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Research Article

Seed Pretreatment with Cinnamic Acid Positively Affects Germination, Metabolite Leakage, Malondialdehyde Content and Heterotrophic Growth of Aging Cowpea (*Vigna unguiculata*) Seeds

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Extended Abstract

Introduction: A wide range of deteriorative conditions (especially moisture content and temperature) may affect seed quality during storage which may lead to seed aging. As the most important component of the phenylpropanoids pathway, trans-cinnamic acid, found abundantly in plants and its endogenous levels is influenced by stress conditions. The present study was conducted to investigate germination features, seed reserve mobilization, electrolyte leakage and malondialdehyde content in aged cowpea seeds affected by different concentrations of cinnamic acid.

Materials and Methods: The research has been performed in the laboratory of Faculty of Agriculture, Shahrood University of Technology, Iran. The experiment was designed as a factorial (two factors of the experiment included two levels of seed quality including non-aged and aged seeds and five levels of cinnamic acid concentrations including 0, 15, 30, 45 and 60 µM) based on a completely randomized design. Accelerated aging was applied as an efficient method to mimic storage conditions in the presence of accelerating factors. Cowpea (*Vigna unguiculata*) seeds (Bastam local variety) were incubated in a relative humidity of 95% and a temperature of 43 °C for 72 h to accelerate aging. Both seed lots were treated with 5 different concentrations of cinnamic acid for 6 h followed by standard germination and vigor tests. Data of germination and vigor tests were processed using the GERMINATOR software. Heterotrophic growth, seed reserves mobilization, electrical conductivity and membrane lipid peroxidation were assessed using the available methods.

Results: In this study, cowpea seeds responded to cinnamic acid differently based on their primary quality. In deteriorated seeds, concentrations of 45 µM and 60 µM could successfully enhance seed germination percentage, as compared with the aged seeds (i.e., control). A concentration of 45 µM also improved the vigor of deteriorated seeds. Seed pretreatment of 15, 30 and 45 µM enhanced seed reserves utilization in non-aged seeds. Aging negatively affected area under curve, germination uniformity and seedling dry weight of the deteriorated seeds. Application of 30 µM cinnamic acid improved germination uniformity. The area under the curve was positively affected by 15µM and 30µM. Concentrations of 45 µM and 60 µM enhanced seedling dry weight. Applying 45 µM cinnamic acid decreased electrolyte leakage by 38% and improved efficiency of seed reserves mobilization. Moreover, seed malondialdehyde content, as an indication of membrane lipid peroxidation, showed a sharp decline by applying increased concentrations of cinnamic acid.

Conclusions: Based on our results, cowpea seeds respond to cinnamic acid differently based on their primary quality. These results imply that seed pretreatment with 45 µM cinnamic acid may successfully invigorate aged cowpea seeds. We also conclude that cinnamic acid application cannot improve physiological traits and can be regarded as a potent antioxidant in the invigoration of the aged seeds.

Keywords:* *Accelerated aging, Lipid peroxidation, Phenylpropanoids, Seed deterioration, Seed enhancement

***Highlights*:**

1- This is the first study focusing on the role of cinnamic acid in alleviating deterioration in aged seeds.
2- Cinnamic acid has been introduced as a robust antioxidant, which is effective in reducing the deleterious effects of seed deterioration.

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Introduction

Based on ISTA (2012) definition, seed vigor, as an important quality parameter, mainly contributes to the properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence and eventually ascertain final crop yield. Therefore, seed vigor, viability and longevity are major challenges for both maintenance of biodiversity and final yield of crop seed. On the other hand, a wide range of deteriorative and unfavorable conditions may affect seed quality during storage which may subsequently lead to seed aging (Bewley *et al*., 2013). Moisture content and temperature at which the seeds are stored are primarily indicative of the rate of loss of seed viability (Bewley *et al*., 2013).

Aging is mainly associated with gradual and progressive seed deterioration ultimately resulting in cell lethal damage, loss of germination, delay in emergence, abnormal seedling growth and poor plant establishment. At the cellular level, it is mainly evidenced by impairment of macromolecules, membrane perturbation and sooner or later programmed cell death (El-Maarouf-Bouteau *et al*., 2011). In recent years, several studies have focused on applying different secondary metabolites possessing antioxidant potential as exogenous seed pretreatment known as seed invigoration or seed enhancement techniques (Hussian *et al*., 2014). These include a range of post-harvest treatments aiming at improving germination and seed vigor through shortening the time between planting and germination.

