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Original article

Germination Test on Native Salt Tolerant Seeds (*Buchanania siamensis* Miq.) Collected from Natural Saline and Non-Saline Soil

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ABSTRACT

Salt-affected areas in northeastern Thailand have been expanding for decades due to human activities including deforestation and salt mining. *Buchanania Siamensis* Miq. from the family Anacardiaceae is one of the salt tolerant species found in northeastern Thailand. Due to benefits such as food, shading, and medicine, *B. siamensis* is a promising species for future restoration in salt-affected areas. Since germination is the most sensitive stage of plant growth, seeds germinated under high saline concentration are preferable. The aim of this study was to investigate the germination of *B. siamensis* seeds at various salt concentration levels and to compare seeds collected from saline and non-saline mother trees. The hypothesis was that seeds collected from saline areas would germinate under higher salt concentration when compared to those collected from non-saline areas. Soil and seed characteristics were investigated. Seeds collected from both locations were tested with NaCl solutions of five concentrations (0, 5, 15, 25, and 35 dS m^{-1}). The soil salt concentration, pH, bulk density, and moisture content in the saline area were significantly greater than those in the non-saline soil. Seeds collected from the non-saline area were larger in size, heavier, and had a greater moisture content when compared to seeds collected from the saline area. The total germination of seeds collected from both locations was not significantly different and was in the range of 80–95%. Germination decreased only at the highest salt concentration (35 dS m^{-1}) which was about 60–80%. High salt concentration delayed the time to germinate from 6 to 13 days after placing in the germination box. However, germination of seeds collected from the non-saline area was more rapid than those collected from the saline area when the salt concentration increased. For restoration purposes, germinated seeds under high salinity are preferred, which can be chosen from large and heavy seeds based on this study.

Keywords: *Buchanania siamensis*, germination percentage, germination rate,
salts concentration, soil salinity

INTRODUCTION

The amount of agricultural land is gradually decreasing due to soil degradation-mainly from nutrient deficiency and salinity. Inland soil salinity has expanded since a few decades ago averaging 180,000 ha per year in northeastern Thailand (Mongkolsawat and Paiboonsak, 2006, Patcharapreecha *et al.*, 1992). Apart from rock salt parent materials in arid areas, changing vegetation from forest that has a deep root system to crop plants with shallow root systems causes the salt water table to rise up close to the soil surface (Barrett-Lennard, 2002). As a result, only salt tolerant plants can survive the high salt concentration near the surface. Even though there have been many attempts to remedy salinity problems, these areas continue to expand over time. The most important problem is that the salt-affected areas are owned by locals not government; therefore, all management must be approved by the owners. Social acceptance is crucial for solving the problem by informing the locals about the benefits that they will obtain from such restoration projects.

Salinity remediation has been done using engineering techniques (such as amendment with calcium chloride, gypsum, and bagasse); installation of subsurface drainage or sprinkler irrigation systems (Jianfeng *et al.*, 2010) and also using biological techniques by planting salt tolerant species (Yuvaniyama *et al.*, 1999). The latter practice is widely recommended for long-term restoration (Biswas and Biswas, 2014, Oo *et al.*, 2011, Pagdee, 2012) because deep-rooted species would help lower the salt water table (Barrett-Lennard, 2002), reduce the soil salinity, and improve the soil bulk density (Oo *et al.*, 2011). Selection of suitable plant species to establish in salt-affected areas is necessary, especially including native species that can adapt to the saline conditions. Most plants found in salt-affected areas in northeastern Thailand are from the families Poaceae and Cyperaceae (Wongwattana *et al.*, 1999) such as *Eriochloa procera* (Retz.) C.E.Hubb., *Chloris barbata* Sw., *Cynodon dactylon* (L.) Pers., *Dactyloctenium aegyptium* (L.) P.B., and *Fimbristylis dichotoma* (L.) Vahl. ect. Thorny shrub species also are common and can be used as an indicator for salt-affected soil including *Gymnosporia mekongensis* Pierre., *Carissa spinarum* L and *Azima sarmentosa* (Blume) Benth. & Hook.f. (Sinanuwong and Takaya, 1974). Moreover, tree species thriving in saline areas include *Careya sphaerica* Roxb., *Acacia harmandiana* (Pierre) Gangnep, *Crateva adansonii* DC., *Azadirachta indica* var. *siamensis* Valeton, *Combretum quadrangulare* Kurz, and *Buchanania siamensis* Miq. (Leksungnoen, 2006).

