Research Article

Enhancement of Tainan 9 Peanut Seed Storability and Germination under Low Temperature

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Low temperature condition during December to January can limit seed emergence and seedling establishment for peanut production in Thailand. The objective of this study was to determine the effects of peanut seed priming on seed germination and vigor under optimal and low temperature conditions before and after 9 months of storage. Tainan 9 peanut seeds were primed with salicylic acid (SA), ascorbate (ASA), CaCl$_2$, or chitosan and tested for germination at 25°C (optimal temperature) and 15°C (low temperature) before and after a 9-month storage period. Seed priming with 50 mg·L^{-1} SA and 50 mg·L^{-1} ASA for 12 hours before germinating improved germination at 15°C when compared to untreated seeds both before and after 9-month storage. The high seed quality, illustrated by high germination percentage, high seed vigor, and low mean germination time related to the low autoxidation substrates: lipoxygenase (LOX), malondialdehyde (MDA), and high antioxidants: superoxide dismutase (SOD) and catalase (CAT). It suggests that peanut seed priming with salicylic acid and/or ascorbate can improve seedling emergence and growth under low temperature conditions.

1. Introduction

Peanut cultivated in north and northeast Thailand may be exposed to soil temperatures below 25°C for 1-2 weeks during the December to January planting season [1]. Temperatures below 16°C are known to reduce peanut germination percentage, germination rate [2], seedling establishment, seed set, and crop yield [3]. No germination was found when peanut germination temperatures fell below 11°C [4]. Temperatures below 10–15°C affect seed imbibition, enzyme activity, metabolism [5], and liquidity of membrane lipid [6]. Low temperatures are also known to induce reactive oxygen species (ROS) and oxidative damage to the membrane system of plants [7] that result in the loss of membrane integrity, solute leakage, and metabolic dysfunction [8]. Superoxide dismutase (SOD) and catalase (CAT) are antioxidants that improve the cold resistance of plants through inhibiting membrane lipid peroxidation [9]

and eliminate ROS and free radicals [10]. Seed priming with some chemicals has been shown to improve seed germination under low temperatures in various crops [10–12]. Most of the priming methods were based on controlling seed hydration and preparing them to germinate before sowing. There are (i) "hydropriming" where seeds are soaked in water; (ii) "halopriming" where seeds are soaked in a solution of inorganic salts, i.e., NaCl, KNO$_3$, CaCl$_2$, CaSO$_4$; (iii) "osmopriming" where seeds are soaked for a certain period in solutions of sugar, sorbitol, or mannitol, polyethylene glycol (PEG) followed by air drying; and (iv) "hormonal priming" where seeds are pretreated with different hormones, i.e., salicylic acid, ascorbate, kinetin, etc. [13]. Guan et al. [14] reported that maize seed priming with 0.50% chitosan for 60–64 hours reduced mean germination time. Under chilling stress of 15°C, maize seed priming with 50 ppm salicylic acid reduced mean germination time and increased emergence percentage, soluble sugars, amylase,

superoxide dismutase, and catalase activities [12]. Additionally, inbred line corn seed primed with 50 ppm ascorbate and 50 mm $CaCl_2$ increased the germination percentage, germination index, and plumule length while reducing mean germination time at 10°C [15]. Seed priming with these chemicals helped to regulate seed metabolism through stimulating catalase and peroxidase enzyme activity, inhibiting leakage of ions through the cell membrane, accelerating water and nutrients uptake in roots, and reducing the reaction of lipid peroxidation [16, 17]. Most of Thai peanut varieties are sensitive to low temperatures, particularly during the germination stage. It is unclear if seed priming can improve peanut seed germination under low temperatures and how each priming agent might affect seed and seedling physiology. The objective of this research is to evaluate the effects of peanut seed priming on seed germination and seed vigor under low temperature conditions before and after 9 months of storage.