Pre-germination metabolism which is physiologically triggered at the first phase of imbibition is induced by seed pretreatment and includes repair responses (activation of DNA and mitochondria repair pathways and intensive antioxidant mechanisms to scavenge free radicals resulting from oxidative stress) to maintain genome integrity, and ensure proper germination and seedling growth (Paparella *et al*., 2015). Furthermore, it is noteworthy to mention that some secondary compounds may exert allelopathic effects on their surrounding plants or even themselves (Saberi *et al*., 2013; Tarayre *et al*., 1995). These compounds leave a kind of "stress memory" in seeds and plants which enables them to select the most appropriate response and withstand future adverse conditions much better (Chen and Arora, 2013; Nemat Alla and Hasan , 2014; Harindrachampa *et al*., 2015). Phenylpropanoids are known as a rich and diverse group of secondary phenolic metabolites derived from phenylalanine and are involved in plant defense mechanisms against both biotic and abiotic stresses, structural support (lignin biosynthesis) and survival (Heldt and Piechulla, 2011). As the most important component of the phenylpropanoids pathway, trans-cinnamic acid (CA), along with its derivative metabolites such as caffeic acid, ferulic acid and salicylic acid are found abundantly in plants and their endogenous levels are influenced by both biotic and abiotic stress conditions (Shalaby and Horwitz, 2014; Singh and Chaturvedi, 2014; Santos and Viera, 2013). Previous studies have elucidated various effects of CA under stressed and unstressed conditions. Singh and Chaturvedi (2014) demonstrated an ameliorating role of CA pretreatment against salt stress in corn seeds. Similar results were obtained from cucumber leaves in chilling stress (Li *et al*., 2011), heat stress (Dai *et al*., 2012) and drought stress (Sun *et al*., 2012). Some recent studies have indicated that exogenous CA application particularly at higher concentrations may exert inhibitory effects, such as benzoic acid and cinnamic acid inhibited seed germination and seedling growth of tomato as allelopathic compounds (Zhang *et al*., 2009).

However, to date no information is available on the possible effects of CA on performance of aged seeds. Hence, the present study was conducted to investigate germination features, seed reserve mobilization and electrolyte leakage in both non-aged and aged cowpea seeds affected by different concentrations of CA. The different kinds of beans as source of protein play an important role in Iranian's diet. On the other hand, many farmers attempt to cultivate beans using stored seed lots from previous years. As stated before, seeds are subjected to aging during storage which may lead to a decrease in seed quality and performance. Most of the farmers in Shahrood use stored cowpea seeds from previous year to cultivate. Therefore, seed deterioration in garners can be a great concern. On the other hand, having a considerable place in the food basket of the households in this region, cowpea (*Vigna unguiculata*) Bastam local variety seeds were selected for the present study.

Materials and Methods

The study has been performed in the research laboratory of faculty of agriculture, Shahrood University of Technology, Iran. Seeds of cowpea (*Vigna unguiculata*) Bastam local variety (Which were produced in the same year) were obtained from Shahrood's Management of Agricultural Department farm. The experiment was designed as a factorial (2 factors of the experiment included two levels of seed quality including non-aged and aged seeds and 5 levels of CA concentrations including 0, 15, 30, 45 and 60 μM) based on a completely randomized design. Accelerated aging (AA) was applied as an efficient method to mimic storage conditions in the presence of accelerating factors (Abreu *et al*, 2013). In order to determine the most appropriate temperature and humidity conditions to artificially deteriorate the seeds, a pilot study had been conducted before the main study. Accordingly, no reduction in germination percentage was detected in 41 °C and near saturation point conditions. Therefore, the temperature was increased by 1 °C. At 42 °C, the germination drop was only 3% in comparison to the control seeds. It was concluded that seeds have not been deteriorated in these conditions. While, by applying a temperature of 43 °C and relative humidity of near saturation point, the germination percentage decreased significantly in comparison to the control (germination of 100%). Increasing the temperature up to 44 and 45 °C led to loss of seeds viability. As a result, suitable conditions for application of aging treatment were regarded as 43 °C and near saturation humidity.

To obtain aged seeds, cowpea seeds were incubated in a relative humidity of 95% and a temperature of 43 °C for 72 h to accelerate aging. Another part of the seed lot was kept at ambient conditions. Both seed lots were treated with 5 different concentrations of CA (with an analytical purity of 97%) (0, 15, 30, 45 and 60 μM) for 6 h followed by standard germination and vigor tests (ISTA, 2012). Data of germination and vigor tests were processed using the GERMINATOR software (Joosen *et al*., 2010) and then analyzed with SAS® 9.5 software.

Germination and seed vigor test

2-mm radicle emergence in seeds was considered as sign of the completion of germination.

Seed vigor index measured using the following equation:

Equation 1: $VI = LS \times MaxG$

Where LS is the mean length of seedling and MaxG indicates germination percentage.

Normal and abnormal seedlings were screened (deformed, unbalanced, fractured, decayed, stunted and missing primary root seedlings were considered as abnormal seedlings) and also dead seeds were omitted. Gmax (maximum germination), U8416 (represents germination uniformity which means the time interval between 16% and 84% of viable seeds to germinate.), t10maxG (time of 10% of viable seeds to germinate), AUC (area under curve) (the integration of the fitted curve between t = 0 and a user-defined endpoint, which results in a parameter that combines information on maximum germination, t50 and U8416 as described by (El-Kassaby *et al*., 2002), t50maxG (time of 50% of viable seeds to germinate) and speed of germination were determined using the GERMINATOR software (Joosen *et al*., 2010). Hardware part of GERMINATOR software included a camera (Nikon D80) connected to a computer (Microsoft Windows XP, Microsoft Office 2003). Germination trays of 15×21 cm were used with a filter paper of 20.3 × 14.2 cm. automatic scoring of germination is done based on the color contrast between the protruding radicle and seed coat. The 8th day was considered as the last day of germination. Vigor index (Agrawal, 2003), number of normal seedlings, seedling dry weight and length of seedlings from both non-aged and aged seeds were calculated and analyzed using SAS® software.