B. siamensis belongs to the family Anacardiaceae which also contains the mango and cashew nut. It is a medium-to-large tree with a height of 5-10 m and can be found in deciduous forest at an elevation of 50-200 m (Chayamarit, 2010). *B. siamensis*, which spreads outside the forest frontier into paddy fields (Natuhara *et al.*, 2012), is also used as a vegetable and medicine for women with leucorrhoea (Chuakul *et al.*, 2002). *B. siamensis* could have been wiped out when the forested land was transformed to agricultural areas but since the locals knew its benefits, which include shading, food, and medicine, they preserved the trees near the paddy fields while razing other species. When the deep-rooted species have been replaced with shallow-rooted ones, salts from the deeper soil column are brought to the surface via capillary forces (Runyan and D'Odorico, 2010) which causes the salinity problem to the plants. *B. siamensis* has gradually adapted to salinity and can thrive in the salt-affected areas. Therefore, establishing *B. siamensis* in the salt-affected soil would help solve the problem not only at a low cost, but also with simple maintenance through social acceptance.

The ability to tolerate salts in *B. siamensis* should be studied in order to quantify its tolerance. Germination is believed to be the first and most sensitive stage of a plant's growth (Schmidt, 2007). Knowledge of germination under different salt concentrations will help to evaluate the salt tolerance of *B. siamensis* and benefit future restoration projects. Therefore, the aim of this study was to investigate the germination of *B. siamensis* seeds at various salt concentration levels. In addition, germination was tested between seeds collected from saline area (where the mother trees had already adapted to the saline conditions) collected from Kham Thale So district, Nakhon Ratchasima province and those collected from non-saline mother trees in Ta Phraya district, Sakaeo province. The hypothesis was that seeds collected from saline area would germinate under higher salt concentration when compared to those collected from non-saline area.

MATERIALS AND METHODS

Study area

The experiment was conducted in areas with varying salinity concentrations consisting of a saline area (Kham Thale So district, Nakhon Ratchasima province: 15° 04' 45.94" N, 101° 54' 2.08" E) and non-saline area (Ta Phraya district, Sakaeo province: 13° 53' 9.09" N, 102° 40' 19.45" E) (Figure 1). The two areas are about 200 kms apart. The total rainfall and average temperature in the non-saline area are greater than those in the saline area in almost every month (Figure 2). Seeds from both study sites were collected from the end of March until early April when the seeds were fully matured. The temperature in the non-saline area was 2-3 °C higher than in the saline area but the rainfall was almost two times lower in the non-saline area when compared to the saline area (Figure 2).

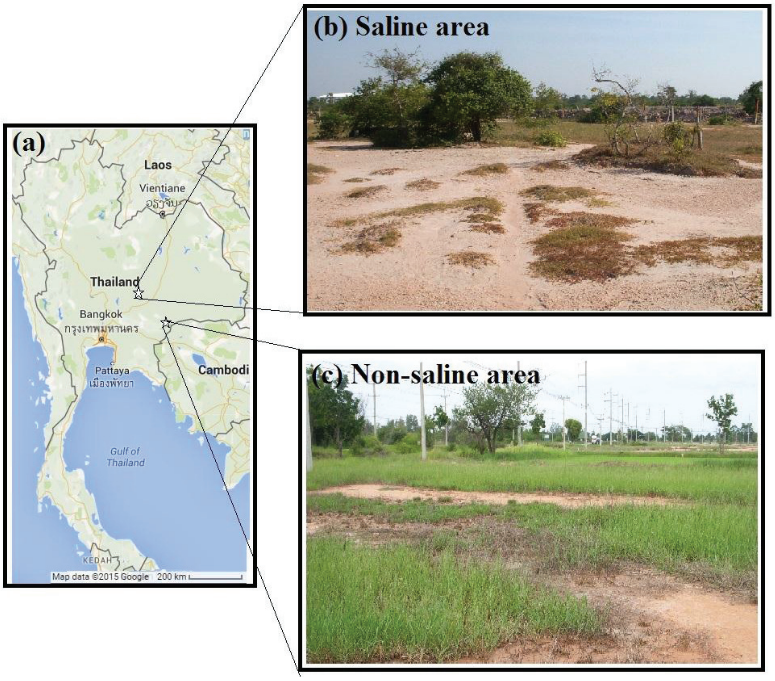

Figure 1 Study areas (a) where seeds were collected from (b) saline area (Kham Thale So district, Nakhon Ratchasima province: 15° 04' 45.94" N, 101° 54' 2.08" E) and (c) non-saline area (Ta Phraya district, Sakaeo province: 13° 53' 9.09" N, 102° 40' 19.45" E).

