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Effect of hydropriming on morphological and physiological performance of aged groundnut (*Arachis hypogaea* L.) seeds

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ABSTRACT

Low viability and poor vigour due to seed deterioration are major problems for successful agricultural production. In this experiment, the ability of hydropriming to ameliorate seed deterioration damage was studied in groundnut. Groundnut seeds were subjected to accelerated ageing for 96 hours at 40 °C and then hydroprimed at 25 °C for 6, 12 and 18 hours. Regardless of duration, hydropriming significantly improved final germination percentage, germination rate, seedling length, vigour index, antioxidant enzyme activities (catalase, superoxide dismutase and ascorbate peroxidase), soluble sugars and proteins of aged seeds. Mean germination time, electrolyte leakage and malondialdehyde content of primed seeds decreased compared to non-primed seeds. Hydropriming for 6, 12 and 18 hours increased final germination to 15.54, 31.56 and 89.7 %, germination rate by 9.42, 66.6 and 95.6 % and vigour index by 29.11, 82.2 and 204.6 % compared to non-primed seed, respectively. Thus, hydropriming for 18 hours is the most suitable priming period to recover loss of seed quality and to improve germination characteristics of aged groundnut seeds.

Keywords: Antioxidant enzyme activities, seed germination, seedling length, soluble sugars.

اثر هیدروپرایمینگ بر کارآیی مورفولوژی و فیزیولوژی بذور زوال یافته بادام زمینی (*Arachis hypogaea* L.)

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چکیده

زوال بذر یکی از مشکلات اصلی در تولید موفق محصولات کشاورزی به علت کاهش قوه‌نامیه و ضعف بنیه بذر است. در این آزمایش توانایی هیدروپرایمینگ بذر در تخفیف خسارت ناشی از زوال بذر بادام زمینی بررسی شد. بذرهای بادام زمینی به روش پیری زودرس به‌مدت ۹٦ ساعت در دمای ٤۰ درجه سانتی‌گراد زوال یافتند. سپس بذور زوال یافته در دمای ۲۵ درجه سانتی‌گراد به‌مدت ٦، ۱۲ و ۱۸ ساعت هیدروپرایم شدند. صرفنظر از مدت‌زمان، هیدروپرایمینگ به‌طور معنی داری منجربه بهبود درصدجوانه‌زنی، سرعت جوانه‌زنی، طول گیاهچه، شاخص بنیه، فعالیت آنزیم‌های آنتی اکسیدانی (کاتالاز، سوپراکسید دیسموتاز و آسکوربات پراکسیداز)، قندها و پروتئین‌های محلول بذور زوال یافته گردید. متوسط زمان جوانه‌زنی، هدایت الکتریکی و محتوای مالون دی‌آلدهید بذور پرایم شده در مقایسه با بذور پرایم نشده کاهش یافت. هیدروپرایمینگ به‌مدت ٦، ۱۲ و ۱۸ ساعت درصد جوانه‌زنی نهایی را ۱۵/٥٤، ۳۱/٥٦، ۸۹/۷، سرعت جوانه‌زنی را ۹/٤۲، ٦٦/٦، ۹۵/٦ و شاخص بنیه را به ترتیب ۲۹/۱۱، ۸۲/۲، ۲۰٤/٦ درصد در مقایسه با بذور پرایم نشده افزایش داد. بنابراین، هیدروپرایمینگ به‌مدت ۱۸ ساعت به‌عنوان مناسب‌ترین زمان پرایمینگ جهت بازیابی کیفیت از دست رفته بذور و بهبود خصوصیات جوانه‌زنی بذور زوال یافته بادام زمینی می‌باشد.

واژه‌های کلیدی: آنزیم های آنتی اکسیدان، جوانه‌زنی بذر، طول گیاهچه، قندهای محلول.