Heterotrophic growth and seed reserves mobilization

Using the following equations, value and efficiency of reserves utilization and dynamic reserves fraction and also seedling heterotrophic growth were estimated (Soltani *et al*., 2006).

(1) Value of reserves utilization = initial seed DW (mg) -final seed DW (mg)

(2) Seed reserves utilization (conversion) efficiency= seedling DW (mg)/value of reserves utilization (mg)

(3) Seed depletion ratio= seed reserves utilization efficiency/ initial seed DW (mg).

Electrical conductivity

After seed pretreatment with CA fifty seeds, both non-aged and aged seeds, for each treatment were soaked in 250 mL of distilled water and incubated at 20 °C for 24 h. Electrical conductivity (EC) was recorded using an EC meter (Analyzer 600) and calculated as $\mu s.cm^{-1}.g^{-1}$ (Hampton and Tekrony, 1995).

Determination of membrane lipid peroxidation

To assess lipid peroxidation, malondialdehyde (MDA) concentration, as a biomarker of lipid peroxidation, was measured following the method of Du and BramLey (1992). 0.25 g of seed sample was homogenized in 5 mL of 0.1% TCA (Trichloroacetic acid). The homogenate was centrifuged at 20 °C and 10000×g for 20 min. 2 mL of 0.25% TBA (Thiobarbituric acid) was added to 250 µL of supernatant. The mixture was placed into a water bath (100°C) for 30 min and immediately cooled off in ice for 15 min and immediately centrifuged at 20 °C and 10000×g for 10 min. Absorbance was recorded at 440, 532 and 600 nm. The MDA content was calculated following equation:

Equation 2: Lipid peroxidation ($nmol.mL^{-1}$) = $[[(A532-A600)-[(A440-A600)\ (MA)]]/155000] \times 10^6$

MA: molar absorption of sucrose in 1 to 10 mM concentrations at 532 and 440 nm which was calculated to be 8.4 and 147, respectively (ratio of 0.0571).

Data analyses and mean comparisons were performed using SAS® software and LSD, respectively.

Results

Germination indices and seed vigor test

Aging affected all germination-related traits, so that the interactive effects of aging and cinnamic acid concentrations were significant for Gmax ($p<0.05$), vigor index ($p<0.01$), t10maxG ($p<0.01$), t50maxG ($p<0.05$) and normal seedling percentage ($p<0.01$). Main effect of cinnamic acid concentrations was significant for U8416 ($p<0.01$), seedling DW ($p<0.05$) and AUC ($p<0.05$). The speed of germination was affected by aging ($p<0.01$) (Table 1).

Seed pretreatment with concentrations of 45 µM and 60 µM CA significantly improved germination percentage of aged seeds while no difference was detected for non-aged seeds, since germination percentage was 100% (Fig. 1.a). In fig. 1.b, the seed vigor index improved after pretreatment of seeds with concentration of 45 µM. In non-aged seeds, concentrations of 15 and 30 µM significantly enhanced the vigor index.

Seed pretreatment with CA of non-aged seeds again had no significant effect on time to 10% and 50% of viable seeds to germinate, whereas in aged seeds deterioration reactions highly delayed both 10% and 50% of viable seeds to germinate (about 3 folds). Amongst different concentrations of CA in aged seeds, application of 15 µM could successfully and significantly decrease time of 10% and 50% of viable seeds to germinate as compared to the control non-aged seeds.

Other concentrations had no significant effect except for concentration of 30 µM which increased t10maxG of aged seeds up to about 10 h compared to the control (Figs. 2.a and 2.b).

Table 1. Analysis of variance for germination, seed vigor index, normal seedlings percentage, t10maxG, t50maxG, germination uniformity, seedling dry weight, AUC, value of reserves utilization, seed reserves utilization efficiency, fraction of utilized seed reserves, electrical conductivity.

Source of variance	df	Germination percentage	Seed vigor index	Normal seedlings percentage	Seed reserves utilization efficiency	Fraction of utilized seed reserves	Electrical conductivity
Seed primary quality (A)	1	4080.4 **	3532482 **	13690 **	0.0372 **	0.00079 **	1354.48 **
Ca concentration (B)	4	136.6 *	9261417 ns	547.40 **	0.0191 **	0.0874 **	2353.84**
A×B	4	105.4 *	250693.5 **	9.7 **	0.0188 **	0.0760 **	1884.79 **
CV (%)		7.8	16.58	12.23	14.29	16.07	6.79

