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Spermine induced protection of onion seed vigour and viability during accelerated ageing

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Abstract

Aim : The aim of the present study was to investigate the role of spermine, a polyamine as a protective agent on accelerated ageing of onion seeds.

Methodology : Onion seeds variety Pusa Riddhi was primed with six concentrations of spermine (0.10 mM to 1.25 mM) and also with hydration and halopriming (2% K₂HPO₄). Hydrated, haloprimed and un-primed seeds were used as control. The primed and control seeds were accelerated aged at 45 °C and 100 % RH for 72 hr. Seed quality was assessed in control, freshly primed seeds, and in primed seeds subjected to accelerate ageing.

Results : All priming treatments enhanced the seed quality, there was 2.34–20.33 % increase in germination. Seed priming with 2% K₂HPO₄ had highest seed quality improvement which was at par with 1.25 mM spermine primed seeds. Enhanced seed vigour and the activity of antioxidant enzymes over un-primed seeds was observed in both 2% K₂HPO₄ and spermine primed seeds over unprimed seeds. Seeds primed with 1.25 mM spermine recorded 66.66 % and 650 % increase in the activity of ROS scavenging enzymes SOD and POD respectively, but they were at par with halopriming. After accelerated ageing, deterioration in seed quality was minimal in seeds primed with spermine. Seeds primed with 1.25 mM spermine recorded 21.33% higher germination, 62.10 % higher speed of germination, 26.56 % longer seedlings, 13.68 % heavier seedlings and 175 % and 200 % higher SOD and POD activity as compared to un-primed seeds. Seeds primed with 1.25 mM spermine also performed better over halopriming and hydropriming treatments.

Interpretation : Onion seed priming with 1.25 mM spermine was most effective treatment in enhancing the seed germination and vigour under accelerated ageing conditions.

Key words: *Allium cepa*, Halopriming, Seed priming, Seed vigour, Spermine

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Introduction

Quality seed is the basic input required to realize the potential productivity of any crop variety. The potential seed longevity of onion during storage is very low (Yalamalle and Kuchlan, 2016). Lipid peroxidation of unsaturated fatty acids in cell membrane is considered as one of the main reasons for loss of storability, which occurs due to fall in the level of antioxidants, reduced activity of ROS and peroxide scavenging enzymes and increased lipid per-oxidation vis-à-vis malondialdehyde content (Chiu *et al.*, 1995). Seed deterioration process reduces seed vigour. Low vigour seeds fail to emerge in field even under minimal stress or produce weak seedlings thus resulting in poor plant growth, and low yield. Seeds have complex system of antioxidant defence mechanism, which helps in overcoming lipid peroxidation and membrane degradation (Draganić *et al.*, 2011). Seed priming is controlled hydration process with the primary aim to improve the vigour, viability and to improve seedling performance under biotic and abiotic stresses (Afzal *et al.*, 2009; Tao *et al.*, 2018). Seed priming provides 'head start' to germinating seeds by prior activation of hydrolytic enzymes, reversion of ageing induced deterioration, repair of damaged protein, RNA and DNA (Taylor *et al.*, 1998; Varier *et al.*, 2010), activation of antioxidant enzymes (Bailly *et al.*, 2000; Chiu *et al.*, 2002). Polyamines (spermine, spermidine and putrescine) have dual role both as a source of ROS, however conjointly as ROS scavengers, and activators of key antioxidant enzymes (Velarde-Buendía *et al.*, 2012; Pottosin *et al.*, 2014). Several studies have reported the role of polyamines in managing the abiotic stress like osmotic and drought (Grzesiak *et al.*, 2013; Yin *et al.*, 2014); salinity and heavy metal toxicity (Gill and Tuteja, 2010; Hussain *et al.*, 2011). Seed priming with polyamines have been reported previously to enhance seed viability and quality but in these reports the effect of spermine was studied after subjecting the seeds to accelerated ageing or after storing the seeds for prolonged durations, but limited information is available how seeds primed with polyamine perform under accelerated ageing conditions. Several reports state that seed priming with polyamines enhance the seed quality but these study did not include the other proven priming techniques like hydro/halo/osmotic priming as control (Iqbal *et al.*, 2005; Farooq *et al.*, 2008; Afzal *et al.*, 2009; Khan *et al.*, 2012). There are also some contradictory reports which suggest that seed priming increases the level of endogenous polyamines (Basra *et al.*, 1994) whereas El-Araby and Hegazi (2004) reported decrease in endogenous levels of polyamine. Thus, in light of the contradictory reports and limited study on the effect of spermine on seed storability, the present study was conducted with the aim to find out how onion seed priming with spermine effects seed storability and quality under accelerated ageing conditions.