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Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in the Fabaceae; however, oil in the seeds are easily oxidized during storage condition, resulting in decreased seed viability and vigour. Low vigour seed is the cause of poor crop establishment and thus a major limitation of grain yield (Jisha *et al.*, 2013). Seed priming has been developed as an essential technique to enhance seed performance against oxidative stress (Ashraf & Foolad, 2005; Jisha *et al.*, 2013; Yan, 2015). It has been reported that hydropriming is the simplest, safest and the most economical technique for increasing seed performance, seedling establishment and crop production under unfavorable conditions (Ashraf & Foolad, 2005; Jisha *et al.*, 2013). The efficacy of hydropriming depends on factors such as plant species, priming period and seed vigour (Ahmad & Lee, 2011). Ghasemi-Golezani *et al*. (2013) showed that hydropriming increased plant height, number of pods and number of seeds per pod, 1000-seed mass, biological yield, grain yield and harvest index of lentil (*Lens culinaris*) seeds when compared to the control group. Ahmad *et al*. (2014) emphasized that hydropriming is the cheapest approach to hydrating seeds and minimizes the use of chemical agents. Further, morphological, physiological and biochemical parameters are enhanced in many crops after seed hydropriming (Wattanakulpakin *et al*., 2012; Yan, 2015; Kamithi *et al*., 2016; Lopez *et al*., 2016). Also, these studies have shown that priming is accompanied with the improvement of antioxidant enzyme systems and the accumulation of soluble sugars and soluble proteins. However, morphological and physiological changes induced by seed priming have been rarely analyzed in seedlings. The objective of this study was to evaluate the effects of hydropriming on the morphological and physiological performance of aged groundnut seeds.

Materials and Methods

Site description and plant material

The current study was carried out at the Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Iran. Groundnut seeds cv. NC2 were attained from Astaneh-Ashrafieh (Guilan Province).

Seed ageing treatment

Seeds were aged with accelerated ageing at 40±1 °C for 96 hours (Delouche & Baskin, 1973).

Seed priming treatment

Groundnut seeds were soaked in distilled water at 25 °C for 6, 12 and 18 hours under dark conditions. The hydroprimed seeds were surface-dried and dried back to their original moisture content at room temperature (25±2 °C for 24 hours), calculated by changes in primed seed mass.

Germination tests

Four replicates of 100 seeds were tested for germination between double layers of papers moistened with 15 ml of distilled water in 15 cm Petri dishes. Seeds were incubated in dark conditions at 25±1 °C in 12 days. Germination was documented to have occurred when the radicles were 2mm long.

Morphological measurements

Germination percentage was recorded every day during the incubation period. Mean germination time (MGT) was calculated using the formula of Ellis & Roberts (1981):

$$MGT = \sum Dn/n$$

Where n is the number of seeds that 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 (Rahnama-Ghahfarokhi & Tavakkol-Afshari, 2007).

$$\text{Vigour Index} = \frac{\text{seedling length} \times \text{germination percentage}}{100}$$

Physiological measurements

Electrolyte leakage was measured according to method of Hampton & TeKrony (1995). After 10 days, seedlings were used to determine malondialdehyde (Cavalcanti *et al.*, 2004), SOD activity (Giannopolitis & Ries, 1977), CAT activity (Cakmak & Horst, 1991), APX activity (Nakano & Asada, 1981), soluble proteins (Bradford, 1976) and soluble sugars (Irigoyen *et al*., 1992).

Statistics

The experiment was a completely randomized design with four replications. Data for germination and abnormal germination percentage were subjected to arcsine transformation before analysis of variance was done with SAS software (version 9.1). Mean comparison was performed with an LSD test if the F - test was significant at 5 and 1% probability levels ($P < 0.05$ and $P < 0.01$). Graphical presentation of data was done with Microsoft Excel program.

Results and Discussion

The initial germination percentage, germination rate and seedling length of groundnut seeds were 89.33 %, 0.286 and 9.18 cm, respectively. After accelerated ageing, the final germination percentage, germination rate and seedling length had diminished to 49.33%, 0.138 and 4.8 cm, respectively (data not shown). All measured parameters were affected by hydropriming treatments (Table 1). Hydropriming increased final germination percentage (Figure 1), germination rate (Figure 3), seedling length (Figure 4), vigour index (Figure 5), total soluble proteins (Figure 7), soluble sugars (Figure 8) and enzyme activities (Figures 10, 11 and 12) but mean germination time, electrical conductivity and malondialdehyde content did not decline (Figures 2, 6 and 9). Final germination percentage and germination rate were significantly increased by increasing priming period (Figures 1 and 3). Hydropriming enhanced the germination percentage and germination rate of pinto bean (*Phaseolus vulgaris*) seeds (Ghasemi-Golezani *et al*., 2010) and the germination rate of rice (*Oryza sativa*) seeds (Matsushima & Sakagami, 2012). Lopez *et al*. (2016) found that hydropriming increased germination rate and synchrony and reduced the lag time for start of germination of *Dodonaea viscosa* seeds, thereby successfully invigorating artificially aged seeds. Sallam (1999) demonstrated that water-soaked seeds of *Vicia faba* exhibited significantly higher germination percentage and rate than those from nontreated seed. They also reported that these effects were correlated with increased amounts of total soluble sugars, lactose and maltose.

