Hormonal priming to overcome drought stress and aging damage in groundnut seed (*Arachis hypogaea* L.)

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Abstract

Groundnut seed priming was performed with an aqueous solution of gibberellic acid (GA3) (0, 50, 100, and 150 ppm for 18h) and accelerated aging (96 h) was done prior to drought stress (-0.4, -0.6, and -0.8 MPa), then recovered. Investigated traits were germination percentage, mean germination time, germination rate, vigor index, plumule length, radicle length, seedling length, malondialdehyde content, antioxidant enzymes activities (CAT, SOD, and APX), soluble sugars, and protein content. In comparison with the control group, hormonal priming mitigated the negative effects of both aging and drought stress significantly. When seeds were recovered, hormonal priming was more effective. The obtained results showed that seed priming with GA3 performed after the accelerated aging had more pronounced effect on all parameters of recovered seeds. Hence, priming with 100 ppm of GA3 is suggested.

Keywords: accelerated ageing; germination; gibberellic acid; groundnut; seed priming,

Abbreviations. APX: Ascorbate peroxidase; CAT: Catalase; GA3: gibberellic acid; SOD: Superoxide dismutase

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Introduction

Groundnut (*Arachis hypogaea* L.) is cultivated predominantly in the tropics and subtropics, mostly under rain-dependent systems, where the availability of water is a major constraint on yield (Nautiyal, 2009; Hou et al., 2014). Groundnut seed, which is an oilseed crop contains about 50% oil. It is deteriorated rapidly during storage conditions which results in loss of viability and vigor (Nautiyal, 2009). In addition, groundnut seed vigor is influenced by environmental conditions during curing/drying (Nautiyal and Zala, 2004) and storage (Nautiyal et al., 2004). Seed deterioration is loss of seed quality, viability, and vigor due to the effects of adverse environmental factors (Kapoor et al., 2010; Eisvand et al., 2010). The increase in seed exposure to external challenges enhances deteriorating changes which result in the decrease in the seed survival ability.

Seed deterioration is an undesirable condition in agriculture (Jyoti and Malik, 2013). Mohammadi et al. (2011) indicated that seed deterioration resulted in the decreasing germination percentage and rate in soybeans as

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well as decreasing normal seedling percentage of the plant. According to Sepehri and Rouhi (2017), seed viability of groundnut was decreased under deteriorating conditions. Hence, poor vigor and weak seedling establishment are big challenges for regeneration and propagation of deteriorated seeds. Reactive oxygen species (ROS) include free radicals which are produced by deteriorated seeds and/or under stress conditions. Antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, and catalase are considered to be the main protective components engaged in the removal of free radicals and activated oxygen species (Sarvajeet Singh and Tuteja, 2010; Jyoti and Malik, 2013).

Interestingly, priming repairs damage of aged seeds or seeds exposed to abiotic stresses, and also improves the germination performance (Jisha et al., 2013). The priming benefits include increased germination rate, consistent emergence under a broad range of environments, and improved seedling vigor and growth (McDonald, 1999; Jisha et al., 2013). Priming could improve germination of different plants under abiotic stresses (Jisha et al., 2013; Sepehri and Rouhi, 2016). In addition, some studies reported the positive effects of priming on deteriorated seeds in different species (Kibinza et al., 2011; Yan, 2015). Plant growth regulators are important parameters in seed germination and seedling growth and development. Gibberellins, e.g., gibberellic acid (GA), are a family of 136 tetracyclic diterpenes, a small subset of which is active as plant hormones and known to stimulate seed germination in a wide range of plant species; the predominant active GA depends on the species (Finkelstein et al., 2008; Rouhi et al., 2010). Gibberellins stimulate germination by inducing hydrolytic enzymes that weaken the barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves, and stimulating expansion of the embryo (Bewley and Black, 1994; Finkelstein et al., 2008). Gibberellins may also stimulate germination via the transition from embryonic to vegetative development, in part mediated by the chromatin remodeling factor PICKLE (PKL) (Henderson et al., 2004; Finkelstein et al., 2008). Eisvand et al. (2010) reported that priming by gibberellin, cytokinin, and abscisic acid improves seed performance of tall wheatgrass under control and drought conditions. Several researchers have also used hormonal priming for improvement of seed performance under accelerated aging, drought, and salt stresses (Jakab et al., 2005; Srivastava et al., 2010; Farahbakhsh, 2012; Draganic and Lekic, 2012; Azadi et al., 2013; Sepehri and Rouhi, 2017). There is no information on the influence of hormonal priming on recovery or repair of groundnut deteriorated seed under drought stress condition. Therefore, the aim of this research was to investigate the effects of hormonal priming with gibberellin on germination parameters, protein, sugar, malondialdehyde, and antioxidant enzymes of aged groundnut seeds during simulated drought stress conditions.