Table 1. Continued

Source of variance	df	t10maxG	t50maxG	Germination uniformity	Seedling dry weight	AUC	Value of reserves utilization
Seed primary quality (A)	1	4080.4 **	3532482.8 **	13690 **	15.95 **	15900.1 **	0.1862 **
Ca concentration (B)	4	136.6 *	9261417.7 ns	547.40 **	0.190 *	53.59 *	0.050 **
A×B	4	105.4 *	250693.5 **	9.7 **	0.0408 ns	28.60 ns	0.0406 **
CV (%)		7.8	16.58	12.23	19.10	7.5	2.97

**: significant at 0.01 probability level
*: significant at 0.05 probability level
ns: not significant.

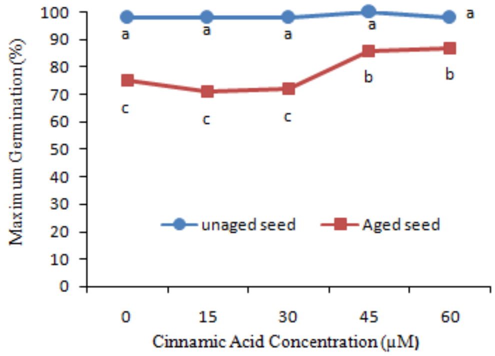

Fig. 1.a. Effect of seed pretreatment with different CA concentrations on maximum germination percentage in unaged and aged seeds. Different letters indicate significant difference at 0.05 probability level by LSD test.

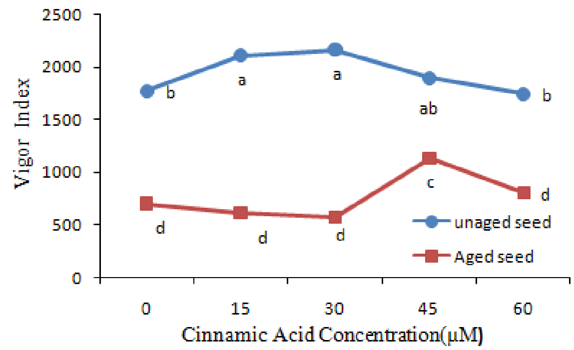

Fig. 1.b. Effect of seed pretreatment with different CA concentrations on vigor index in unaged and aged seeds. Different letters indicate significant difference at 0.05 among treatments as determined by LSD test.

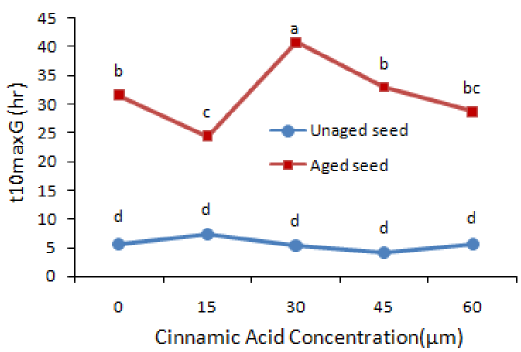

Fig. 2.a. Effect of seed pretreatment with different CA concentrations on t10maxG in unaged and aged seeds. Different letters indicate significant difference at 0.05 among treatments as determined by LSD test.

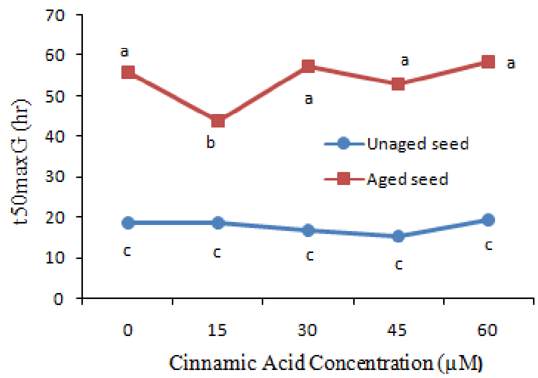

Fig 2.b. Effect of seed pretreatment with different CA concentrations on t50maxG unaged and aged seeds. Different letters indicate significant difference at 0.05 among treatments as determined by LSD test

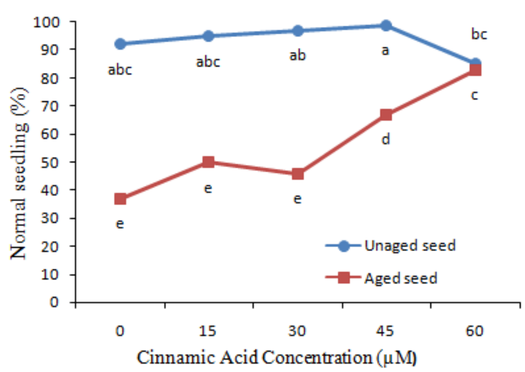

Fig. 3.a. Effect of seed pretreatment with different CA concentrations on normal seedling percentage in unaged and aged seeds. Different letters indicate significant difference at 0.05 probability level by LSD test.