2. Materials and Methods

2.1. Plant Materials. Randomized Complete Block Designs (RCBD) were used in these experiments conducted at the Seed Laboratory, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Thailand. The experiment included five priming methods with four replications. Tainan 9 peanut seeds at 6% moisture and 90% germination were soaked in (i) distilled water, (ii) 50 mg·L^{-1} salicylic acid, (iii) 50 mg L^{-1} ascorbate, (iv) 50 mm $CaCl_2$, and (v) 0.5% w/v chitosan at 27°C for 12 hours. The concentration of each priming agent was determined based on preliminary experiments (Tables S1–S8). After soaking, the seeds were dried back to 6% moisture at room temperature. Unprimed seeds were used as control. The primed and unprimed seeds were kept in plastic bags, sealed, and stored at 27°C. After 9 months of storage, primed and unprimed seeds were analyzed.

2.2. Seed Germination and Seedling Vigor Evaluation. Seed germination was tested before and after the 9-month storage following the methods of ISTA [18]. One hundred seeds were used in the germination test for each replication. Moist sand (70% moisture content and 3 cm deep) was put in an $18 \times 27 \times 10$ cm plastic box. Fifty peanut seeds were then planted on the moist sand of each box and covered with 1 cm of uncompressed sand. The germination boxes were then incubated in a germination chamber at 25°C (optimal temperature) and 15°C (low temperature) under alternating cycle of 12-hour illumination and 12-hour darkness for 10 days. Germination counts were made at 5 days after seeding (DAS) and 10 DAS. Normal seedlings were those with emergence of true leaves from the sand and a normal shoot. Germination percentage was calculated from the number of normal seedlings.

Mean germination time (MGT) was conducted following the same general procedure used in the seed germination test, yet seedling evaluations were done every day. MGT was calculated according to the equation of Matthews and Khajeh-Hosseini [19]:

$$MGT = \left(\frac{\sum (G_t \times T_t)}{\sum G_t} \right), \tag{1}$$

where G_t is the number of germinated seeds on day t, and T_t is time corresponding to G_t in days. Shoot height and root length were measured manually with a ruler. Dry weights of shoot and root were determined after oven-drying at 80°C for 72 hours.

2.3. Electrolyte Leakage Measurement. Electrolyte leakage to evaluate membrane permeability was done according to ISTA [18]. Twenty-five seeds were separately placed in containers with 75 ml of deionized water at 25°C for 24 hours. The electrical conductivity of solution ($\mu s \cdot cm^{-1}$) was measured using EC meter.

2.4. Enzyme Analysis. One gram of ground peanut seeds was mixed with buffers for enzyme and substrate assays: Tris-HCl buffer (100 mm, pH 8.0) for lipoxygenase (LOX), 50 mm sodium phosphate (pH 7.0) for catalase (CAT), 100 mm sodium phosphate buffer (pH 6.4) for superoxide dismutase (SOD), and 0.1% trichloroacetic acid (TCA) for malondialdehyde (MDA). The samples were centrifuged at 4°C for $20,000 \times g$ and 30 min. Supernatants after centrifuging were used for the assay.

LOX activity was determined using the modified method of Wang et al. [20]. To 0.1 ml supernatants we added 2.85 ml sodium phosphate buffer (100 mm, pH 6.0) and 0.05 ml mixture substrates (0.01 ml linoleic acid, 1 ml NaOH (0.1 N), 0.005 ml Tween-20, and 4 ml distilled water). Blank is the 2.85 ml sodium phosphate buffer (100 mm, pH 6.0) mixed with the 0.05 ml mixture substrates. Increase in absorbance at 234 nm and 30°C was measured using a UV-160 Spectrophotometer (Shimadzu, Japan). The LOX specific activity was expressed as U/mg protein, where one unit was expressed as 1 μmol hydroperoxide formed per min at 30°C.

SOD activity was determined using the method suggested by Ukeda et al. [21]. To the 1 ml of supernatants we added 0.1 ml of each substrate: sodium carbonate (50 mm, pH 8), 3 mm xanthine, 3 mm EDTA, 0.75 mm nitroblue tetrazolium salt (NBT), 15% BSA, and SOD solution or water. The absorbance change at 560 nm was measured at 25°C for 20 min. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as U/mg protein.

CAT activity was measured according to Beers and Sizer [22] with some modifications. The analysis mixture consisted of 1 ml supernatants, 2 ml sodium phosphate buffer (50 mm, pH 7.0), 0.5 ml H_2O_2 (40 mm), and 0.5 ml of enzyme. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. The specific activity was expressed as U/mg protein, where one unit of catalase converts one μmol of H_2O_2 per min.