By increasing the period of hydropriming, mean germination time of groundnut significantly decreased. The lowest MGT value was observed after hydropriming for 18 hours and the highest was detected in non-primed seeds (Figure 2). The reduction of MGT via seed priming agrees with the results obtained by Yan (2016), who reported that hydropriming reduce MGT and enhanced seedling growth in two napa cabbage cultivars. Sung and Chiu (1995) reported that hydropriming decreased MGT without changing the amount of water imbibed by watermelon (*Citrullus lanatus*) seeds. The extended priming period positively affected length of seedlings from aged seeds (Figure 4). Seedling length increased significantly with an increase in the period of priming from 6 to 18 hours. Ghasemi-Golezani *et al*. (2008) found that hydropriming treatment increased the germination rate and seedling dry weight of lentil (*Lens culinaris*) seeds.

Table 1. Analysis of variance for hydropriming treatment effects on germination traits of aged groundnut seeds (cv. NC2)

Source of variation	df	Mean Squares											
		FGP	MGT	GR	SL	VI	EL	SS	SP	MDA	CAT	SOD	APX
Treatment	3	428.885**	8.838**	0.012**	13.793**	13.755**	26.077**	87.44**	12.298**	305.372**	0.004**	32.272**	0.010**
Error	8	8.886	0.031	0.00005	0.096	0.113	0.472	6.789	0.101	1.191	0.00003	0.457	0.00004
C.V. (%)	-	4.79	3.60	3.24	4.84	7.92	2.83	10.24	3.83	3.12	2.23	2.76	1.80

ns,**,* non-significant and significant at 1 and 5 percent of probability, respectively.

FGP: Final Germination Percentage, MGT: Mean Germination Time, GR: Germination Rate, SL: Seedling Length, VI: Vigour Index, EL: Electrolyte leakage, SS: Soluble Sugars, SP: Soluble Proteins, MDA: Malondialdehyde content, CAT: Catalase, SOD: Superoxide dismutase, APX: Ascorbate peroxidase.

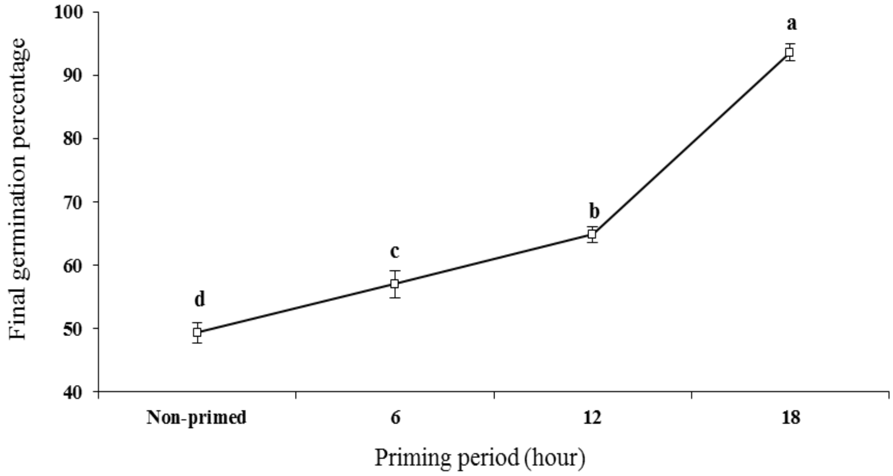

Figure 1. Final germination percentage of aged groundnut seeds in different period of hydropriming