Deterioration reactions led to a substantial decline (about 60%) in the percentage of normal seedlings from aged seeds as compared to the non-aged seeds (figs. 3.a and 3.b (images a, and f)). Seed pretreatment with different

a. unaged seed, 0µM CA

b. unaged seed, 15µM CA

c. unaged seed, 30µM CA

d. unaged seed, 45µM CA

e. unaged seed, 60µM CA

f.aged seed, 0µM CA

g. aged seed, 15µM CA

h. aged seed, 30µM CA

i. aged seed, 45µM CA

j. aged seed, 60µM CA

Fig. 3.b. Effect of seed pretreatment with different cinnamic acid concentrations on the morphology of seedlings from unaged and aged seeds.

concentrations of CA did not affect the percentage of normal seedlings in seedlings from non-aged seeds, while in aged seeds concentrations of 45 µM and 60 µM CA significantly improved normal seedling emergence from aged seeds by about 81% and 124%, respectively, so that at the highest concentration the percentage of normal seedlings reached the number for control normal seedlings. It is also clear from the seedling images (fig. 3.b) that application of 45 µM and 60 µM CA successfully compensated the deleterious consequences of aging.

Seed primary quality affected the uniformity of germination (fig. 4.a). Between, seed pretreatment with concentration of 30 µM CA significantly enhanced germination uniformity as compared to the control while other concentrations had no significant impact (fig. 4.b).

As shown in Fig. 5.a, aging drastically declined the AUC of deteriorated seeds compared to the non-aged seeds. On the other hand, concentrations of 15 µM and 45 µM improved AUC (fig 5.b).

Fig. 6.a shows that aging processes resulted in a considerable decline of seedling dry weight from deteriorated seeds (about 3-fold). On the other hand, concentrations of 45 µM and 60 µM CA significantly improved the trait with about 33.5% compared to the control (Fig. 6.b).

Electrical conductivity

Aging resulted in a significant increase in electrolyte leakage of control aged seeds as compared to the control non-aged seeds (about 41%). Seed pretreatment did not affect non-aged seeds except for 30 µM CA which increased the EC by about 56% as compared to the control non-aged seeds. On the other hand, in case of aged seeds different responses to CA concentrations were detected, so that application of 15 µM CA significantly increased electrolyte leakage whereas the concentration of 45 µM could significantly and effectively decrease electrolyte leakage (roughly 38%) to the rate of leakage recorded for control non-aged seeds (Fig. 7).

Heterotrophic growth and seed reserves mobilization

Assessing the heterotrophic growth of cowpea seedlings from both non-aged and aged seeds revealed that unlike the seed reserve utilization efficiency (Fig. 8.b) and fraction of utilized seed reserves (Fig. 8.c), aging significantly affected the reserves utilization (Fig. 8.a) compared to the control non-aged seeds.

Fig. 8.a shows that seed pretreatment with 15, 30 and 45 µM CA significantly enhanced the reserves utilization of non-aged seeds, whereas a concentration of 60 µM did not change it comparing to the control. In case of aged seeds some fluctuations were detected regarding application of the various CA concentrations; concentrations of 30 µM and 60 µM increased the trait while application of 45 µM resulted in a significant decline compared to the control aged seeds. Fig. 8.b shows that seed pretreatment with CA concentrations did not affect the efficiency of seed reserves utilization in non-aged seeds whereas application of CA significantly enhanced seed reserves utilization efficiency up to 45%. In non-aged seeds CA pretreatment with 30 µM and 45 µM improved the fraction of utilized seed reserves, while CA concentrations enhanced this fraction comparing to the aged control seeds (approximately 48%)(Fig. 8.c).

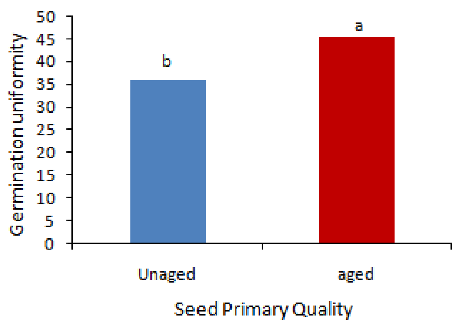

Fig. 4.a. Effect of seed primary quality on germination uniformity. Different letters indicate significant difference at 0.05 probability level by LSD test.

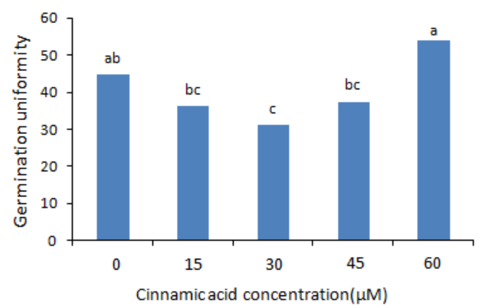

Fig. 4.b. Effect of seed pretreatment with different CA concentrations on germination uniformity in cowpea seeds. Different letters indicate significant difference at 0.05 probability level by LSD test.

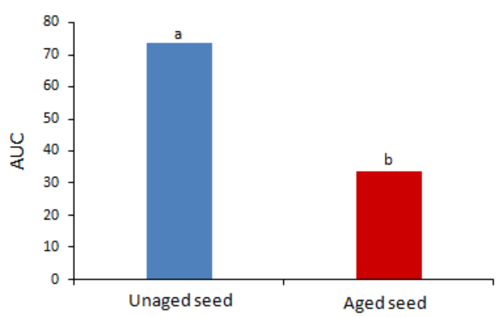

Fig. 5.a. Effect of seed primary quality on area under curve. Different letters indicate significant difference at 0.05 probability level by LSD test.