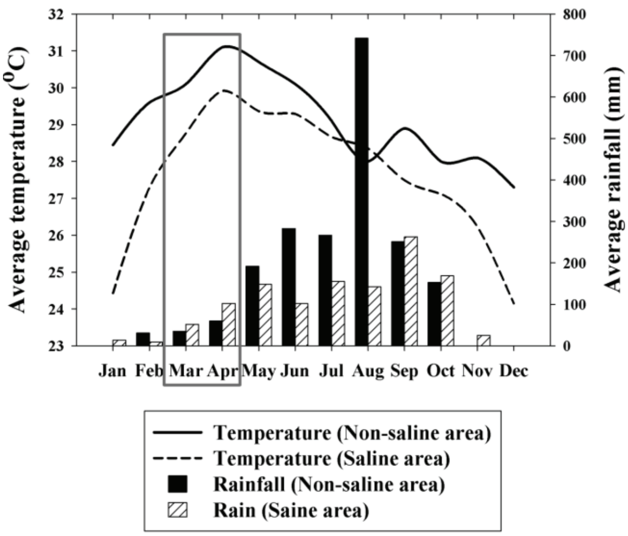

Figure 2 Monthly climatic data for 30-year period (1983-2013) of both study areas consisting of average temperature (°C) in non-saline area (Sakeao) as solid line, average temperature (°C) in the saline area (Nakhon Ratchasima) as short dashed line, rainfall (mm) in the non-saline area (Sakeao) as solid black bar and rainfall (mm) in the saline area (Nakhon Ratchasima) as cross-hatched bar. The boxed area indicates the period of seed collection in the two areas.

Seed collection and preparation

Seeds were randomly collected by hand from five trees (Figure 3a) within a radius of 1 km from the center at each location (15° 04’ 45.94” N, 101° 54’ 2.08” E and 13° 53’ 9.09” N, 102° 40’ 19.45” E). Fruit of *B. siamensis* is indehiscent with a mesocarp and only one seed (drupe). Only fully matured fruits were selected based on the dark red color of the exocarp (Figure 3e). Then, fruits were cleaned immediately with water to remove the green flesh (Figure 3f) and air-dried under the shade for 1–2 days until seeds were completely dry and ready for germination testing.

Measurements

1. Soil characteristics

Surface soil at a depth of 0-20 cm at each selected tree was sampled using a soil destructive method near the base of the tree. Soil was mixed from 0-20 cm and collected to measure the saturated soil-paste extraction electrical conductivity (EC_e), pH, and moisture content. The EC_e from the sieved soil was measured using a meter (Model Edge HI 2030 with Electrode HI 763100, Hanna Instruments Inc., Woonsocket, RI, USA), following the US Salinity Laboratory (1954) method. Distilled water was added to 50 g of sieved soil until the soil was saturated, indicated by the glittering of the soil solution and the mixture was left to saturate overnight. The saturated soil paste was passed through filter paper (Whatman® qualitative filter paper, grade 1, GE Healthcare Bio-Science, Pittsburgh, PA, USA) and only the clear liquid was used to measure the electrical conductivity using a hand-held meter (Edge®, Hanna instruments Inc., RI, USA). The pH of each soil sample was measured using a 1:1 water-to-soil ratio, using a hand-held pH/Conductivity/TDS/Salinity/Temperature meter (Model multi-parameter PCTestr™ 35, Oakton Instruments, Vernon Hills, IL, USA). The soil moisture content was measured using the weight method. Soil was weighed before and after being oven-dried at 110 °C for 48 hours. Then, the soil moisture content was calculated from the equation; (wet weight – dry weight) / dry weight x 100. The soil bulk density was measured using a soil core with a diameter of 5.7 cm and a length of 6 cm, which was inserted into the soil at a depth of 10 cm and soil was collected from a depth of 10–16 cm. Soil was weighed before and after being oven-dried at 110 °C for 48 hours. Then, the soil bulk density was calculated from the equation; (dry weight /volume of the soil core).

