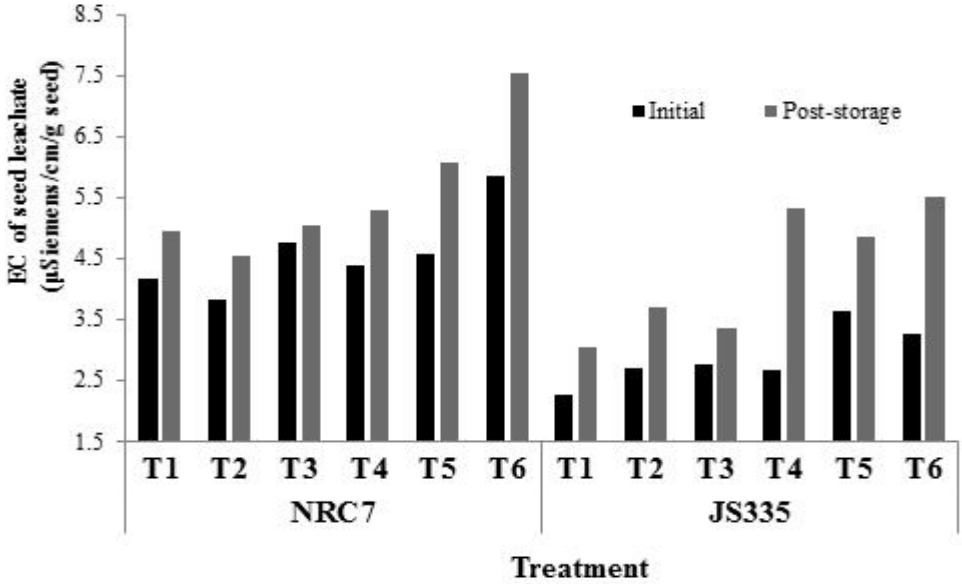
Fig 2: Storability of soybean seeds as influenced by foliar application of growth activator and antioxidant

higher seedling vigour before and after storage. Higher seed vigour (V.I. 2820) was found with the application of salicylic acid and lowest with the untreated control (V.I. 1827). After six month of storage the seed vigour was maximum with α-tocopherol (V.I. 1780) followed by salicylic acid (V.I. 1710) (Table 2). The reduction in deterioration of seedling vigour due to α-Tocopherol treatment could be attributed to the protection of polyunsaturated fatty acid of the cell membrane from oxidative damage.

The electrical conductivity of seed leachate is the indicator of seed membrane stability as well as the quality of seed. The foliar application of different chemicals on plant had significant effect on membrane integrity of soybean seeds which was reflected as lower electrical conductivity of seed harvested from α-tocopherol (2.55 µsiemens/cm/g seed), K_2HPO_4 (2.76) and salicylic acid (2.71) in the variety JS335 (Fig 3). Similar trend for these treatments was also found in case of NRC 7. There was a significant deterioration of seed membrane after six month of storage in all the treatments in both the varieties, but the rate of membrane degradation was lowest in α tocopherol (3.03µsiemens/cm/g seed) and K_2HPO_4 (3.34 µsiemens/cm/g seed) treatments compared to control (5.50 µsiemens/cm/g seed) (Fig 3). Foliar application of different chemicals on plant had significant effect on membrane integrity of soybean seeds which was reflected as lower electrical conductivity of seed leachate after storage.

Malondialdehyde (MDA), product of lipid peroxidation is an indicator of biochemical degradation of phospholipids of cell membrane and unit membrane of cell macromolecules. Higher the MDA content higher is the degree of seed deterioration. Antioxidant, α-tocopherol and K_2HPO_4 treatments significantly controlled the lipid peroxidation of cells thus protected it from biochemical degradation. MDA production of seeds harvested from α-tocopherol and K_2HPO_4 treated plots was significantly lower (61.4978 nM/g seed and 46.3834 nM/g seed) than the control (90.0868 nM/g seed) (Fig 4). α -tocopherol was proposed to function in relation to their antioxidant properties being prominent in protection of polyunsaturated fatty acids from lipid peroxidation (Munne-Bosch, 2005). Similarly Bassiouny *et al*. (2005) reported that lipid peroxidation was decreased with response to application of different concentration of α-tocopherol on *Vicia faba* L.