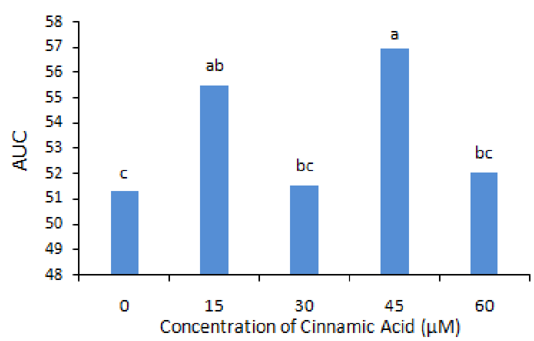

Fig. 5.b. Effect of seed pretreatment with different CA concentrations on area under curve. Different letters indicate significant difference at 0.05 probability level by LSD test.

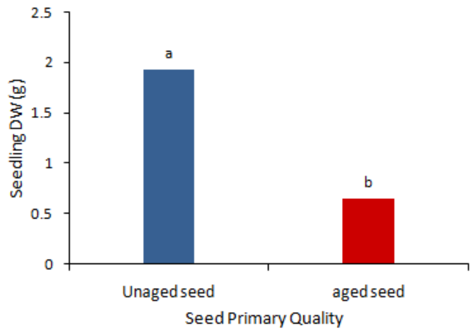

Fig. 6.a. Effect of seed primary quality on seedling dry weight. Different letters indicate significant difference at 0.05 probability level by LSD test.

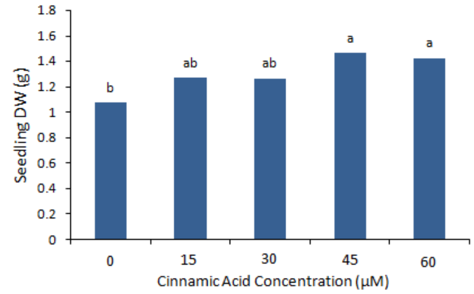

Fig. 6.b. Effect of different CA concentrations on seedling dry weight. Different letters indicate significant difference at 0.05 probability level by LSD test.

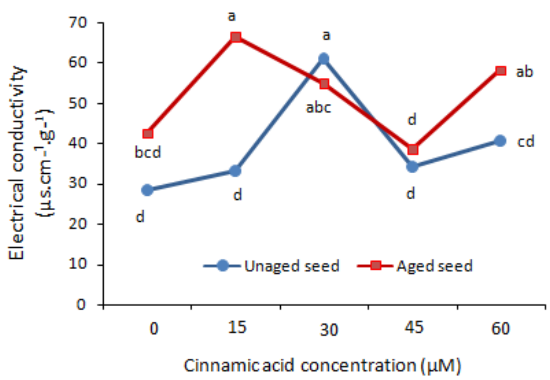

Fig. 7. Effect of seed pretreatment with different cinnamic acid concentrations on electrical conductivity in unaged and aged seeds. Different letters indicate significant difference at 0.05 probability level by LSD test.

Malondialdehyde

Deteriorative reactions during aging caused a drastic increase of about 2.8 fold as compared to the non-aged seeds MDA content, whereas, MDA content in aged seeds sharply decreased as concentrations of CA increased, so that a concentration of 60 μM reduced MDA content by 98%. However, in non-aged seeds applying concentrations of 15 and 30 μM increased MDA content compared to the control. But higher concentrations of CA reduced its content (Fig. 9).

Discussion

Seed deterioration is generally characterized by vigor reduction (Gupta and Aneja, 2004), germination retardation (Arefi and Abdi, 2003) and an increase in metabolite leakage (Basra, 2003). As expected, aging processes negatively affected these characteristics in cowpea seeds, as well. Based on our results showed here (and also consistent with our other results including seed antioxidant enzymes, yield and yield components (data not shown here)) cowpea seeds responded to CA very differently based on their primary seed quality. Obviously, most of the studied traits in non-aged seeds did not respond to CA application very likely because the non-aged seeds already display a maximum response that cannot get any

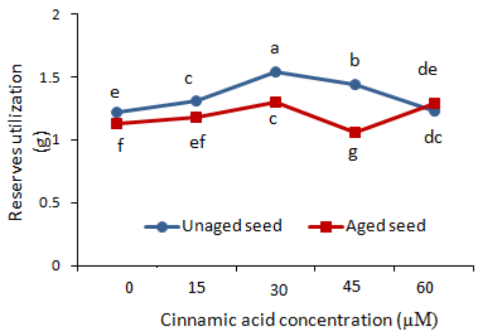

Fig. 8a. Effect of seed pretreatment with different CA concentrations on reserves utilization. Different letters indicate significant difference at 0.05 probability level by LSD test.

