Enhancement of *Acacia cyanophylla* Seed Germinability and Vigor through salt Hardening Technique

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ABSTRACT

Hardening is a seed technique that has been successfully used for enhancing seed vigor for many crops. However, there is dearth information on the influence of hardening treatments on tree and shrub seeds. Thus, this experiment was conducted to investigate the effects of salt-hardening treatments on germination and seed vigor of *Acacia cyanophylla* seeds. The seeds were presoaked in 10 mmole $CaCl_2$, NaCl or KCl for 16 h followed by drying back for 16 h (approximately to their original weight). Untreated seeds were used as a control. The experiment was laid out in randomized complete block design (RCBD) with three replications. As compared to untreated seeds, results indicated that salt-hardening treatments improved final germination percentage (FGP) to 100% in $CaCl_2$-HD treatment. KCl-HD and CaCl2-HD treatments decreased significantly mean germination time (MGT), increased mean germination rate (MGR) and coefficient of velocity of the germination (CVG). Salt-hardening treatments also increased significantly the germination index and germination rate index. However, the values of these measured indices were higher in KCl-HD treatment. Consequently, the application of KCl-HD resulted in germination value (GV), significantly greater than all applied treatments. This study demonstrates that KCl-HD treatment is effective for enhancing seed vigor of *Acacia cyanophylla* seeds.

Keywords: seed hardening, seed germination, seed vigor, *Acacia cyanophylla*

INTRODUCTION

Seed invigoration techniques are developed to enhance seed performance during germination and emergence (Yari.Zareyan, *et al.,* 2012).Hydration-dehydration is one of these techniques. The technique is also known as seed hardening. Seed hardening is a pre-sowing treatment in which seeds are hydrated for a given time followed by drying the seeds back (Rehman *et al*. ,1998b, Andoha and Kobata 2001 and Ashraf *et al*., 2008). Treated seeds may be exposed to a single cycle of wetting and drying (Barsa *et al*., 2005, Farooq *et al*., 2007,Yari *et al*., 2011, Ganesh *et al*., 2013 and Matsushima and Sakagami 2013) or two cycles of hydration and dehydration (Farooq *et al*., 2004, 2006a, 2006b; Barsa, *et al*. ,2003) or more than twice (Lee and Kim, 1999; 2000). The most often dehydrating regime is to dry the treated seeds approximately to their initial weight (Rehman *et al*., 1998b, Andoha and Kobata 2001, Demir and Mavi 2004). Re-drying the treated seeds allows pre-germinative metabolic activities to start, but radicle emergence are prevented (Khan, 1992).

Hardening has been reported to be effective treatment in the enhancement of germination, emergence, mean germination time, seedling vigor of two rice (*Oryza sativa*) cultivars (Farooq *et al*., 2004). Hardening has also resulted in the enhancement of final germination and germination rate of bay wood (*Guazuma ulmifolia*) (Brancalion *et al*., 2010), two varieties of parsley (*Petroselinum sativum*) (Podlasky *et al*. ,2003), as well as seedling vigor of rice (Barsa, *et al.* ,2003, Farooq *et al*., 2006a), and wooly brome(*Bromustomentellus*) and smooth brome (*B. inermis*) (Tavili *et al*., 2010). In addition, hardening is found to be effective in obtaining a healthy crop and increasing salt tolerance of wheat (*Triticumae stivum*) (Barsa *et al*., 2005), of mustard (*Brassica juncea*) (Bharti and Malik 2013) and four coriander cultivars (*Coriandrum sativum*) (Ben Fredj *et al*., 2013) and improving drought tolerance of several crops (Hanson 1973,Khan 1992, Halmer 2004, Matsushima and Sakagami 2013). Hardening has also been found to be more effective for seed invigoration than traditional seed soaking (Barsa, *et al*., 2003, Basra *et al*., 2005), Manjunath and Dhanoji (2011). The technique has also been claimed to be an easy, cheap and low risk technique and nowdays is being used as an alternative approach to overcome abiotic stress (Ganesh *et al.* ,2013).

Although, the available information indicates that hardening treatments are beneficial in enhancing seed vigor for many species, there is dearth information about the germination response of *Acacia cyanophylla* seeds to hardening.Thus, this present research was conducted to evaluate the effects of hardening (hydration-dehydration) treatments with different agents on seed germination behavior of *A. cyanophylla* to determine the most effective treatment that can be used locally or globally.