Materials and Methods

Plant materials

This study was done at the Crop Physiology laboratory, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Iran. Groundnut seeds (cv. NC2) were provided from Astaneh-Ashrafieh, Gilan province, North of Iran. Viability of the seeds was 90-95% using tetrazolium and standard germination tests.

Steps of experiment

Seeds were aged with accelerated ageing test at 40±1 °C and 96 - 100% relative humidity for 96 hours (Delouche and Baskin, 1973) before they were primed with aqueous solution of gibberellic acid and put into the simulated drought stress levels by Poly Ethylene Glycol 6000. After 5 days, half of the seeds in each Petri dish were transferred to optimum condition in distilled water at 25 °C until final germination day (10 days).

Seed priming

The optimal priming duration was determined in preliminary experiments (data not shown). After optimization, seeds were primed in gibberellic acid ($C_{19}H_{22}O_6$, Merck) solutions with four concentrations of 0 (distilled water), 50, 100, and 150ppm at 25±1 °C. After 18 hours, they were

removed from the priming solutions, surface-dried, and dried back to their original moisture content via experience at room temperature. Treated seeds were equilibrated at room temperature (about 25±2 °C) for 24 hours. The non-primed seeds were considered as control.

Drought stress

For simulating drought stress in laboratory conditions, groundnut seeds were immersed in osmotic potentials of −0.4, −0.6, and −0.8 MPa with polyethylene glycol 6000 (PEG 6000) at 25 °C for 10 days under dark conditions (Michel and Kaufmann, 1973).

Germination tests

Before starting this study, seeds were surface-sterilized by soaking in 1% (v/v) sodium hypochlorite (NaOCl) for three minutes and washed thoroughly with sterilized water. Seeds were germinated between double layered papers (ISTA, 2007) with 15 ml water in 15 cm petri dishes. The petri dishes containing seeds were put into plastic bags to keep moisture content. Seeds were allowed to germinate at 25±1 °C in the dark condition for 10 days (ISTA, 2007). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was investigated every 24 h for 10 days. Mean germination time (MGT) was calculated by following formula (Ellis and Roberts, 1981):

$$MGT: \Sigma Dn/n$$

where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of germination. Germination rate (GR) was calculated by reversing MGT formula:

$$GR = 1/MGT$$

The seed vigor index was calculated as following (Sepehri and Rouhii, 2016):

$$VI = SL \times GP/100$$

where VI is vigor index, SL is the mean seedling length (cm), and GP is germination percentage.

Enzymes assay

Ground tissues (0. 5 g) were mixed with 1 ml of 100 mM potassium phosphate buffer (pH 7.8), and centrifuged at 15000 × g for 30 min at 4 °C. Supernatant was collected and used for enzyme assay. Activity of enzymes was then determined spectrophotometrically (Cary 100 UV-Vis., Australia).

Superoxide dismutase (SOD, 1.15.1.1) activity was assayed according to the method of Giannopolitis and Ries (1997). The reaction mixture contained 100 µL of 1µM riboflavin, 100 µL of 12 mM L-methionine, 100 µL of 0.1 mM EDTA (pH 7.8), 100 µL of 50 mM Na_2CO_3 (pH 10.2), 100 µL of 75 µM nitroblue tetrazolium (NBT), 2300 µL of 25 mM sodium phosphate buffer (pH 6.8) and 200 µL of crude enzyme extract with a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photo reduction of NBT. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. The SOD activity of the extract was expressed as U mg^{-1} protein. One unit of SOD (U) was defined as the amount of enzyme activity that was able to inhibit the photo reduction of NBT to blue formazan by 50%.

Catalase (CAT, 1.11.1.6) activity was assayed by the method of Cakmak and Horst (1991). Frozen tissues (0.5 g) were homogenized in a mortar and pestle with 3 mL of ice-cold extraction buffer (25 mM sodium phosphate; pH 7.8). The reaction mixture contained 100 µL of crude enzyme extract, 500µL of 10 mM H_2O_2 and 1400 µL of 25 mM sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded. The CAT activity was calculated with an extinction coefficient (39.4 mM^{-1} cm^{-1}) and was expressed as units (1 µM of H_2O_2 decomposed per minute) per mg protein.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by monitoring the decrease in absorbance at 290 nm from ascorbate oxidation (Nakano and Asada, 1981). The assay mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 100 µl of enzyme extract. The level of activity was calculated with an extinction

coefficient (2.8 mM^{-1} cm^{-1}) and expressed as (1 µM of ascorbate oxidized per minute) per mg protein.