Protein content was determined following the method of Bradford [23] which used bovine serum albumin (BSA) as the standard protein.

2.5. Determination of the Malondialdehyde (MDA) Content.
The assay of MDA as an end product of lipid peroxidation was measured using the method described by Jiang et al. [24]. To 1 ml supernatants of the samples we added 2 ml of 0.5% thiobarbituric acid (TBA) in 15% trichloroacetic acid. The solution was heated at 95°C for 20 min, quickly cooled in an ice bath for 5 min, and then centrifuged at 12,000 g for 10 min to clarify the solution. Absorbance at 532 nm was measured and subtracted from the absorbance at 600 nm. The amount of MDA-TBA complex (red pigment) was calculated with an extinction coefficient of 155 mm/cm.

2.6. Statistical Analysis. The data were analyzed using analysis of variance (ANOVA). Mean comparison among priming treatments calculated using Fisher's LSD. The comparisons of seed quality from two germination conditions or from two storage months were determined using a *t*-test.

3. Results

3.1. Effects of Seed Priming on Germination. Peanut seed priming with $50 \, mg \cdot L^{-1}$ salicylic acid (SA), $50 \, mg \cdot L^{-1}$ ascorbate (ASA), 50 mm $CaCl_2$, and 0.5% chitosan was significantly different from the control in germination and mean germination time (MGT) when germinated at both 25°C and 15°C (Table 1). Primed seeds with SA and ASA germinated 100% and reduced mean germination time from untreated seeds about 1 day at 25°C (Table 1). At 15°C, the germination of primed seeds with SA, ASA, and $CaCl_2$ was 97%, 98%, and 98%, respectively. The control was significantly different at 86% germination. Seed priming with ASA showed the lowest MGT (Table 1). All the priming treatments stimulated seed germination and germinated faster than the untreated seeds (Table 1).

3.2. Effects of Seed Priming on Seedling Growth. Priming treatments with SA, ASA, $CaCl_2$, and chitosan significantly increased seedling growth rate, shoot height, and root length of seedlings when compared to the untreated control (Table 2). The SA and ASA treatments outperformed the other priming treatments. Seed priming with SA showed the highest seedling shoot height when tested at both 25°C and 15°C. Both SA- and ASA-primed seeds were the highest in seedling root length and seedling growth rate at 15°C (Table 2).

3.3. Effects of Seed Priming on Electrolyte Leakage (EL), Lipoxygenase (LOX) Activity, and Malondialdehyde (MDA) Content. All the primed seeds were significantly lower in electrolyte leakage, lipoxygenase activity, and malondialdehyde content than the untreated seeds (Table 3). The lowest electrolyte leakage was observed in SA-primed seeds, whereas seed priming with SA and ASA was the lowest in lipoxygenase activity and malondialdehyde content (Table 3). This information suggests that low free radicals (LOX, MDA) relate to high seed germination.

3.4. Effects of Seed Priming on Superoxide Dismutase (SOD) and Catalase (CAT) Activities. Superoxide dismutase and catalase are antioxidants. All the primed seeds were significantly higher in superoxide dismutase and catalase activities than those in the untreated seeds (Table 4). Seed priming with SA, ASA, $CaCl_2$, and chitosan resulted in higher SOD than the untreated and hydroprimed seeds (Table 4). The highest CAT was detected in primed seeds with SA and ASA (Table 4). The high SOD and CAT in SA- and ASA-primed seeds related to high seed germination.

3.5. Effects of Seed Priming on Germination and Seedling Vigor of Tainan 9 Peanut Seeds after Nine Months of Storage. After 9 months of storage, peanut seed priming with SA, ASA, $CaCl_2$, and chitosan was significantly different in germination, mean germination time, seedling shoot height, seedling root length, and seedling growth rate at both 25°C and 15°C conditions (Figure 1). Seed priming with SA and ASA showed the highest germination percentages (Figure 1(a)) and seedling growth rates (Figure 1(e)) at 25°C and 15°C for both before and after 9 months of storage. Seed priming with ASA resulted in the lowest mean germination time (Figure 1(b)), whereas seed priming with SA resulted in the highest seedling shoot height at low temperature of 15°C after 9 months of storage (Figure 1(c)). Additionally, seed priming with SA showed greater seedling root length than that of primed seed with ASA at both 25°C and 15°C (Figure 1(d)). Notably, the primed seeds with SA and ASA after 9 months of storage maintained seed quality similar to seeds at 0 months of storage.