SOD is a critical component of the active oxygen scavenging system (Gupta *et al.*, 1993). Higher the SOD

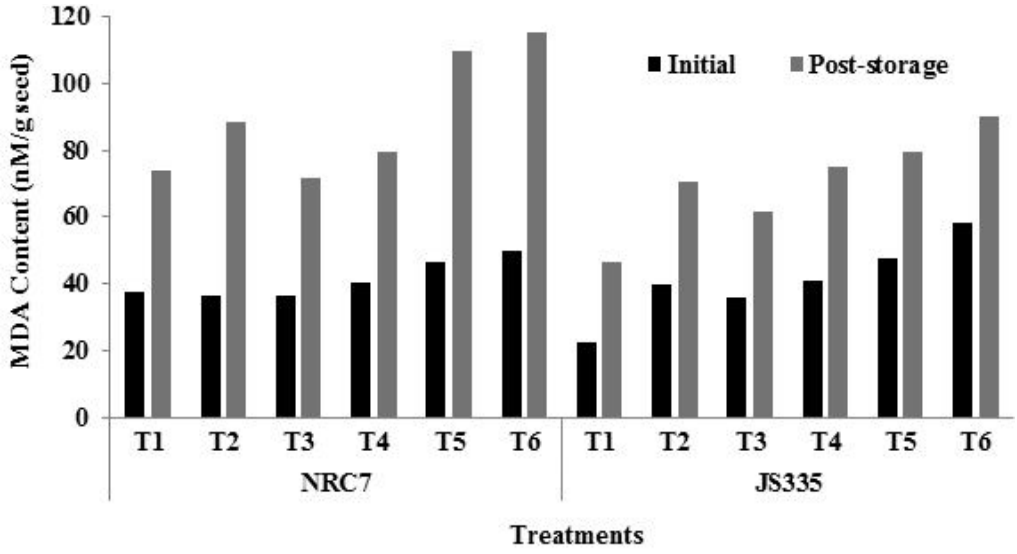


T1- α-Tocopherol, **T2**- SA, **T3**- K_2HPO_4, **T4**-ASA ,**T5**-GA_3, **T6**- Control

Fig 3: Electrical conductivity of seed leachate as influenced by the foliar application of growth activator and antioxidant

Table 2: Effect of foliar applications on post harvest seed germination and seed vigour in NRC 7 and JS 335

Treatments	NRC7				JS 335			
	Germination		Vigour Index		Germination		Vigour Index	
	Initial	Post storage	Initial	Post storage	Initial	Post storage	Initial	Post storage
SA	79.5	74.0	1872	1444	94.0	83.5	2820	1710
GA_3	79.0	69.0	1885	1071	90.0	84.0	2346	1322
K_2HPO_4	79.0	74.0	2184	1658	89.5	84.0	2186	1665
ASA	79.0	71.0	1703	1427	91.0	86.0	2194	1219
á-tocopherol	80.5	75.0	1896	1571	94.5	88.0	2198	1780
Control	73.5	62.0	1508.5	1101	85.5	75.0	1827	1230
CD at 5% t*d	7.81		347.89		13.65		460.96	