2. Seed characteristics

Seeds from both locations were measured for seed size, weight, and moisture content. A digital caliper (AD-5765A-150, LEGA Corporation Co., Ltd., Japan) was used to measure the width and length of 100 seeds of each species and the results were averaged. Seed weight was obtained from 100 seeds of each species using an analytical balance with a readability of 1 mg (XT620M, Precisa, Dietikok, Switzerland) with 5 replicates. The seed moisture content was measured on a wet weight basis, according to the International Seed Testing Association (ISTA) (2015). Twenty five seeds from each location were cut or ground roughly to allow the water to escape and they were then placed in a container and weighed, in order to get the wet weight. Containers were then oven-dried at 103 ± 3 °C for 17 ± 1 hr and allowed to cool down in a desiccant chamber before taking the dry weight measurement using an analytical balance with a readability of 0.1 mg (Mettler AE200, Mettler-Toledo International Inc., Leicester, UK). The seed moisture content was then calculated from the equation; (wet weight – dry weight) / wet weight × 100

3. Seed germination

Clean and dry seeds were germinated at five different salt concentrations (0, 5, 15, 25, and 35 dS m^{-1}) of NaCl solution in mid April, a week after seed collection. Salt solutions were prepared from NaCl dissolved in deionized water to obtain the target solutions using the EC meter to measure the EC_e. A clear, plastic box sized 10.5 cm × 10.5 cm (0.5 L) lined with filter papers (Whatman® qualitative filter paper, grade 1, GE Healthcare Bio-Science, Pittsburgh, PA, USA) was used to store 25 seeds from each location. Each box was saturated with target salt solutions of 20 mL. Five replicates of each location were used in this study. All germination boxes were stored in a germination chamber with the temperature maintained at 25± 3°C and relative humidity (RH) at 60-70% with no light supply for the entire experimental period. Germinated seeds were counted daily for 34 days. In this study, the seeds were considered germinated when a radicle had developed (ISTA, 2015) and was visible to the naked eye. The total germination percentage and daily germination accumulation were then calculated. The germination rate was calculated from the equation;

$$\text{Germination rate (seed d}^{-1}\text{)} = \frac{\text{Numbers of daily germation}}{\text{Number of the day}}$$

Statistical analysis

Means of soil and seed characteristics at each location were separated using the t-test method at a significance level <0.05. The experiments were established using a completely randomized design (CRD) with five replications. The total germination percentage and germination rate were subjected to testing for mean differences using analysis of variance (ANOVA) and the GLM procedure (SAS version 9.0, SAS Institute, Cary, NC). The experiment was developed as a two-way factorial with completely randomized design with five replicates within each location (two levels) and salt concentration (five levels), as the main factors. Differences among the means of main factors and interaction of locations x salt concentrations were tested using the least square difference (LSD) method at a probability level of 0.05 (SAS version 9.0; SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Genetics and the environments play crucial roles in plant behavior. Plants can acclimatize under different environments leading to genotypic variation within the same species (Van Rooijen *et al.*, 2015). *B. siamensis* is native to deciduous forest but can also adapt to saline areas. In this study, *B. siamensis* was found in saline areas with an average salt concentration of about 13 dS m^{-1} (Table 1) and it was the only salt tolerant species able to survive at such a high concentration (threshold 8 dS m^{-1})

(US Salinity Laboratory, 1954). In contrast, in the non-saline area, the salt concentration was significantly lower than in the saline area (0.3 dS m^{-1}) (Table 1). Moreover, the soil pH, bulk density, and moisture content in the saline area were also significantly greater than those in the non-saline soil (Table 1). Due to these different conditions, the seed characteristics at each location greatly varied (Table 2). Seeds collected from the non-saline area were significantly larger in size, heavier, and contained more water inside the seeds when compared to those collected from the saline area (Table 2).

Table 1 Mean soil physical and chemical analyses from saline area (Nakhon Ratchasima province) and non-saline area (Sakeao Province).

Soil characteristic	Saline area	Non-saline area	P-value
Bulk density (g m^{-3})	1.60 ± 0.01 a*	1.48 ± 0.02 b	<0.001
EC_e (dS m^{-1})	12.69 ± 4.55 a	0.3 ± 0.01 b	<0.001
pH	8.9 ± 0.5 a	6.4 ± 0.2 b	<0.001
Moisture content (% dry basis)	6.87 ± 1.20 a	4.43 ± 1.59 b	<0.001

Remark: * Mean values with different lower case superscript letters in each row are significant at $P < 0.05$.