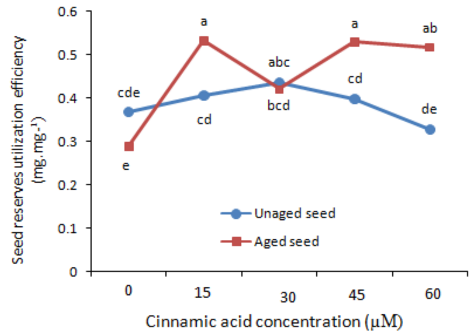

Fig. 8b. Effect of seed pretreatment with different CA concentrations on seed reserves utilization efficiency. Different letters indicate significant difference at 0.05 probability level by LSD test.

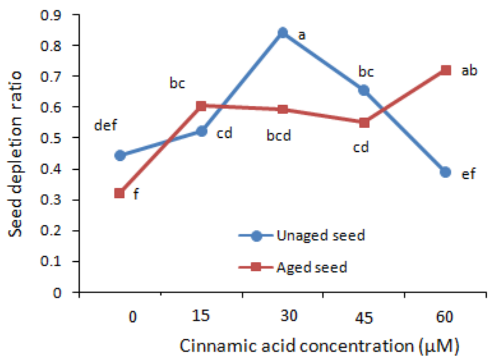

Fig. 8.c. Effect of seed pretreatment with different cinnamic acid concentrations on fraction of utilized seed reserves in unaged and aged seeds. Different letters indicate significant difference at 0.05 probability level by LSD test.

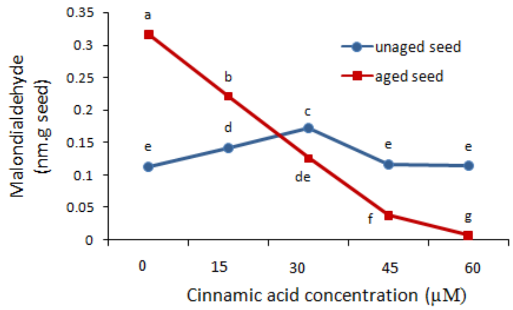

Fig. 9. Effect of different CA concentrations on malondialdehyde content. Different letters indicate significant difference at 0.05 probability level by LSD test.

higher, whereas application of CA enhanced Gmax, seed vigor index, t10maxG, t50maxG, normal seedling percentage, and seed reserves utilization efficiency in aged seeds. Previous studies on application of exogenous cinnamic acid on plants under various stress conditions revealed that CA may ameliorate deleterious effects of both biotic and abiotic stresses in different plant species (Singh and Chaturvedi, 2014; Dai *et al*., 2012). On the other hand, a wide range of other studies provide arguments for allelopathic effects of CA on plants (Li *et al*., 2017; Singh *et al*., 2013). According to these findings, CA retards seed germination and root growth through induction of lignification (Salvador *et al*., 2013). In addition, our other study on effects of CA on some physiological traits of non-aged cowpea seeds (unpublished data) revealed its inhibitory effects on non-aged seeds.

Seedling heterotrophic growth includes 3 main components: (1) reserves utilization, (2) reserves utilization (conversion) efficiency, (3) fraction of seed depletion (Soltani *et al*., 2006). Growth of seedlings from aged seeds may be affected by a decrease in these components. Therefore, understanding the relative sensitivity of these components to deterioration can be helpful to identify the sensitive components of seedling growth to ageing and to plan subsequent invigoration treatments. Thus, mobilization of seed storage compounds during imbibition is a crucial process to set proper conditions for seed germination and seedling establishment (Weitbrecht *et al*., 2011). All CA concentrations in our study resulted in a significant decrease in electrolyte leakage and also increase in seed reserves utilization efficiency and fraction of utilized seed reserves. Thus, improvement of the germination indices of the aged seeds may result in the enhancement of heterotrophic growth and retardation of the metabolite leakage since reserve depletion generates energy to fuel germination. These results may imply that seed pretreatment with 45 µM CA could successfully invigorate aged cowpea seeds.

Conclusions

Cowpea seeds responded to CA very differently based on their primary seed quality. Non-aged seeds did not respond to CA application whereas application of CA enhanced Gmax, seed vigor index, t10maxG, t50maxG, normal seedling percentage, and seed reserves utilization efficiency in aged seeds. CA treatment also blocked membrane degradation of aged seeds. Results indicate that seed pretreatment with 45 µM CA could successfully invigorate aged cowpea seeds.

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مقاله پژوهشی

تاثیر پیش تیمار با اسید سینامیک بر خصوصیات جوانه‌زنی، نشت الکترولیت‌ها، میزان مالون دی آلدهید و رشد هتروتروفیک بذرهای فرسوده لوبیا چشم بلبلی (*Vigna unguiculata*)

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چکیده مبسوط

مقدمه: طیف وسیعی از شرایط نامطلوب محیطی به ویژه دما و رطوبت بالا طی دوره انبارداری بذر می‌توانند بر کیفیت آن تأثیر نامطلوب بگذارد. اسید سینامیک به عنوان مهمترین ترکیب مسیر فنیل پروپانوئید در گیاهان به وفور یافت می‌شود و سطوح داخلی آن در مواجهه با انواع تنش‌های زنده و غیرزنده تغییر می‌کند. از این رو، این مطالعه به منظور بررسی ویژگی‌های جوانه‌زنی، پویایی ذخایر بذر، نشت الکترولیت‌ها و محتوای مالون دی آلدهید در بذرهای فرسوده لوبیا چشم بلبلی تحت تأثیر غلظت‌های مختلف اسید سینامیک انجام گرفته است.