4. Discussion

Seed priming improved peanut seed germination. Hormonal priming peanut seeds with salicylic acid (SA) and ascorbate (ASA) enhanced seed germination (Table 1), seedling shoot height, seedling root length, and seedling growth rate (Table 2) and also reduced mean germination time (MGT) under low temperatures (15°C) when compared to the control (Table 1). The priming may control hydration levels in seeds that generate metabolic activities necessary for germination [25]. The SA, ASA, and $CaCl_2$ primed seed showed high germination percentages and low MGT under low temperature stress conditions, similar to the studies in rice [26, 27], corn [12, 15, 28], and *S. miltiorrhiza* [29]. Salicylic acid is an antioxidant that inhibits oxidative reactions, helps regulate cell growth (cell enlargement and division), and activates amino acids and proteins [30] that help protect the cell structure [31]. ASA may protect and prevent oxidative deterioration of lipids and maintain structural and functional integrity of membrane cells [16].

Low electrolyte leakage (EL) in SA-primed seeds indicates lower membrane damage under low temperature than untreated seeds (Table 3). Enhanced electrolyte leakage is considered to be a symptom of stress-induced membrane damage and deterioration [32, 33]. Chilling conditions may cause an increase in reactive oxygen species (ROS), inducing oxidative damage to the membrane system of plants [7].

TABLE 1: Effects of seed priming on germination percentage and mean germination time of Tainan 9 peanut seeds at 25°C and 15°C before 9 months of storage.

Treatments	Germination (%)			Mean germination time (days)		
	25°C	15°C	Average	25°C	15°C	Average
Untreated	A 90.00d	A 86.50c	88.25c	A 8.06b	B 9.25e	8.66d
Hydropriming	A 92.50c	B 88.00c	90.25c	A 8.05b	B 8.84d	8.45cd
SA (50 mg·L^{-1})	A 100a	B 96.75a	98.38a	A 6.80a	B 7.64b	7.22a
ASA (50 mg·L^{-1})	A 100a	A 98.50a	99.25a	A 6.83a	B 7.22a	7.03a
CaCl$_2$ (50 mm)	A 96.00b	A 98.00a	97.00ab	A 8.00b	B 8.56c	8.28b
0.5% chitosan	A 97.50b	B 92.00b	94.75b	A 8.03b	B 8.49c	8.26b
Average	A 96.00	B 93.29		A 7.63	B 8.33	
LSD$_{0.05}$	2.44	3.79		0.19	0.25	
CV (%)	1.69	2.69		1.65	2.01	

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by t-test.

TABLE 2: Effects of seed priming on shoot height, root length, and seedling growth rate of Tainan 9 peanut seeds at 25°C and 15°C before 9 months of storage.

Treatments	Shoot height (cm)			Root length (cm)			Seedling growth rate (mg/seedling)		
	25°C	15°C	Average	25°C	15°C	Average	25°C	15°C	Average
Untreated	A 4.58f	A 3.11f	3.85f	A 4.67d	B 3.56d	4.12d	A 172c	B 42b	107.00c
Hydropriming	A 4.88e	B 3.29e	4.09e	A 7.00c	B 3.65d	5.56c	A 212bc	B 48b	130.00b
SA (50 mg·L^{-1})	A 6.08a	B 4.55a	5.32a	A7.65ab	B 4.79a	6.22a	A 324a	B 87a	205.50a
ASA (50 mg·L^{-1})	A 5.75b	B 4.16b	4.96b	A 7.85a	B 4.55b	6.20a	A 321a	B 82a	201.50a
CaCl$_2$ (50 mm)	A 5.08d	B 3.55d	4.32d	A 6.78c	B 4.00c	5.39c	A 256b	B 55b	155.50b
0.5% chitosan	A 5.37c	B 4.07c	4.72c	A 7.48b	B4.65ab	5.83b	A 240b	B 50b	145.00b
Average	A 5.29	B 3.79		A 6.91	B 4.20		A 254.17	B 60.67	
LSD$_{0.05}$	0.18	0.06		0.27	0.23		59.61	15.77	
CV (%)	2.32	1.05		2.59	3.70		15.56	17.24	

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by t-test.