Table 2 Seeds characteristics from saline area (Nakhon Ratchasima province) and non-saline area (Sakeao Province).

Seed characteristic	Saline area	Non-saline area	P-value
Width (mm)	10.25 ± 0.80 b*	10.81 ± 0.79 a	0.023
Length (mm)	9.41 ± 0.48 b	9.87 ± 0.69 a	0.017
Weight (g per 100 seeds)	31.24 ± 0.61 b	42.2 ± 0.80 a	<0.001
Moisture content (% wet basis)	8.59 ± 0.09 b	8.79 ± 0.12 a	0.007

Remark: * Mean values with different lower case superscript letters in each row are significant at $P < 0.05$.

Naturally, after being sown for 5 days, seeds of *B. siamensis* germinate. This germination occurred at the no salt concentration (0 dS m^{-1}) at both locations (Figures 3g and 4) because the thick seed coat (about 1–2 mm) allowed low water and gas permeability into the seed. Total seed germination was about 80–95% which is promising for future reproduction in salt-affected area revegetation. Germination is classified as being of the hypogeal type, as the cotyledons or storage tissues remain beneath the soil and the epicotyl rapidly elongates to bring the plumule above the ground. After 10-12 days, germinated seeds were ready to be transferred to the emergent stage (Figure 3g). According to the hypothesis, the germination percentage of *B. saimensis* in a saline habitat should be higher than in the natural habitat due to the adjustment to the high salt concentration. However, the results diverged from this hypothesis. The germination percentages in seeds collected from both locations were not significantly different (Figure 4 and 5a). Moreover, at higher salt concentration levels (> 15 dS m^{-1}), the total germination percentages of seeds collected from the non-saline area was slightly higher than those collected from saline area (Figure 5a).