مواد و روش‌ها: این آزمایش به صورت فاکتوریل ۲ عاملی بر پایه طرح کاملاً تصادفی در آزمایشگاه بذر گروه فیزیولوژی گیاهی دانشگاه وخنینگن هلند انجام شد. فاکتورها به صورت دو سطح از کیفیت اولیه بذر (بذر غیرفرسوده و بذر فرسوده) و ۵ سطح غلظت‌های اسید سینامیک (۰، ۱۵، ۳۰، ۴۵ و ۶۰ میکرومولار) بودند. از پیری تسهیل شده به عنوان روشی کارامد جهت شبیه‌سازی شرایط نگهداری بذر در حضور عوامل تسهیل کننده فرسودگی استفاده شد. به منظور فراهم آوردن بذرهای فرسوده، بذرها در معرض دمای ۴۳ درجه سلسیوس، رطوبت نسبی ۹۵٪ به مدت ۷۲ ساعت در انکوباتور قرار گرفتند. برای هر دو گروه بذری فرسوده و غیرفرسوده پس از پیش تیمار غلظت های CA به مدت ۶ ساعت، آزمون جوانه‌زنی استاندارد و بنیه بذر در چهار تکرار انجام شد. صفات مربوط به جوانه‌زنی با استفاده از نرم‌افزار جرمیناتور برآورد گردید. رشد هتروتروفیک، پویایی ذخایر بذر، هدایت الکتریکی و پراکسیداسیون لیپیدهای غشاء (میزان تجمع مالون دی آلدهید) نیز با استفاده از روش‌های موجود بررسی شدند.

نتایج: بذرهای لوبیا چشم بلبلی بر حسب کیفیت اولیه خود پاسخ متفاوتی به پیش تیمار اسید سینامیک دادند. بذرهای فرسوده پیش تیمار شده با غلظت‌های ۴۵ و ۶۰ میکرومولار به طور معنی‌داری درصد جوانه‌زنی بالاتری در مقایسه با شاهد داشتند. همچنین غلظت ۴۵ میکرومولار سبب بهبود بنیه این بذرها گردید. پیش تیمار بذرهای غیرفرسوده با غلظت های ۱۵، ۳۰ و ۴۵ میکرومولار میزان استفاده از ذخایر بذر را افزایش داد. کاربرد غلظت ۳۰ میکرومولار اسید سینامیک یکنواختی جوانه زنی بذرهای فرسوده را بهبود بخشید. پیش تیمار با غلظت های ۱۵ و ۳۰ میکرو مولار، سطح زیر منحنی به طور مثبت و معنی‌دار تحت تاثیر قرار داد. پیش تیمار با غلظت‌های ۴۵ و ۶۰ میکرومولار افزایش معنی‌دار وزن خشک گیاهچه حاصل از بذرهای فرسوده را در پی داشتند. به‌کارگیری غلظت ۴۵ میکرومولار سبب کاهش نشت الکترولیت‌ها تا حدود ۳۸ درصد و بهبود کارایی استفاده از ذخایر بذرهای فرسوده گردید. به علاوه، محتوای مالون دی‌آلدهید بذر به عنوان شاخصی از پراکسیداسیون لیپیدهای غشایی با کاربرد اسید سینامیک و افزایش غلظت کاربرد آن به شدت در بذرهای فرسوده کاهش نشان داد.

نتیجه‌گیری: بر اساس نتایج این بررسی، بذرهای لوبیا چشم بلبلی برحسب کیفیت اولیه خود واکنش متفاوتی به اسید سینامیک نشان می‌دهند. در مجموع نتایج این مطالعه نشان داد پیش تیمار بذرهای فرسوده با اسید سینامیک به ویژه غلظت ۴۵ میکرومولار به‌خوبی قادر است سبب بهبود و تقویت بذرهای فرسوده لوبیا چشم بلبلی گردد. می‌توان این متابولیت را به عنوان یک آنتی اکسیدان قوی در شرایط تنش فرسودگی بذر در نظر گرفت که قادر به بهبود بذرهای فرسوده لوبیا چشم بلبلی می باشد.

واژه‌های کلیدی: پراکسیداسیون لیپیدی، پیری تسهیل شده، تکنیک‌های بهبوددهنده بذر، فنیل پروپانوئیدها، فرسودگی بذر

جنبه‌های نوآوری:

۱- مطالعه پیش رو اولین مطالعه روی اثرات اسید سینامیک به عنوان یک آنتی اکسیدان قوی بر بذرهای فرسوده می باشد.
۲- اسید سینامیک به عنوان آنتی اکسیدان موثر بر کاهش اثرات مخرب فرسودگی بذر معرفی گردیده است.

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