Materials and Methods

The present study was conducted at the Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi using seeds of onion variety Pusa Riddhi.

Freshly harvested seeds were allowed to age naturally for six months by storing under ambient condition in cloth bag. The moisture content of seed sample before treatment was around 8.1 %. The seed quality parameters were assessed as per ISTA guidelines (ISTA, 2015).

Seed priming: There were 9 treatments with seed priming agents, namely, spermine (0.10 mM, 0.25 mM, 0.50 mM, 0.75 mM, 1.0 mM and 1.25 mM); halopriming (2 % K_2HPO_4), hydropriming and un-primed seeds as control. Spermine 99 % gas chromatographic grade was sourced from Sigma-Aldrich, Co., (MO. USA) and K_2HPO_4 99 % analytical grade was sourced from HiMedia Laboratories (Mumbai, India). Five gram seeds were surface sterilized by immersing seeds in 5% sodium hypochlorite for 10 min followed by rinsing in distilled water for three times. Washed seeds were placed on a twin layer of Watman No.10 filter paper, pre-moistened in 10 ml solution of priming agent. There after petri plates were kept in dark in BOD incubator set at 15 °C for 24 hrs. After priming, the seeds were thoroughly washed with distilled water to remove the priming agents. Washed seeds were dried at ambient laboratory temperature (< 30 °C) for 72 hr till seeds had reached the initial moisture content (Jao *et al.*, 2018; Khan *et al.*, 2012).

Accelerated ageing: Accelerated ageing expose the seeds to two seed deteriorating variables *i.e.*, high temperature and high relative humidity, which cause rapid loss in seed vigour and viability. Seeds with high vigour deteriorate at a slower rate as compared to low vigour seeds. Onion seeds were accelerated aged following the method of Delouch and Baskin (1973). Five gram seeds were packed in muslin cloth bag and placed on a wire mesh in an air tight desiccator filled with demineralized water. Care was taken to ensure that seeds did not come in direct contact with water. A desiccator was placed in an incubator maintained at 45°C and 100 % RH for 72 hr. The accelerated ageing was done on control *i.e.*, hydroprimed, haloprimed (2 % K_2HPO_4), un-primed and spermine primed seeds.

Germination and seed vigour: Hundred seeds in three replication were placed on moistened Watman No. 10 filter paper in 15 cm petri plates and placed in walk-in germinator at 20 ± 1 °C for 12 days. Speed germination was calculated by following the formula (Czabator, 1962): Speed of germination = $\sum n1/d1 + n2/d2 + n3/d3 + \dots$, where, n is the number of germinated seeds; d is the number of days. A seed was scored germinated when root length had reached 2 mm. The final germination per cent was calculated based on the number of normal seedling on 12th day of germination. Ten normal seedlings were randomly selected at the final count, seedling length and seedling dry weight was calculated by drying seedling at 65 °C for 24 hrs. The seedling vigour index I and II were calculated as per Abdul-baki and Anderson (1973).

Estimation of ROS scavenging enzymes: SOD activity was estimated by the method of Dhindsa *et al.* (1981). One gram of onion seeds was homogenized with the help of liquid nitrogen in 6