TABLE 3: Effects of seed priming on electrolyte leakage (EL), lipoxygenase activity (LOX), and malondialdehyde (MDA) content of peanut seed priming before 9 months of storage.

Treatments	EL (μs/cm)	LOX (units/mg protein)	MDA (nmol^{-1}/ml)
Untreated	19.64d	1.41c	3.91d
Hydropriming	15.65c	1.44c	2.52c
SA (50 mg L^{-1})	7.93a	0.97a	1.58ab
ASA (50 mg L^{-1})	13.48b	0.95a	1.47a
CaCl$_2$ (50 mM)	14.92bc	1.19b	2.25bc
Chitosan (0.5% w/v)	13.33b	1.26bc	2.18bc
LSD$_{0.05}$	1.89	0.20	0.72
CV (%)	8.84	9.17	17.25

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD.

Excessive reactive oxygen has negative effects on cell structure and metabolism and also causes damage to proteins, DNA, and membrane lipids [34]. Chilling conditions also result in membrane integrity losses leading to solute leakage and metabolic dysfunction. The integrity of intracellular organelles is also disrupted and leads to the loss of compartmentalization, reduction, and impairing of protein assembly and other general metabolic processes [8].

Peanut seed priming with SA and ASA showed the lowest lipoxygenase activity (LOX) and malondialdehyde content (MDA) (Table 3). This suggests the treatments may help maintain cell membrane stability through restriction of lipid peroxidation [35]. The concentration of MDA content is an indicator of lipid peroxidation in plant cells [32, 36]. The decline of the MDA concentrations in the Tainan 9 peanut seed under low-temperature stress indicates that seed priming with SA and ASA reduced lipid peroxidation. Similar to the study by Elkelish et al. [37], ASA mediated oxidative damage by lowering the electrolyte leakage and lipid peroxidation. Li et al. [38] reported that coating maize

TABLE 4: Effects of seed priming on superoxide dismutase (SOD) and catalase (CAT) activities before 9 months of storage.

Treatments	SOD (units/mg protein)	CAT (units/mg protein)
Untreated	0.067c	0.63d
Hydropriming	0.096bc	1.23c
SA (50 mgL^{-1})	0.132ab	2.34a
ASA (50 mgL^{-1})	0.139a	2.40a
CaCl$_2$ (50 mm)	0.123ab	1.55b
Chitosan (0.5% w/v)	0.118ab	1.17c
LSD$_{0.05}$	0.04	0.29
CV (%)	18.67	10.39