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MATERIALS AND METHODS

i. Germination experiment

Prior to hardening treatments, seeds of *A. cyanophylla* were mechanically scarified to overcome physical dormancy. For salt-hardening, adequate numbers of seeds were immersed in 100 ml of 10 mmole NaCl, $CaCl_2$ or KCl solutions. Soaking of seeds was conducted at room temperature for 16h. Prior to drying, salt treated seeds were washed with distilled water for five minutes to eliminate traces of salts. These applied regimes were based on the previous work done by Eshkab *et al* (2014). After soaking, the treated seeds were dried back for 16 h (approximately to their initial weight) under ambient conditions. Untreated seeds were used as a control. Hardened and unhardened seeds were placed in 9 cm diameter petri dishes on two layers of Whatman No. 1 filter papers and irrigated with 10 ml of distilled water. The Petri dishes were placed in an incubator at 25 °C and 16:8 h day / night regime. The experiment was arranged in a completely randomized design with three replications and 25 seeds per replicate. Germination was recorded every 24 h for 14 days. Germination was considered to have occurred when the radicles were 2 mm long.

ii. Germination measurements

The following are the germination parameters measured to evaluate the effects of hardening on germination characteristics of *Acacia cyanophylla*: Final germination percentage (FGP), mean germination time (MGT), mean germination rate (MGR), coefficient of velocity of the germination (CVG), germination rate index (GRI), germination index (GI).All germination indices were calculated according to formulas given by Ranal and Santana (2006) except (GI) was measured according to Arnold *et al*. (1991). Significance of differences between treatments were tested by ANOVA and means separated by Tukey's Honestly Significant Difference Test (HSD), allowing all pairs comparison for significance.

RESULTS AND DISCUSSION

The effect of hardening treatments on the germination characteristics of *A. cyanophylla* seeds is presented in table (1). The analysis of variance revealed that there were significant effects of treatments on all measured germination parameters at ($P \leq 0.05$). Among all treatments, $CaCl_2$-HD gave the highest germination percentage (100%), but this value was similar to those recorded in Nacl-HD and KCl-HD treatments, where the germination capacity of each treatment is (98.7%).

Further more MGT is the reciprocal of the rate of the germination and is used as a measure of seed vigor (Mavi, *et al*., 2010). The mean germination time (MGT) illustrates that the average length of time needed for maximum seed germination (Bewley and Black, 1994). Results clearly exhibit that salt hardening treatments decreased the mean germination time compared to untreated seeds. The lowest germination process expressed by mean germination time (MGT) reported in KCl-HD treatment. The treatment achieved their maximum germination within 4.3 days compared to 5 days in untreated seeds. Therefore, KCl-HD seeds are anticipated to have the highest seedling vigor (Ranal and Santana, 2006 and Homrani-Bakali, 2015).

The results indicate that all salt-hardening treatments increased mean germination rate (MGR) and coefficient velocity of germination (CVG) compared to unhardened seeds. The highest MGR and CVG were observed in potassium-hardened seeds. According to Ranal and Santana (2006), coefficient velocity of germination explains precisely the velocity and the spreading of germination during the whole period of germination, whereas mean germination rate is the reciprocal of the mean germination time (Ranal *et al.* ,2009). These parameters are particularly good measures when seed lots germinate equally. In addition, it has been claimed that seeds with higher MGR and CVG are anticipated to induce better seedling vigor (Ranal and Santana, 2006). Thus, although the final germination of salt hardening treatments was statistically similar, it is expected that KCl-HD seeds would produce higher seedling vigor.

Compared to untreated seeds, salt-hardening treatments also improved both germination rate index (GRI)and germination index (GI). The highest values of these indices were recorded in KCl-HD seeds (6.9 and 165.7, respectively). Germination rate index (GRI) represents the germination capacity and germination rate and is beneficial parameter for comparing samples with an equal germinability (Homrani-Bakali, 2015). Similarly, germination index (GI) measures both germination capacity and its speed. A higher GI reflects a higher germinability and germination rate (Arnold, *et al*., 1991). The treatment KCl-HD increased the germination value (GV) about 234, 149.8 and 149% of control, Na-HD and $CaCl_2$-HD seeds, respectively. Therefore, KCl-HD is the most effective treatment in invigorating *A. cyanophylla* seeds.

The obtained beneficial effect of hardening *A. cyanophylla* seeds is in line with the findings of Farooq *et al*. (2004) who observed that hardening of two cvs of rice increased final germination compared to the control. Demir and Mavi (2004) also found that seed hardening of two watermelon cultivars with KNO_3 salt effectively enhanced the final emergence and emergence rate.

Table 1. The effect of hydration-dehydration treatments on the germination indices of *Acacia cyanophylla* seeds

Germination indices	Treatments				P-value
	Control	NaCl-HD	$CaCl_2$-HD	KCl-HD	
FGP (%)	92 B	98.7 AB	100 A	98.7 AB	0.025
SE	2.31	1.33	0.00	1.33	
MGT	5.042 A	4.727 AB	4.613 BC	4.287 C	0.002
SE	0.076	0.099	0.027	0.107	
MGR	0.198 C	0.212 BC	0.217 AB	0.234 A	0.002
SE	0.003	0.004	0.001	0.006	
CVG	19.84 C	21.17 BC	21.68 AB	23.36 A	0.002
SE	0.30	0.45	0.13	0.57	
GI	137 B	154.7 A	159.7 A	165.7 A	<0.001
SE	3.00	0.882	0.667	4.84	
GRI	5.023 B	6.419 A	6.542 A	6.911 A	<0.001
SE	0.102	0.143	0.09	0.202	
GV	12.9 C	19.22 B	19.33 B	30.21 A	<0.001
SE	0.52	1.8	1.5	0.601	

The different letters within the row are statistically different at $P \leq 0.05$.
SE is standard errors.
FGP; Final germination percentage, MGT; Mean germination time, MGR; Mean germination rate, CVG; Coefficient of velocity of the germination, GRI; Germination rate index, GI; Germination index.