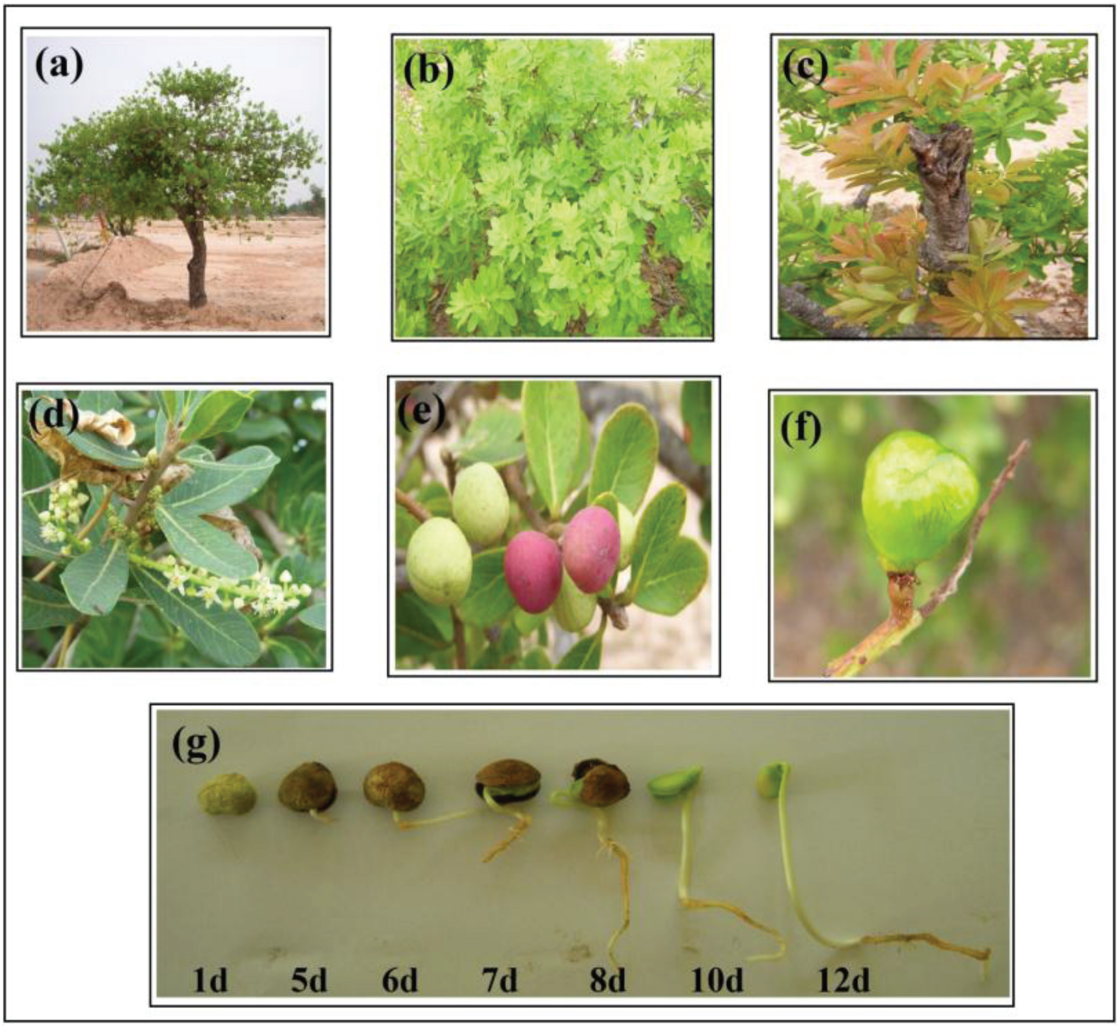

Figure 3 *Buchanania siamensis* Miq. (a) whole plant shape with a large round crown cover that is suitable for landscape decoration (b) simple leaf with alternate arrangement sized 4-8 cm in length and 2-3.5 cm in width (c) new, red young leaves that provide food for local people (d) axillary inflorescencs 4-8 cm in length and white flowers (e) mature fruit (green color) and ripening fruit (red color) with green mesocarp and only one seed (drupe) (f) green mesocarp and heart-shaped seed (g) stages of germination from days 1 to 12 (hypogeal germination).