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD.
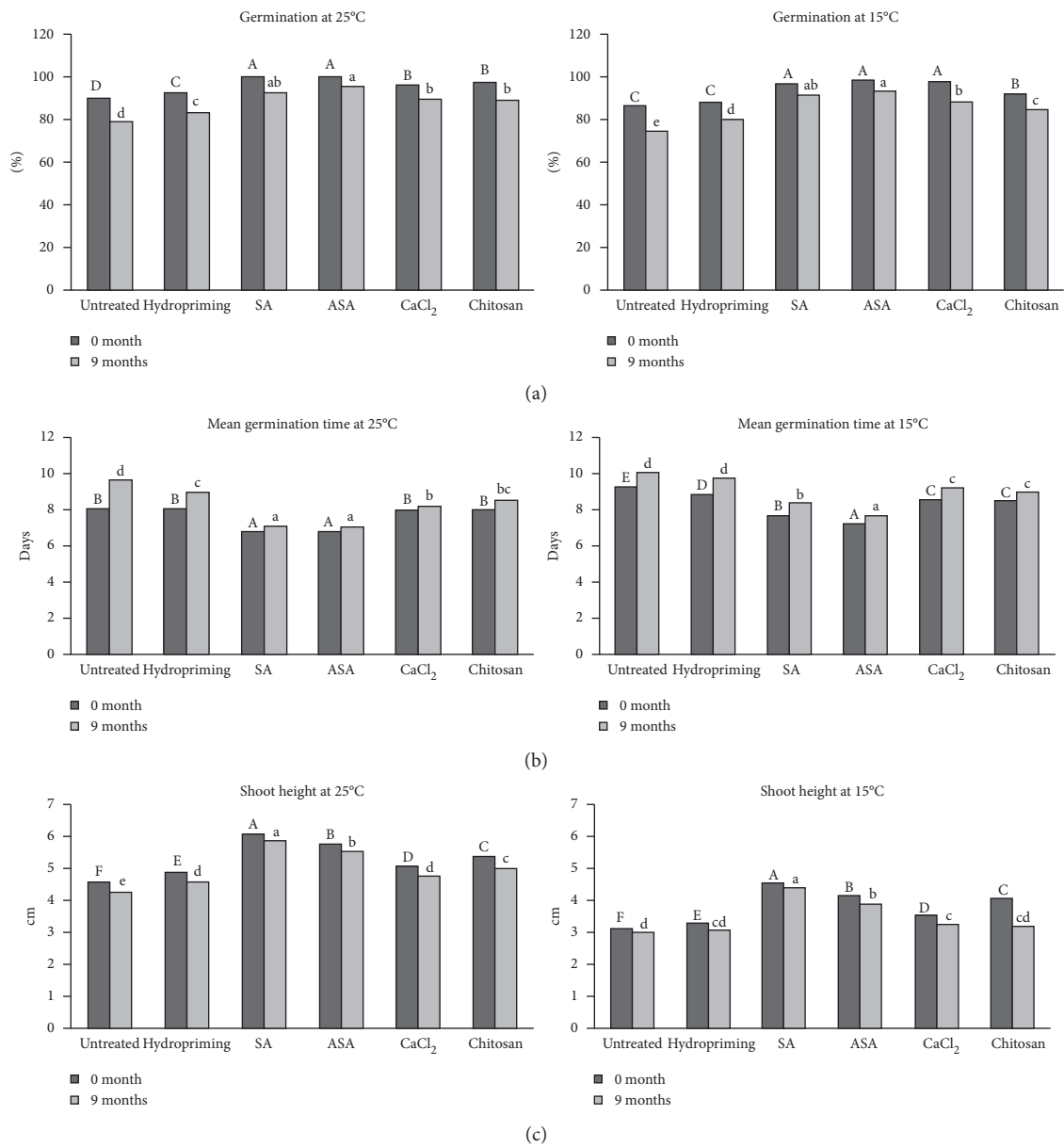

(a)