ml of 50 mM phosphate buffer (pH 7.0). The extract was centrifuged at 16000 rpm for 20 min. A 3 ml of reaction mixture contained 100 µl enzyme, 50 mM phosphate buffer (pH 7.8), 75 µM NBT, 13 mM L-methionine, 0.1 mM EDTA and 0.5 mM riboflavin. The samples were illuminated for 20 min under 20 W fluorescent lamp. One unit of enzyme activity was defined as the amount of enzyme required for 50% inhibition of nitro blue tetrazolium at 560 nm using spectrophotometer (Shimadzu UV-1800 UV-Vis). Peroxidase activity (POD) was estimated by the method of Rao (1996). One gram seed was homogenized in liquid nitrogen in 6 ml 50 mM phosphate buffer (pH 7.0). The extract was centrifuged at 16000 rpm for 20 min, the supernatant was used for estimation of peroxidase, 3 ml reaction mixture consisting of 1 ml phosphate buffer (100 mM, pH 6.1), 0.5 ml of guaiacol, 0.5 ml H₂O₂, 0.9 ml water. The absorbance was measured using spectrophotometer (Shimadzu UV-1800 UV-Vis) at 470 nm every 15 sec for 3 min. Peroxidase activity was expressed as $\Delta OD \text{ min}^{-1} \text{ g}^{-1} \text{ seed}$.

Statistical analyses: The analysis of quantitative data generated was performed by using statistical analysis system (SAS). The data collected was subjected to analysis of variance and means were separated by least significant difference (at $P = 0.05$). To normalize variance, the percent data were arcsin transformed before analysis. Grouping letters on treatment means was assigned using Fishers Least Significant Difference. Means with at least one common letter were not statistically significant.

Results and Discussion

Seed priming is a cost effective technique which not only improves germination but also reduces seedling emergence duration and improves stand establishment (Singh *et al.*, 2018). All seed priming treatments improved the seed quality traits over

un primed seeds (Table 1). Seed priming increased the germination rate by 2.34 % to 20.34 % over un primed seed. Highest germination (74.67 %) recorded in seeds primed with 2% K₂HPO₄ which is at par with 0.50 mM and 1.25 mM spermine seed priming. Seed priming with 1.25 mM spermine recorded the highest speed of germination (1.82) which is on par with 1.25 mM spermine seed priming. Seed priming with 2% K₂HPO₄ recorded the highest seedling length (9.68 cm) and seedling dry weight (21.07 mg) which is at par with spermine seed priming concentrations- 0.5 mM, 1.00 mM and 1.25 mM and hydropriming. Seedling vigour index-I (722.80) and seedling vigour index-II (1570.93) was highest in onion seeds primed with 2% K₂HPO₄ which is at par with 1.25 mM spermine seed priming. Seeds primed with 1.25 mM spermine recorded 66.66 % - 650 % increase in the activity of ROS scavenging enzymes SOD and POD, respectively, over un primed seeds. The activity of reactive oxygen scavenging enzymes increased due to seed priming and the highest increase in SOD and POD was reported in 1.25 mM spermine seed priming. Seed priming including hydration is known to improve the seed quality parameters by activation of hydrolytic enzymes, reversion of ageing induced deterioration, repair of damaged protein, RNA and DNA (Taylor *et al.*, 1998; Varier *et al.*, 2010), activation of antioxidant enzymes (Chiu *et al.*, 2002). Thus, all seed priming treatments performed well and showed improvement in seed quality parameters over un primed seeds. In freshly primed seeds, priming with spermine was as effective as halopriming. In contrary, previous studies (Iqbal *et al.*, 2005; Afzal *et al.*, 2009; Khan *et al.*, 2012) have attributed improvement in seed quality due to polyamine. These studies compared the polyamine primed seeds with un primed seeds and improvement in seed quality may be due to hydration treatment.

Seed priming involves controlled hydration which lengthens the lag phase of germination (stage -II) which allows the seeds to go through pre-germination physiological and

Table 1 : Effect of seed priming with spermine on seed quality of onion seeds variety Pusa Riddhi in non-accelerated ageing