Total soluble proteins

For protein extraction, about 0.5 g of groundnut tissue was ground with liquid nitrogen and then re-suspended in extraction buffer (50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 1 mM leupeptin, 1 mM pepstatin, and 1 mM phenylmethylsulfonyl fluoride). After centrifuging at 12000 × g for 30 min at 4° C, the protein content in the supernatant was determined according to the method of Bradford (1976) with bovine serum albumin (BSA) as a standard.

Soluble sugar assay

Soluble sugar content was measured based on the anthrone method (Irigoyen et al., 1992).

Determination of lipid peroxidation

Lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances (TBARS) concentration based on the method of Cavalcanti et al. (2004). Three hundred mg of fresh sample was homogenized in 3 ml 1.0% (w/v) TCA at 5° C. The homogenate was centrifuged at 12000 × g for 20 min and 1 ml of the supernatant was added to 3 ml 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was incubated at 95° C for 30 min and the reaction was stopped by quickly placing it in an ice bath. The cooled mixture was centrifuged at 11000 × g for 10 min, and the absorbance of the supernatant was read at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the TBARS concentration was determined by its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Statistical Analysis

The statistical analysis was done based on a completely randomized design with four replications, and 100 seeds per replicate, with treatments arranged in a 5 x 4 factorial scheme. Accordingly, 5 priming levels viz. 0, 50, 100, and 150 ppm GA3 for 18 h and non-primed seeds as a control group x 4 drought stress potentials, viz. 0, -0.4, -0.6, and -0.8 MPa were used. Data for germination percentage was subjected to arcsine transformation before analysis of variance. Statistical analysis was carried out using SAS 9.2 software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513). Mean comparison was performed using a least significant differences (LSD) test at a 5% probability level.

Results

Analysis of variance showed that double interaction effects on traits were significant (data not shown). Therefore, it was decided to avoid explaining and discussing main interaction effects.

Final germination percentage (FGP*)*

The maximum germination percentage of the recovered seeds was observed in hydropriming treatment at 0 MPa level (Table 1). Hydropriming of seeds at 0 MPa was not significantly different from the 50 and 100 ppm of GA3 but it was higher than 150 ppm of GA3 and non-primed seeds (Table 1). In comparison with recovered seeds at -0.4, -0.6, and -0.8 MPa in all priming treatments, the germination percentage in 100 ppm of GA3 was the highest whereas germination percentage decreased gradually with increasing drought stress concentration (Table 1). Seed recovery in non-primed seeds also had a positive effect (Table 1). Apparently, presoaking the seeds with exogenous GA3 was favorable for groundnut seed germination after aging. However, this treatment could enhance seed germination capacity under recovery similar to normal condition.

Table 1
Effect of hormonal priming treatment on germination and seedling characteristics of groundnut recovered seed after accelerated aging test