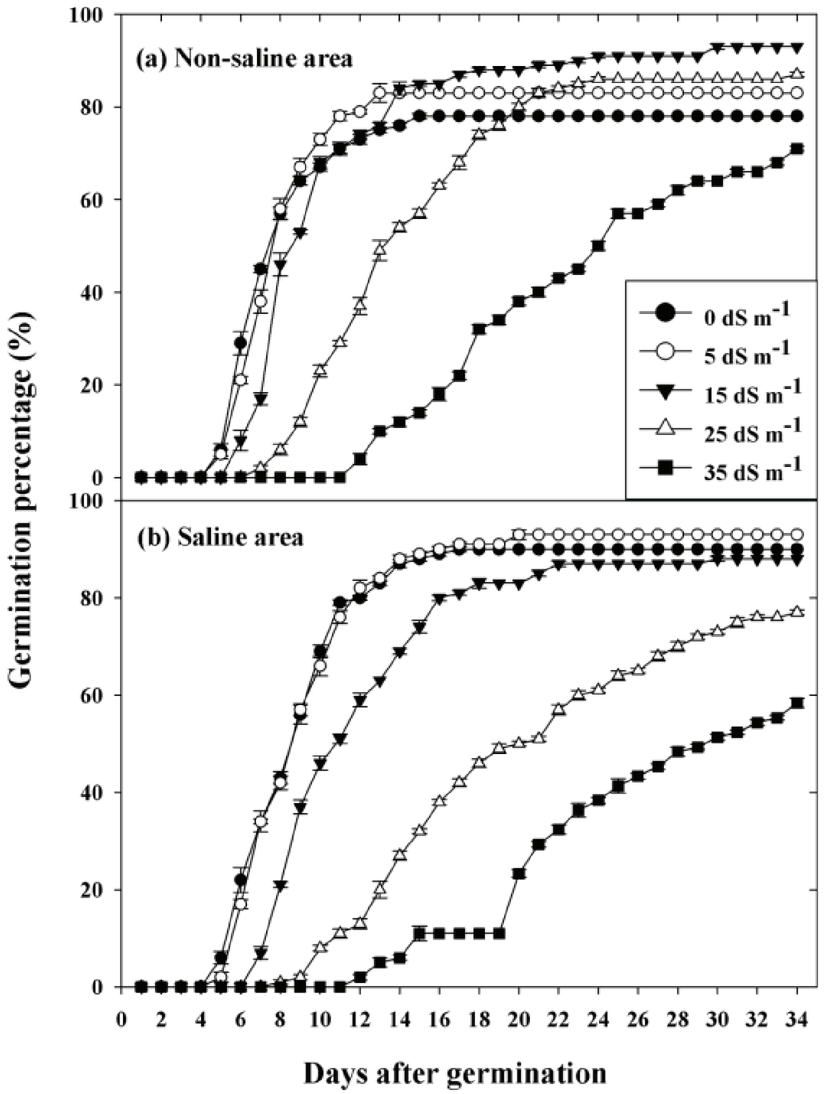

Figure 4 Accumulated germination percentage under five salt concentrations of seed collected from (a) non-saline area (Sakeao) and (b) saline soil (Nakhon Ratchasima) for 34 days (no germinated seed after day 34th).

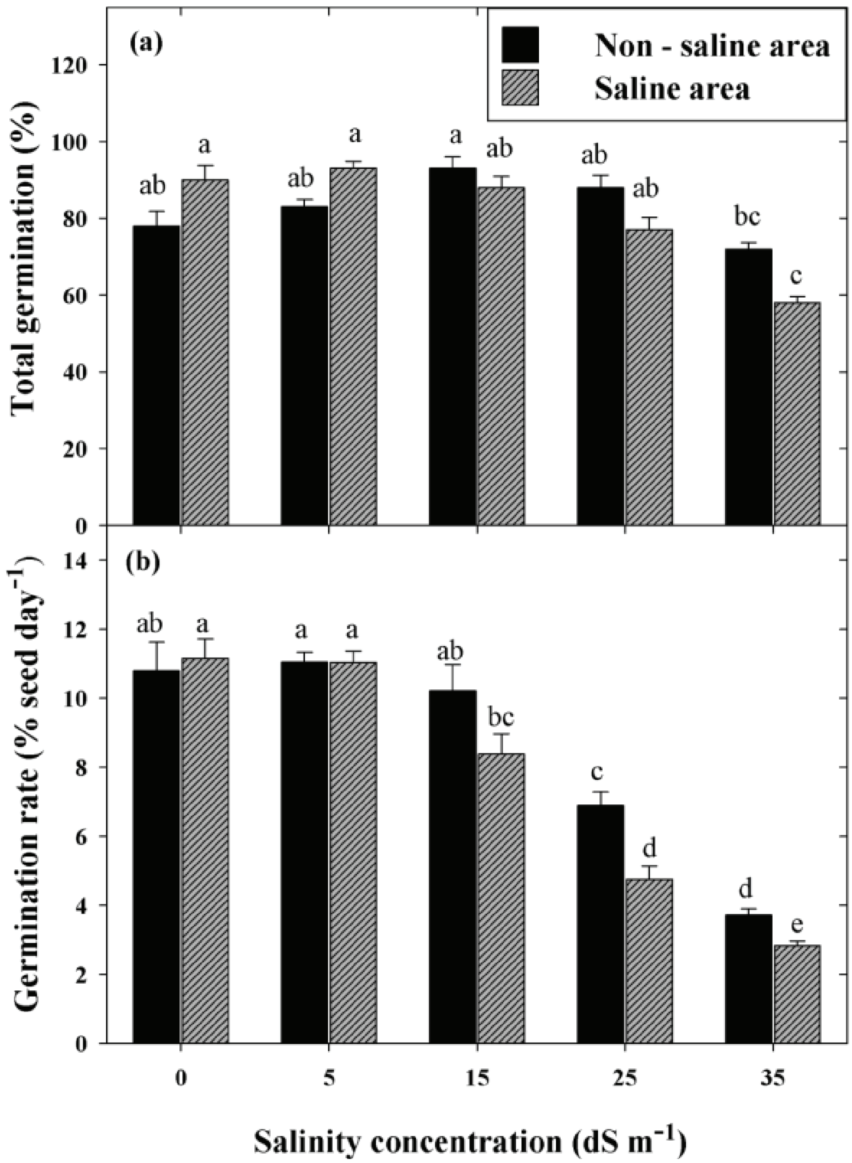

Figure 5 Derivative calculation from accumulated germination percentage (Figure 4) of seed collected from non-saline area (Sakeao) and saline soil (Nakhon Ratchasima) (a) total germination percentage based on the accumulation germination from day 1 to 34 and (b) germination rate calculated from the slope of accumulated germination percentage which it indicates the speed of seed germination. Values with Different lower case letters in each figure (a and b) are statistically differenes in means of interaction between locations (non-saline and saline areas) and salt concentration levels (0, 5, 15, 25, and 35 dS m^{-1}) at $P < 0.05$.