(b)
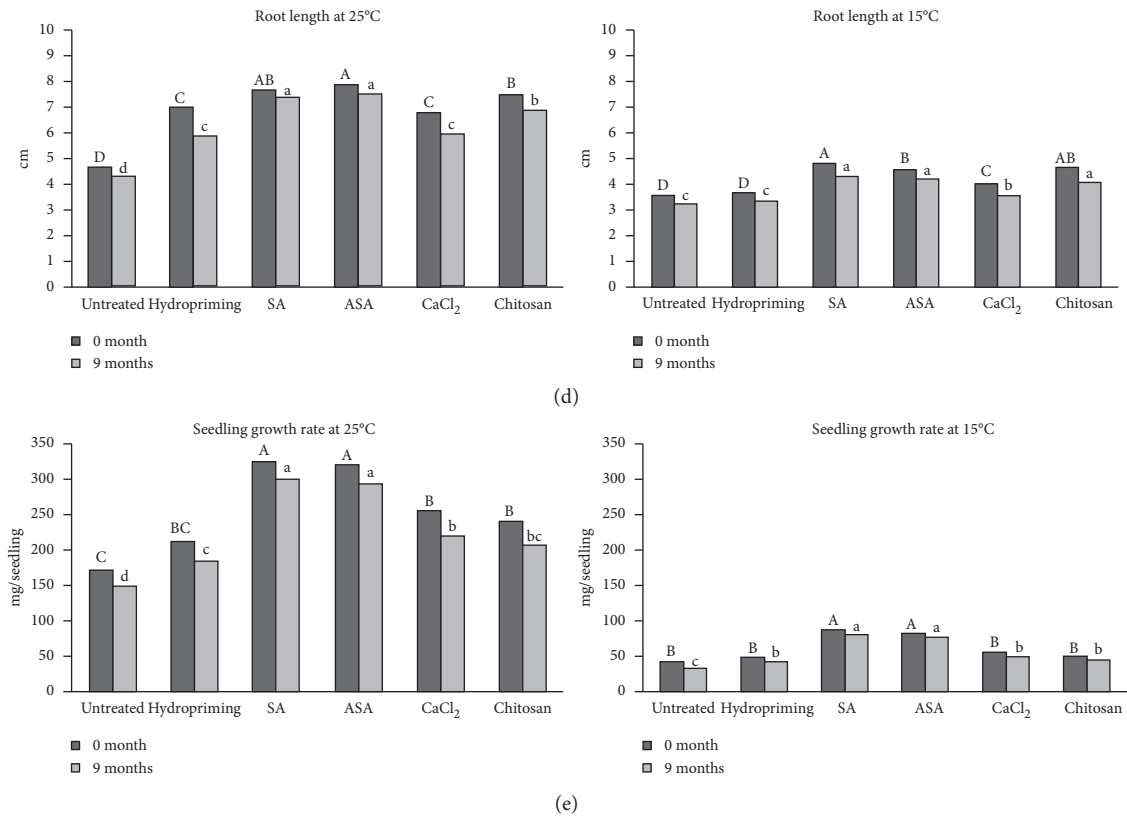

(c)

FIGURE 1: Continued.

(d)

(e)

FIGURE 1: Effects of seed priming on germination, mean germination time, shoot height, root length, and seedling growth rate of Tainan 9 peanut seeds after nine months of storage when germinated at 25°C and 15°C. Means among bar charts followed by the same uppercase (lowercase) letters are not significantly different at $P < 0.05$ by LSD for 0-month (9-month) storage.

seed with a cold-tolerant agent reduced MDA accumulation in seeds geminated under low temperatures. The accumulation of MDA is related to superoxide dismutase (SOD) and catalase (CAT) enzyme activities, decreases in membrane damage (as indicated by low MDA concentration), and the higher activities of antioxidative enzymes [36].

Seed priming with SA and ASA was the highest in SOD and CAT activities under low temperature (Table 4) indicating an efficient scavenging system. SA-induced intrinsic heat or cold tolerance may be aided by maintenance of relatively higher activities of antioxidants [39]. Our results are similar to Farooq et al. [12] who noted that maize seed priming with $50 \, mg \cdot L^{-1}$ salicylic acid solution increased emergence percentage, reduced mean germination time, and increased amylase activity, soluble sugars, superoxide dismutase, and catalase activity at 27°C (optimal temperature) and at 15°C (chilling stress). The SOD and CAT helped to eliminate reactive oxygen species (ROS) and free radicals during stress [10, 16, 40, 41]. These antioxidants potentially inhibited membrane lipid peroxidation [9] and protected the plant from oxidative damage under chilling stress [39–41]. Nonetheless, the physiological response of plants to cold stress condition is complex. Our research only evaluated SOD as the first and second enzymes involved in the antioxidant defense process [16, 42, 43]. Other enzymatic antioxidants (i.e., ascorbate peroxides, APX; peroxidase, POD; and glutathione reductase, GR) and nonenzymatic

antioxidants (i.e., ascorbate, ASA; glutathione, GSH; and guaiacol peroxidase, POX) also improved seed germination under cold stress which these antioxidant contents related to the increase of SOD and CAT [12, 44–51].

5. Conclusions

Tainan 9 peanut seed priming with $50 \, mg \cdot L^{-1}$ salicylic acid and $50 \, mg \cdot L^{-1}$ ascorbate for 12 hours improved seed quality when germinated at low temperature of 15°C compared to untreated seeds and other treatments. The primed seeds had the highest germination percentage, seedling shoot height, seedling root length, and seedling growth rate and the lowest in mean germination time. The high-quality primed seed also had low electrolyte leakage, lipoxygenase activity, malondialdehyde content, and high superoxide dismutase and catalase activities.

Data Availability

The complete dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

Supplementary Materials

The supporting information about the effects of different concentrations of each priming agent on seed quality is given in Supplementary Tables S1–S8. (*Supplementary Materials*)

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