Priming Treatments	Drought Stress (MPa)	FGP (%)	MGT (day)	GR (1/day)	PL (cm)	RL (cm)	SL (cm)	VI	SS (mg.[g/fw]$^{-1}$)	Pr (mg.[g/fw]$^{-1}$)	MDA (nmol.[g/fw]$^{-1}$)	CAT (Units[mg/pr]$^{-1}$)	SOD (Units[mg/pr]$^{-1}$)	APX (Units[mg/pr]$^{-1}$)
GA3 50 ppm	0	71.00 b	4.08 j	0.245 c	4.94 a	3.93 a	8.87 b	6.47 b	48.30 c	10.96 k	26.45 j	0.300 b	28.15 e	0.410 b
	-0.4	51.00 e	7.15 fg	0.140 f	3.10 c	2.00 c	5.10 d	2.59 e	18.29 f	12.58 g	46.47 fg	0.250 c	23.05 f	0.343 c
	-0.6	45.66 fg	8.05 d	0.124 gh	2.75 de	1.64 de	4.39 ef	2.00 f	14.58 g	13.56 e	52.32 d	0.230 de	21.85 g	0.320 de
	-0.8	40.66 hij	8.40 c	0.119 hi	2.59 ef	1.49 ef	4.08 fg	1.66 gh	11.57 h	14.31 b	54.60 c	0.220 efg	20.92 h	0.310 efg
GA3 100 ppm	0	78.33 a	3.55 k	0.282 b	5.07 a	4.01 a	9.08 ab	7.12 a	56.59 b	11.60 j	23.15 k	0.320 a	30.86 a	0.450 a
	-0.4	56.33 d	6.35 h	0.157 e	3.19 c	2.09 c	5.28 d	2.97 d	22.81 e	12.85 f	41.27 h	0.250 c	30.82 a	0.343 c
	-0.6	51.66 e	7.05 g	0.142 f	3.09 c	1.99 c	5.08 d	2.62 e	18.09 f	13.78 d	45.82 g	0.240 cd	30.31 b	0.330 cd
	-0.8	45.66 fg	8.07 d	0.124 gh	2.84 d	1.75 d	4.59 e	2.09 f	14.51g	14.80 a	52.45 d	0.230 e	28.66 c	0.313 def
GA3 150 ppm	0	68.33 c	4.50 i	0.222 d	4.72 b	3.60 b	8.32 c	5.68 c	37.75 d	10.00 m	29.85 i	0.302 b	28.22 de	0.410 b
	-0.4	43.66 ghi	7.85 e	0.127 g	2.85 d	1.74 d	4.59 e	2.00 f	15.02 g	11.95 i	51.02 e	0.226 ef	21.03 h	0.313 def
	-0.6	39.66 ij	8.45 c	0.118 i	2.58 ef	1.49 ef	4.07 fg	1.62 h	11.82 h	13.03 f	54.92 c	0.220 efg	20.23 i	0.300 fgh
	-0.8	35.33 kl	9.30 a	0.107 j	2.34 gh	1.24 gh	3.58 h	1.26 ij	9.48 i	14.00 c	59.47 b	0.213 fgh	18.93 i	0.283 hi
Hydro-priming	0	76.00 ab	3.45 k	0.290 a	5.12 a	4.10 a	9.22 a	7.01 a	58.52 a	10.32 l	22.99 k	0.301 b	28.46 cd	0.400 b
	-0.4	51.66 e	7.20 f	0.139 f	3.09 c	1.99 c	5.08 d	2.62 e	18.29 f	12.28 h	46.80 f	0.225 efg	20.93 h	0.313 def
	-0.6	44.66 gh	8.15 d	0.122 ghi	2.84 d	1.74 d	4.58 e	2.05 f	15.08 g	13.40 e	52.97 d	0.210 gh	19.55 k	0.293 gh
	-0.8	40.33 ij	8.44 c	0.118 i	2.60 ef	1.48 ef	4.08 fg	1.64 gh	11.94 h	14.20 bc	54.92 c	0.200 h	19.86 j	0.293 gh
nonprime	0	49.33 ef	7.10 fg	0.141 f	3.28 c	1.96 c	5.24 d	2.59 e	18.92 f	8.18 p	45.87 g	0.223 efg	20.85 h	0.310 efg
	-0.4	43.00 ghi	8.42 c	0.118 i	2.74 de	1.65 de	4.39 ef	1.88 fg	14.56 g	8.78 o	54.92 c	0.200 h	18.12 m	0.270 i
	-0.6	38.66 jk	8.40 c	0.119 hi	2.49 fg	1.39 fg	3.88 g	1.50 hi	11.56 h	9.26 n	54.60 c	0.180 i	16.84 n	0.253 j
	-0.8	32.66 l	9.15 b	0.109 j	2.24 h	1.14 h	3.38 h	1.10 j	9.69 i	9.40 n	60.45 a	0.170 j	15.34 o	0.220 k

In each column means followed by the same letter are not significantly different at p≤0.01. FGP: final germination percentage; MGT: mean germination time; Pr protein content; MDA: malondialdehyde content; CAT: catalase; SOD: superoxide dismutase; APX: ascorbate peroxidase

Mean germination time (MGT)

Seed recovery decreased MGT values in both primed and non-primed seeds but the positive effect of recovery was observed in primed seeds (Table 1). The lowest MGT was detected for hormonal priming with 100 ppm of GA3 (Table 1).

Germination rate (GR)

Among all aged seeds, after recovery from drought stress conditions, maximum amount of germination rate was achieved with 100 ppm of GA3. The lowest value of this trait was detected in non-primed seeds (Table 1).

Plumule length (PL)

According to Table 1, seed recovery improved plumule length of groundnut in primed and non-primed groups. The longest plumule length was detected for hydropriming under non-stress conditions. However, it was not significantly different from treatments such as those involving priming with 50 and 100 ppm GA3 under similar conditions as shown in Table 1. Seed recovery from drought stress increased plumule length and 100 ppm of GA3 had the highest effect at all levels (Table 1).

Radicle length (RL)

The obtained results showed that seed recovery had a positive effect on radicle length especially after drought conditions. Recovered seeds from 100 ppm of GA3 in all drought levels were higher than the other treatments although they were not significantly different from hydropriming treatment at -0.4MPa (Table 1).