Salt decreases the water potential (more negative) in solution resulting in difficulty for seeds to absorb water during the imbibition stage (first stage of seed germination). Various studies reported that a high salt concentration delays germination (Katembe *et al.*, 1998, Nizam, 2011, Ye *et al.*, 2005) which also occurred in this study (Figure 4). At the highest concentration in this study (35 dS m^{-1}), seeds needed 12–13 days to germinate while a lower concentration of 15 and 25 dS m^{-1} required 6–8 days to germinate (Figure 4). The seed germination percentage of *B. siamensis* is considered high (being above 80%) at most salt concentration levels (Figure 5a). The germination rate (Figure 5b) indicates how fast the seeds germinate and can be calculated from the slope of the germination percentage curve (Figure 4). Even though the germination percentage was not statistically different until it reached the highest salt concentration level, the germination rate of seeds collected from both locations decreased rapidly at a salt concentration above 15 dS m^{-1} (Figure 5b). High salt concentration not only delayed the germination but also reduced the speed of germination (Nizam, 2011). Seeds collected from the saline area required more time to germinate (lower speed) at a high salt concentration, as indicated by the lower germination rate as the salt concentration increased when compared to the seeds collected from the non-saline area (Figure 5b).

Overall, seeds collected from the non-saline area were superior to those collected from the saline area in terms of their greater total germination percentage and more rapid germination rate under higher salt concentration levels (Figure 4 and 5). Soil and seed characteristic differences affected the germination behavior of seeds collected from both locations. Larger seeds from the non-saline area contain more storage food when compared to smaller seeds from the saline area resulting in the more energy to lower their water potential to take up water from the salt solution during the imbibition stage (Croser *et al.*, 2001). As a consequence, the germination rate of non-saline seeds was more rapid than that of saline seeds due to more energy being needed to complete the germination processes. Additionally, a greater moisture content in the non-saline seeds helped with rapid germination because recalcitrant seed, like *B. siamensis*, needs to germinate right after ripening time when the moisture content is still high (Schmidt, 2007). Also the large, heavy seed contributed partially to the high moisture content as shown in the non-saline seeds (Table 2).

Smaller seeds in the saline area (Table 2) could be a result of the high salt concentration in the soil and sodium toxicity (Table 1) that directly affected the growth of plants and also seeds (Mer *et al.*, 2000). Plants grown in a saline area spend most of their energy in their roots in order to extract the water from the high salt concentration soil solution with less energy to the shoot, leading to a high root:shoot ratio (Tuna *et al.*, 2007). Along with high soil bulk density, which explains how compact the soil is, the roots of plant grown in saline soil required more energy to penetrate the compact soil to access the water and nutrient in the soil solution. A high pH also affects soil nutrition. The soil pH for optimum plant growth is between 6.2 and 7.3 (Havlin *et al*., 2005). Alkaline (high pH) or acid (low pH) causes either nutrient deficiency or toxicity, with a high pH leading to phosphorus, iron, manganese, zinc, and copper deficiency (Rengasamy *et al.*, 2003). Toxicity of boron and molybdenum also occurs when the pH is higher than 8.5 (Havlin *et al.*, 2005). Consequently, plants grown in the saline area experienced nutrient deficiency and toxicity leading to adverse plant and seed growth.

CONCLUSION

B. siamensis seeds germinated after sowing for 5 days and the germination was classified as being of the hypogeal type, as the cotyledons or storage tissues remain beneath the soil, while the epicotyl rapidly elongates to bring the plumule above the ground. After 10–12 days, the germinated seeds were ready to be transplanted. The total germination of seeds collected from the non-saline (0.3 dS $m^{-1}$and pH 6.4) and saline areas (13 dS m^{-1} and pH 8.9) was not significantly different, ranging from 80 to 95%. Germination decreased only at the highest salt concentration (35 dS m^{-1}) which was about 60–80%, but the time for germination increased also as the salt concentration increased from 6 to 13 days after placing the seed in the germination box. However, the germination rate or the speed of seed germination of seeds collected from the non-saline area were more rapid than for those collected from the saline area when the salt concentration increased. Seeds collected from the non-saline area were larger in size, heavier, and had a greater moisture content when compared to seeds collected from the saline area.

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