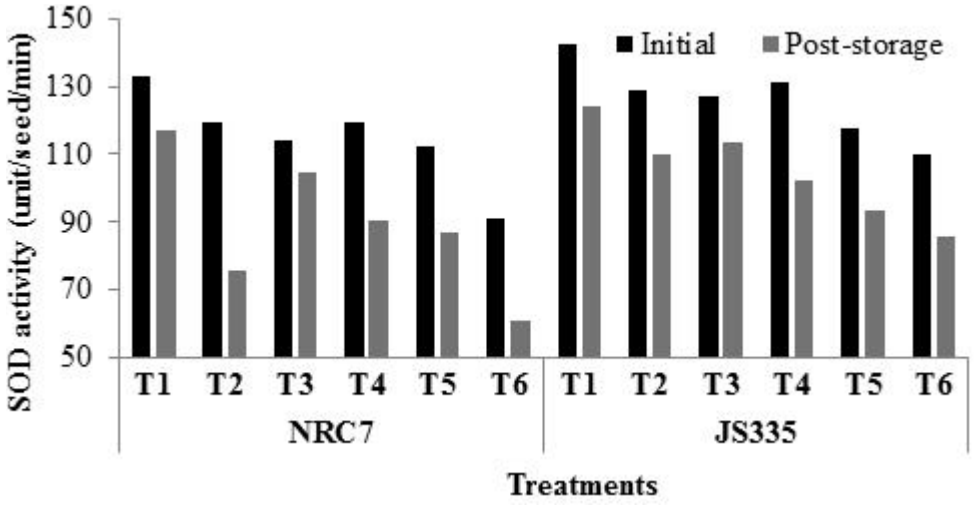
T1- α-Tocopherol, **T2**- SA, **T3**- K_2HPO_4, **T4**-ASA ,**T5**-GA_3, **T6**- Control

Fig 4: Lipid peroxidation(MDA content) during storage of seeds as influenced by the foliar application of growth activator and antioxidant

activity betters the seed quality. Application of α-tocopherol, K_2HPO_4 and salicylic acid improved the storage or keeping quality of soybean seed by maintaining higher SOD activity in both the varieties. α- Tocopherol treatment maintained maximum super oxide dismutase activity (124.4unit/g seed /min) as compared to control (85.69unit / g seed/min) after storage (Fig 5). α-Tocopherol is low molecular weight lipophillic antioxidant which mainly protect membrane from oxidative damage (Asada, 1999).). Lipid peroxidation of seeds during storage can be reduced by α-tocopherol spray.

It was found that among all the foliar treatments, salicylic spray treatment controlled the transmission of fungal pathogen to a great extent. In control plot's seed lot of NRC7, *Macrophomina phaseolina* (10%), *Fusarium sp* (17%) *and Cercospora kikuchi.* (5%) infections were observed, whereas with salicylic acid treatment less infection of *Macrophomina sp.* (0.5%) was found. In JS 335 control plots seed infection *of Fusarium sp.* (11%), *Cercospora sp.* (5%) and *Phomopsis sp*. (6%) was observed. SA reduced the infection level of *Cercospora sp.* and *Fusarium sp.* to 0.61 and 2.0% only respectively. Seed health test established the effect of salicylic acid for its systemic acquired resistance (Shah, 2003) and defence mechanism against fungal diseases. Manikandan and Sathiyabatma (2014) reported that SA treatment to the groundnut leaves induced defence protein in intercellular washing fluid and promotes resistance towards *Puccinia arachis*. Salicylic acid had been hypothesized to be a translocable signal compound in SIR (Yalpani *et al*., 1991).

There was a great prospect of application of SA to protect the seedling from field pathogens and production of healthy and quality seeds as well as better plant stand. Foliar application of α-tocopherol (100 ppm) at flowering and pod filling stage improved yield as well as storability or keeping quality of soybean seeds. Therefore, it would be of great advantage in the seed quality if seed production crop may be applied with SA and α-tocopherol as foliar application during specific crop stage.



T1- α-Tocopherol, **T2**- SA, **T3**- K_2HPO_4, **T4**-ASA ,**T5**-GA_3, **T6**- Control

Fig 5: Superoxide dismutase activity during storage of seeds as influenced by the foliar application of growth activator and antioxidant

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