Seedling length (SL)

Since seedling length is comprised of the plumule plus radicle length, the pattern for seedling is similar to plumule and radicle length. Therefore, we avoid explaining it (Table 1).

Vigor index (VI)

Recovered seeds from hormonal priming with 100 ppm of GA3 were the best in stress and non-stress conditions; therefore, it can be regarded as superior treatment (Table 1). Recovery had positive effect on the vigor of non-primed seeds but it was not higher than that of primed seeds.

Soluble sugars (SS)

Seed recovery significantly enhanced the content of total soluble sugars in primed and non-primed seeds whereas this positive effect was higher in primed seeds compared with the others under stress conditions, especially in seed priming with GA3 at 100 ppm concentration (Table 1). Under non-stress conditions, hydropriming was the best treatment in comparison with hormonal priming and the control group (Table 1). Seed priming significantly enhanced total soluble sugars in the aged groundnut seeds under study.

Total soluble proteins

The best reaction to seed recovery was observed in hormonal priming with 100 ppm of GA3 at all levels of stress conditions (Table 1). The lowest reaction to seed recovery was observed in non-primed seeds.

MDA content

Seed recovery reduced contents of MDA in both primed and non-primed seeds. The positive effect of recovery was observed in primed seeds (Table 1). The lowest content of MDA was detected under hormonal priming with 100 ppm of GA3 (Table 1). Lipid peroxidation can result in not only destruction of the lipid itself, but also damage to cell membranes and other cellular components.

Antioxidative enzymes

A relationship between enhanced activities of antioxidant enzymes and decreased lipid peroxidation has been found in the recovered seeds (Table 1). For catalase, maximum amount of recovered seeds under stress condition was observed in hormonal priming with 100 ppm GA3 (Table 1). For superoxide dismutase, maximum amount of recovered seeds under stress condition was obtained in hormonal priming with 100 ppm GA3. This was higher than other treatments and the control group (Table 1). Activity of ascorbate peroxidase after recovery increased, and response of hormonal priming was better than hydropriming especially the priming with 50 and 100 ppm of GA3 (Table 1).

Discussion

The effect of aged seed recovery from drought stress on morphological and physiological traits of primed seeds was higher than that of non-primed seeds. According to Butler et al. (2009), priming repair is possible if accumulated damage is not irreversible. Irreversible damage at cellular level was designated as non-return point in which the cell becomes irreversibly committed to die (Kibinza et al., 2011). Seeds that had been irreversibly damaged due to ageing and stress conditions were not repaired by priming or recovery after priming. In fresh seeds, free radicals and peroxides are generally kept at low levels by cooperative reactions of enzymatic and non-enzymatic anti-oxidative systems (McDonald, 1999). If the production of free radicals and peroxides exceeds the rate of their removal, seeds suffer oxidative stress and their viability is reduced. It seems that, recovery of primed seeds from -0.4 MPa incurred soft damage due to the low accumulating ROS and -0.6 or -0.8MPa that had accumulated important. Also, without seed priming treatment, the seed is not able to be fully repaired. De novo synthesis of antioxidant enzyme transcripts and proteins resumed in primed seeds recovered from ageing and drought stress conditions. Although the involvement of protective enzymes like catalase is suggested as a key enzyme for seed repair against aging (Kibinza et al., 2011), our data demonstrated that catalase, ascorbate peroxidase, and superoxide dismutase are key enzymes for seed repair against aging ROS-induced damage during recovery. Tabatabaei (2013) showed that catalase and ascorbate peroxidase activity decreased in seeds after aging while hormonal priming with 50 ppm of GA3 increased enzyme activity in sesame seed in comparison to non-primed seeds. In the present experiment, priming improved the morphological

and physiological traits of groundnut seeds, which is in agreement with Eisvand et al. (2010), Kibinza et al. (2011), and Yan (2015). It is concluded that both aging and drought stress inhibit seed germination. The low activity of enzymes resulted in low germination. Thus, the antioxidant protection of a plant becomes largely restrained under the effect of aging and drought stress. Therefore, it seems that a high oxidation-reduction potential during the post-stress recovery can maintain proper homeostasis of oxidation and reduction reactions. This eventually enables plants to survive under unfavorable environmental conditions.

In conclusion, exogenous GA3 could significantly alleviate the growth inhibition of groundnut seedlings. It also protected morphological and physiological parameters from oxidative damage under aging and drought stress, which might depend on the higher antioxidant capacity modulated by GA3. Molecular study of synergistic effects between seed deterioration and drought stress would increase our understanding of the gene expression mechanisms in groundnut seeds.

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