Treatment	Germination %	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seedling vigour index-I	Seedling vigour index-II	SOD (units min ⁻¹ g ⁻¹ seed)	POD ($\Delta \text{ g}^{-1} \text{ seed}$)
Hydropriming	59.33 (51.16) ^{abc}	1.57 ^{bs}	9.35 ^{ab}	18.70 ^{abc}	554.57 ^{bc}	1108.33 ^{cd}	0.10 ^{de}	0.06 ^d
Priming- spermine 0.10 mM	56.67 (50.40) ^{bc}	1.36 ^b	9.05 ^b	16.93 ^{cd}	511.65 ^{cd}	958.73 ^{cd}	0.12 ^{bcd}	0.06 ^{cd}
Priming- Spermine 0.25 mM	61.33 (51.56) ^{bc}	1.40 ^b	9.00 ^b	18.90 ^{abc}	551.57 ^{bc}	1157.60 ^c	0.11 ^{cde}	0.14 ^{bcd}
Priming- Spermine 0.50 mM	62.67 (53.93) ^{ab}	1.40 ^b	9.11 ^{ab}	19.23 ^{abc}	595.73 ^b	1205.90 ^{bc}	0.12 ^{bcd}	0.20 ^{abc}
Priming- Spermine 0.75 mM	66.67 (58.44) ^{bc}	1.40 ^b	9.26 ^{ab}	17.37 ^{bcd}	603.20 ^b	1168.53 ^c	0.14 ^{ab}	0.23 ^{ab}
Priming- Spermine 1.00 mM	60.67 (50.39) ^{bc}	1.42 ^b	9.48 ^{ab}	18.53 ^{abcd}	561.21 ^{bc}	1128.80 ^{cd}	0.13 ^{abc}	0.18 ^{abcd}
Priming- Spermine 1.25 mM	74.00 (56.90) ^{bc}	1.82 ^a	9.51 ^{ab}	19.87 ^{ab}	701.34 ^a	1473.20 ^{ab}	0.15 ^a	0.30 ^a
Un-primed	54.33 (48.06) ^c	1.37 ^b	8.25 ^c	15.90 ^d	448.90 ^d	861.67 ^d	0.09 ^e	0.04 ^d
Halopriming-2% K ₂ HPO ₄	74.67 (59.56) ^a	1.78 ^a	9.68 ^a	21.07 ^a	722.80 ^a	1570.93 ^a	0.13 ^{abc}	0.20 ^{ab}
General mean	53.38	1.50	9.19	18.50	583.44	1181.52	0.12	0.16
CD (0.05)	6.21*	0.214**	0.620**	2.72*	82.58**	279.42**	0.023**	0.139**

^aValues in the parentheses are arcsine transformed values; \$-Values with at least one common letter are not statistically significant using Fisher's least significant difference

Table 2 : Effect of seed priming with spermine and accelerated ageing on seed quality attributes in onion seeds variety Pusa Riddhi

Treatment	Germination %	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seedling vigour index-I	Seedling vigour index-II	SOD (units min ⁻¹ g ⁻¹ seed)	POD (Δ g ⁻¹ seed)
Hydropriming	50 (45.00) ^{ba}	1.21 ^{cs}	7.48 ^b	18.27 ^{bc}	377.63 ^b	800.33 ^c	0.05 ^{cd}	0.02 ^{cd}
Spermine 0.10 mM	50.6 (45.38) ^b	1.22 ^c	8.68 ^a	18.67 ^{bc}	441.21 ^{ab}	825.07 ^c	0.07 ^{bc}	0.02 ^{bcd}
Spermine 0.25 mM	51.33 (45.76) ^{ab}	1.39 ^b	8.67 ^a	18.69 ^{bc}	443.53 ^{ab}	852.00 ^{bc}	0.09 ^{ab}	0.01 ^{de}
Spermine 0.50 mM	53.33 (46.91) ^{ab}	1.27 ^c	8.96 ^a	18.97 ^{bc}	478.45 ^{ab}	890.97 ^{bc}	0.09 ^{ab}	0.03 ^{abc}
Spermine 0.75 mM	55.33 (48.08) ^{ab}	1.40 ^b	9.71 ^a	19.30 ^{ab}	535.63 ^a	937.40 ^{abc}	0.04 ^{cd}	0.03 ^{ab}
Spermine 1.00 mM	58 (49.61) ^{ab}	1.45 ^{ab}	8.72 ^a	19.37 ^{ab}	508.07 ^a	1008.13 ^{ab}	0.10 ^a	0.03 ^{abc}
Spermine 1.25 mM	59.33 (50.43) ^a	1.54 ^a	9.10 ^a	20.43 ^a	541.03 ^a	1072.47 ^a	0.11 ^a	0.03 ^a
Un-primed	38 (38.08) ^c	0.95 ^d	7.19 ^b	17.97 ^c	274.15 ^c	533.13 ^d	0.04 ^d	0.01 ^e
Halopriming- 2% K ₂ HPO ₄	50.67 (45.38) ^b	1.40 ^b	8.64 ^a	18.69 ^{bc}	442.04 ^{ab}	844.51 ^c	0.09 ^{ab}	0.02 ^{abc}
Treatment mean	46.07	1.32	8.58	18.93	449.08	862.67	0.07	0.02
CD (0.05)	4.90**	0.11**	1.101**	1.17*	103.38**	158.81**	0.024**	0.0103**