Salt hardening with 10 or 50 mM of $CaCl_2$, $Ca(NO_3)_2$, KCl or KNO_3 resulted in enhancement of germination percentage and rate of *Acacia tortilis* under both normal and salt stress conditions (Rehman, *et al.* ,1998a). Similarly, Barsa *et al.* (2005) reported that salt-hardening using NaCl or $CaCl_2$ improved the emergence rate and mean emergence time and seedling vigor of wheat (*Triticumae stivium* L.)cv Auqab-2000 compared to untreated control. Treating rice (*Oryza sativa*) seeds with KNO_3 for 24 or 28h and drying them back to their original weight improved the germination rate and mean germination time (Barsa, *et al.*, 2003). However, $CaCl_2$ and KNO_3 significantly increased germination percentage, germination rate, crops establishment, vigor and productivity of cotton (Patil, *et al.* ,2013). The germination rate of two wheat cultivars were also significantly enhanced by salt hardening treatments with 50 mmol solutions of $CaCl_2$, NaCl or $CaSO_4$ (Afzal *et al.* ,2008).

The improved seed vigor of *A. cyanophylla* as explained by improved final germination percentage, low mean germination time, greater mean germination rate, germination index, and germination rate index and germination value could be attributed to increased metabolic activities in the hardened seeds as suggested by Lee and Kim (2000) and Farooq *et al.* (2004), or because of positive effect of salt hardening treatments on the replications of the root tips (Barsa *et al.* ,2005; and Barsa *et al.* (2002). In addition, the improved germination percentage and seed vigor could be also attributed to better membrane repair occurred during seed hardening as suggested by Farooq *et al.* (2004), due to embryo enlargement (Austin *et al.* 1969), enzyme activation (Basra *et al.* 2005), enhancement of respiration activity (Podlasky, *et al.* ,2003).

CONCLUSION

From the present investigation, it might be concluded that seed salt-hardening particularly KCl-HD is an effective as a vigor enhancement technique for *A. cyanophylla* seeds. This regime could be adopted by nurseries locally or globally as it is effective, simple, easy and cheap method.

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الملخص العربي

زيادة قابلية وقوة إنبات بذور السنط الحقيقى بواسطة تقنية التقسية الملحية

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تقسية البذور seed hardening عبارة عن تقنية يتم إستخدامها بنجاح لتحسين قوة البذور seed vigour لكثير من المحاصيل الزراعية. ولكن هناك ندرة فى المعلومات المتعلقة بتأثير معاملات التقسية على بذور الأشجار والشجيرات. ولذلك، فقد أجريت هذه التجربة لدراسة آثار معاملات التقسية بالأملاح على إنبات وقوة بذور أشجار السنط الحقيقى *Acacia cyanophylla*. تم نقع البذور فى ١٠ مللى مول كلوريد الكالسيوم، كلوريد الصوديوم أو كلوريد البوتاسيوم لمدة ١٦ ساعة واعقب ذلك عملية تجفيف لمدة ١٦ ساعة (تقريباً للوزن الأصلى للبذور). واعتبرت البذور غير المعالجة كشاهد للمقارنة. نفذت التجربة حسب التصميم الكامل العشوائية (RCBD) مع ثلاثة مكررات. بالمقارنة مع البذور غير المعالجة تشير النتائج إلى أن معاملات التقسية بالأملاح حسنت نسبة الإنبات النهائية(FGP) حيث تم تسجيل نسبة ١٠٠% عند معاملة البذور بكلوريد الكالسيوم $CaCl_2$-HD. معاملات التقسية بواسطة كلوريد البوتاسيوم KCl-HD وكلوريد الكالسيوم $CaCl_2$-HD أدت إلى إنخفاضاً معنوياً فى متوسط زمن الإنبات(MGT)، كما أدت إلى زيادة معنوية فى كل من متوسط معدل الإنبات (MGR) ومعامل سرعة الإنبات(CVG).

معاملات التقسية بالأملاح رفعت أيضاً من قيم دليل الإنبات (GI) ودليل معدل الإنبات (GRI) بشكل معنوى، غير أن قيم هذه المؤشرات كانت أعلى فى البذور التى تم تقسيتها بإستخدام كلوريد البوتاسيوم. وبالتالى فإن معاملة وتقسية بذور السنط الحقيقى بملح كلوريد البوتاسيوم نتج عنه قيمة إنبات (GV) أعلى بكثير (بشكل معنوى) من كل المعاملات الأخرى.

هذه الدراسة أوضحت أن تقسية البذور بإستخدام كلوريد البوتاسيوم معاملة فعالة لتحسين قوة البذور فى أشجار السنط الحقيقى.

الكلمات المفتاحية: تقسية البذور، إنبات البذور، قوة البذور، السنط الحقيقى.