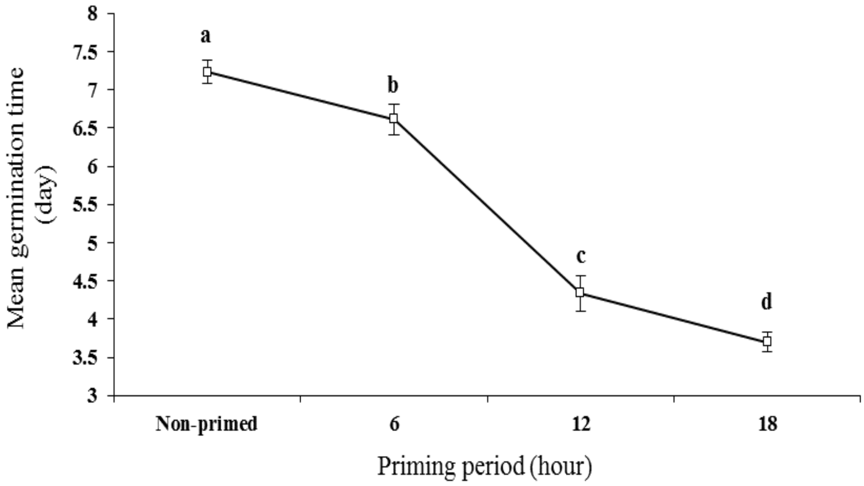

Figure 2. Mean germination time of aged groundnut seeds in different period of hydropriming.

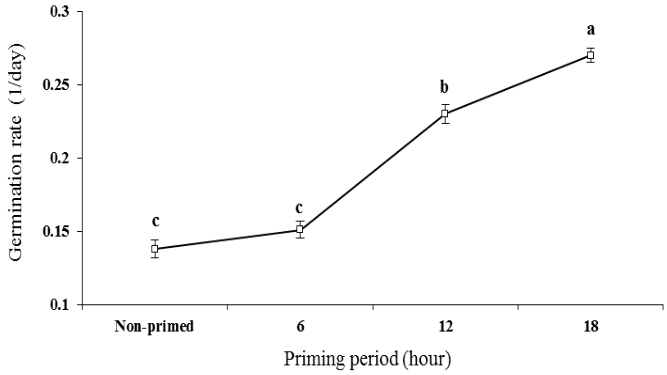

Figure 3. Germination rate of aged groundnut seeds in different period of hydropriming.

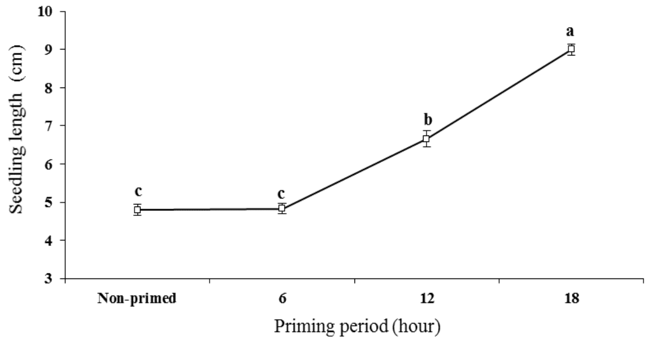

Figure 4. Seedling length of aged groundnut seeds in different period of hydropriming.

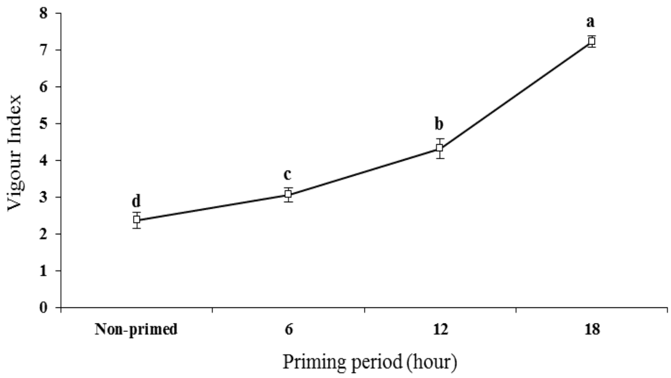

Figure 5. Vigour index of aged groundnut seeds in different period of hydropriming.

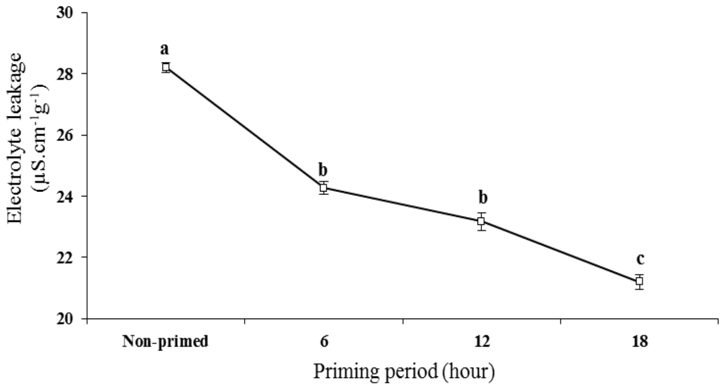

Figure 6. Electrolyte leakage of aged groundnut seeds in different period of hydropriming.