^aValues in the parentheses are arcsine transformed values; \$-Values with at least one common letter are not statistically significant using Fisher's least significant difference

biochemical changes, which improves seed germination and other seed quality parameters (Lamichaney *et al.*, 2017). Iqbal *et al.* (2006) reported the superior performance of KCl priming over polyamines in wheat. Seed priming is reported to increase the endogenous polyamine levels (Basma *et al.*, 1994), thus it explains the superior performance of haloprimed vis-à-vis spermine primed seeds. Thus, the present study propose that beneficial effects of polyamine seed priming reported in the previous studies is only due to controlled hydration and not due to exogenous application of polyamines. The real effect of spermine priming was realized when primed seeds were subjected to accelerated ageing. Spermine slowed down the ageing process and recorded the highest seed quality attributes (Table 2).

Highest germination (59.33 %) was recorded in seeds primed with 1.25 mM spermine which is at par with other spermine priming concentrations- 0.25 mM, 0.50 mM, 0.75 mM and 1.00 mM. Seed priming with 1.25 mM spermine recorded the highest speed of germination (1.54) which is at par with 1.00 mM spermine seed priming. Seed priming with 1.25 mM spermine recorded the highest seedling length (1.54 cm) which is at par with other spermine priming concentrations- 0.25 mM, 0.50 mM, 0.75 mM and 1.00 mM. Onion seeds primed with 1.25 mM spermine recorded highest seedling vigour index-I (541.03) which is at par with other spermine priming concentrations- 0.25 mM, 0.50 mM, 0.75 mM and 1.00 mM. Seedling vigour index-II (1072.47) was highest in onion seeds primed with 1.25 mM spermine which is at par with other spermine priming concentrations- 0.75 mM and 1.00 mM. SOD activity (0.03 units min⁻¹ g⁻¹ seed) was highest in onion seeds primed with 1.25 mM spermine which was at par with K₂HPO₄ primed seeds and spermine priming concentrations- 0.25 mM, 0.50 mM and 1.00 mM. Most of spermine seed priming concentrations in freshly primed and accelerated aged seeds were at par for seed quality parameters. They were also collectively superior over the control (Table 1 & 2). This indicates that application of spermine even under low

concentration i.e. 0.50 mM and 0.25 mM in freshly primed seeds and accelerated aged seeds respectively was equally effective as highest concentration (1.25 mM), in enhancing the seed quality parameters. This indicates that addition of even small quantity of spermine is effective in enhancing the survival rate of onion seeds under adverse storage condition. Among all the treatments onion seeds primed with 1.25 mM spermine consistently performed better under both stress free and accelerated ageing conditions. The improvement in seed quality traits after seed priming with spermine and accelerated ageing vis-à-vis halopriming shows that the spermine seed priming provides protection to onion seeds during accelerated ageing, but it is also interesting to note that the level of ROS scavenging enzymes did not increase accordingly. Plants generated ROS in response to abiotic stress; polyamines reduces the ROS level by activating the antioxidant defense mechanism of plants. Polyamines are reported to have dual role of both as a source of ROS as well as ROS scavengers and activators of key antioxidant enzymes (Kusano *et al.*, 2008; Velarde-Buendía *et al.*, 2012; Pottosin *et al.*, 2014). Several studies have indicated that polyamines can themselves act as antioxidants and inhibit lipid peroxidation (Velikova *et al.*, 2000; Ahn and Jin, 2004; Kim and Jin, 2006). Hence in the present study, the level of ROS scavenging enzymes did not increase and spermine itself acted as an antioxidants. In conclusion, seed priming with 1.25 mM spermine is most effective treatment in enhancing seed germination and vigour under accelerated ageing conditions. The present study has demonstrated the beneficial role of spermine in slowing down the effects of accelerated ageing, which will be helpful in enhancing the storability and performance of onion seeds under adverse storage conditions.

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