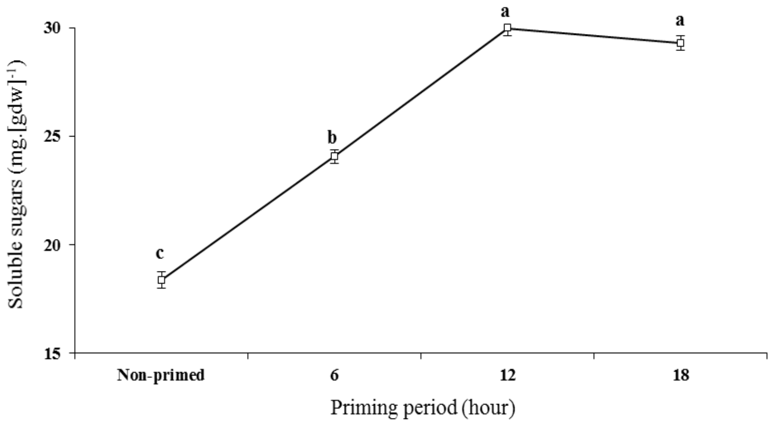

Figure 7. Soluble sugars of aged groundnut seeds in different period of hydropriming

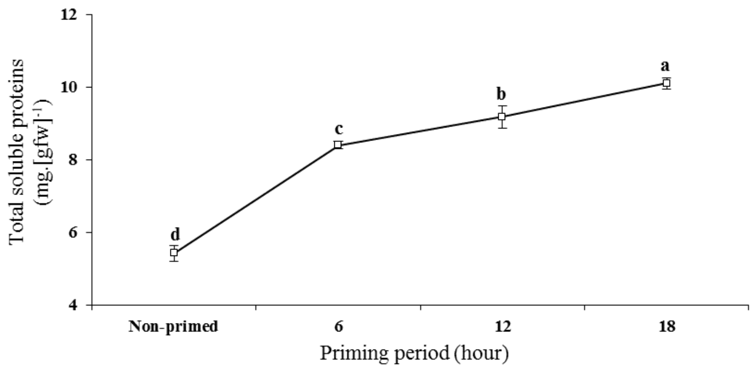

Figure 8. Total soluble proteins of aged groundnut seeds in different period of hydropriming

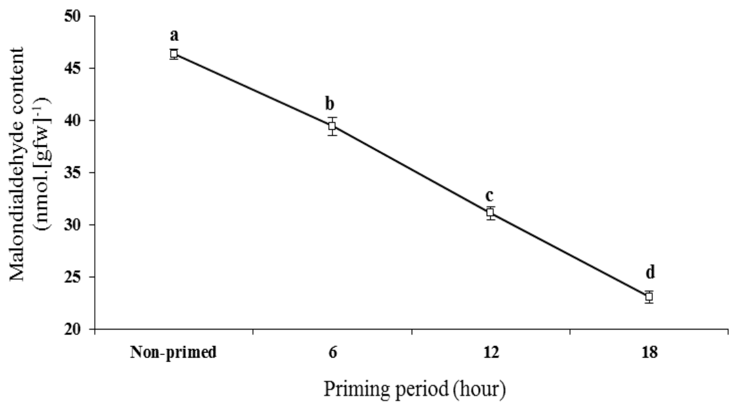

Figure 9. Malondialdehyde content of aged groundnut seeds in different period of hydropriming

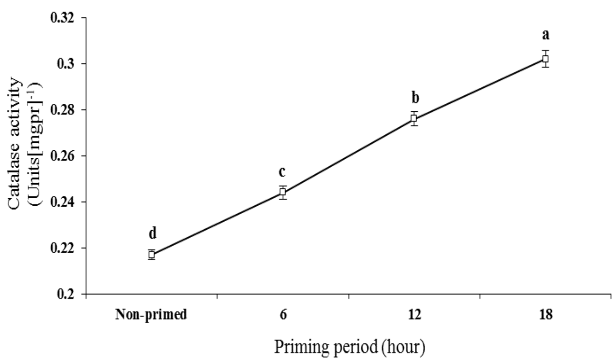

Figure 10. Activity of catalase enzyme in aged groundnut seeds in different period of hydropriming

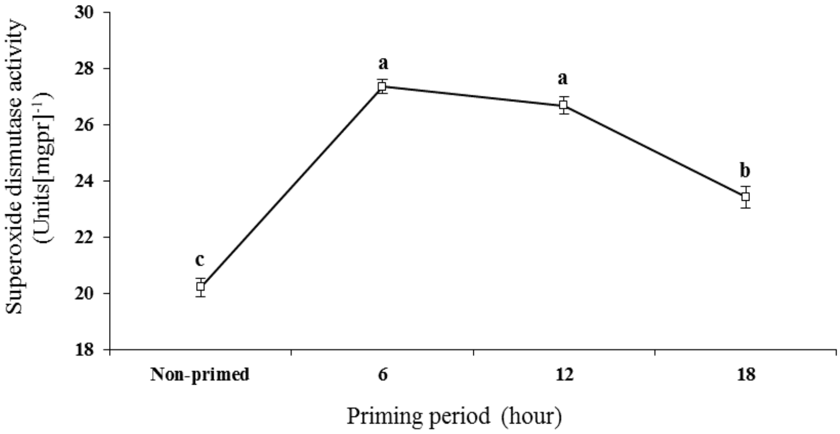

Figure 11. Activity of superoxide dismutase enzyme in aged groundnut seeds in different period of hydropriming

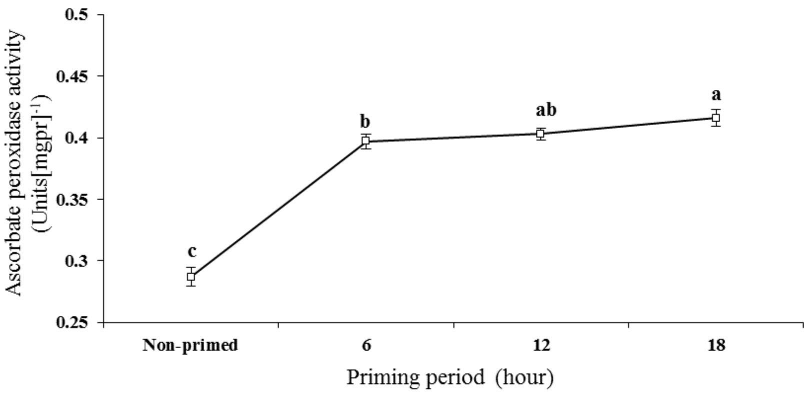

Figure 12. Activity of ascorbate peroxidase enzyme in aged groundnut seeds in different period of hydropriming

In seeds of some plant species, trypsin-like proteolytic enzymes that are produced during seed development are important during germination (Ashraf & Foolad, 2005; Matsushima & Sakagami, 2012; Yan, 2015). The activity of such enzymes, however, is often prevented by trypsin inhibitors, which may be present in the seed and play regulatory roles in protein mobilization during germination (Ashraf & Foolad, 2005). Priming, however, may reduce the inhibitory activities of such enzymes and promote germination as well as cell elongation.

Submersing seeds in water for 18 hours caused a significant increase in the vigour index compared to other priming periods and non-primed (Figure 5). Sharma *et al.* (2014) reported that hydropriming improved the germination traits especially the vigour index of okra seeds. Priming usually induces faster and more uniform seed germination and seedling vigour index (Nascimento, 2012). Matsushima & Sakagami (2012) demonstrated that hydropriming for 12 h enhanced emergence and seedling vigour of rice seeds under different soil moisture conditions.

Electrolyte leakage decreased after all priming periods (Figure 6). Chiu *et al*. (1995) indicated that improved membrane repair in primed seeds might cause the better performance of primed seeds because of lower leakge of electrolytes from the cells. Fujikura *et al.* (1993) also reported the positive effects of hydropriming on electrical conductivity of aged cauliflower

(*Brassica oleracea*) seeds. Yan (2015) noted that seed ageing induces the accumulation of reactive oxygen species (ROS), which degrade or damage nucleic acids and hydropriming can reduce the negative effects by promoting expression of repaire genes. Soluble sugars and proteins in seedlings from primed seeds were higher than those of the non-primed, while MDA content of seedlings was lower than that of seedlings from non-primed seeds (Figures 7, 8 and 9). At the cellular level, seed deterioration is correlated with reduced energy metabolism (Kibinza *et al*., 2006). During priming, carbohydrate reserves in the seed are transformed into simple carbohydrates by hydrolysis of starch. These components are the source of plant energy and are involved in building units (Langens-Grrits *et al*., 2003; Matsushima & Sakagami, 2012). Thus, the effect of hydropriming on improving seed germination is closely related to the solubilization of b-subunit of 11-S globulin storage protein (Capron *et al.*, 2000). Similarly, in pigeonpea (*Cajanus cajan*), hydropriming was very effective in the mobilization of compounds such as proteins, free amino acids, and soluble sugars from storage organs to growing embryonic tissues (Jyotsna & Srivastava, 1998).

It seems that free radical production is the leading candidate that causes increasing seed deterioration. Free radical production, primarily triggered by oxygen, has been related to the peroxidation of lipids and degradation of soluble proteins found in cells. This causes a host of undesirable events, including decreased lipid content, reduced respiratory competence, and increased evaluation of volatile compounds such as aldehyde (Wilson & McDonald, 1986). The slow repair of damage membranes during imbibition may cause low germination percentage and seedling vigour (Abdulrahmani *et al*., 2007). Lowered MDA and EC of primed seed were in accordance with previous studies (Abdulrahmani *et al*., 2007; Yan, 2015). Reduction in MDA content has been detected by El-Araby & Hegazi (2004) in hydroprimed seeds of tomato. Kamithi *et al*. (2016) found several processes, including activation and synthesis of a number of antioxidant enzymes and nucleic acids, repair and build up, ATP synthesis, and the cytoplasmic membrane repair, begin during priming. Thus, during priming the content of MDA began to decrease. CAT activity in seeds increased significantly after 6, 12 and 18 hours of priming (Figure 10). The maximum activity of SOD in groundnut seeds was observed after 6 hours of priming (Figure 11). The activity of APX was induced by seed priming, but there was no significant difference between 12 and 18 hours priming (Figure 12).

The deterioration of the aged seed is a natural phenomenon, and seeds tend to lose quality and viability even under optimal storage conditions. There is a correlation between deterioration of seeds during storage and enzymes degradation and inactivation (Bailly, 2004). It is evident that priming can increase antioxidative enzymes such as CAT, APX and SOD in seedlings (Chiu *et al.,* 1995; Sepehri & Rouhi, 2016). A correlation between enhanced activities of antioxidative enzymes and decreased lipid peroxidation has been reported in primed seeds of purple coneflower, bitter gourd and napa cabbage (Hsu *et al*., 2003; Yan, 2015). Kibinza *et al.* (2011) noted that CAT is a key enzyme for repairing seeds during priming, and Yan (2015) suggested that CAT activity was correlated with the improvement in germination of primed napa cabbage seeds. The present study, demonstrated that hydropriming improved morphological traits as well as CAT, APX and SOD activities in seedlings, resulting in a higher final germination and vigour index in aged groundnut seeds. Seed with poor germination parameters showed reduced antioxidant enzyme activity and increased electrolyte leakage associated with cell damage and necrosis, growth of pathogenic fungus, and necrotization of whole seed (Ashraf & Foolad, 2005; Sepehri & Rouhi, 2016). Results from various studies indicate that hydropriming is a useful method for improving the quality of aged seeds, if seed deterioration has not gone too far (Kibinza *et al.*, 2011; Yan, 2015; Kamithi *et al*., 2016; Yan, 2016). Many investigations have suggested that priming is a practical treatment for increasing germination parameters (Ahmad & Lee, 2011; Ghasemi-Golezani *et al*., 2013; Ahmad *et al*., 2014; Yan, 2015). Kaya *et al.* (2006) found that increased time of hydropriming resulted in higher cumulative germination in sunflower. Also, Caseiro *et al.* (2004) reported that hydropriming was the most effective technique for enhancing germination traits of onion seed, especially when seeds were hydrated for 96 h compared to 48 h. According to Sacala & Demczuk (2016), higher time of hydropriming enables seed cells to respond to very low levels of a stimulus in a more rapid and robust manner comparing to non-primed cells.

The results of the current study showed that hydropriming accelerates the germination of groundnut with higher antioxidant enzyme activities, soluble proteins and sugars in seedlings of primed seeds than in those of non-primed seeds. Priming period had a significant effect on the morphological and physiological traits of groundnut seedlings. The best results were obtained from 18 hours of hydropriming. Therefore, hydropriming for 18 hours can be successfully applied to enhanced seed germination and seedling vigour of aged groundnut seeds.

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