Germination Traits of *Millettia Pinnata (L.)* in Response to Salinity

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Abstract: *Millettia pinnata* is a mangrove associates and found to occur in the Sundarbans mangrove forest of Bangladesh. We have examined the effect of salinity on seed germination of this species. Seed germination experiment was conducted with different salinity levels (0 to 35 ppt) at 5 ppt interval. Throughout the experiment Cumulative Germination Percentage (CGP) varied from 73.89% to 27.78% among 0 to 10 ppt and from 17.22% to 4.44% between 15 to 20 ppt salinity. Final germination percentage (FGP) of *M. pinnatta* seeds varied significantly ($p<0.05$) among saline treatments and comparatively higher (73.89%) final germination percentage was observed at non saline condition (0 ppt) and germination percentage was sharply decreased to 32.78% and 22.78% for salinity of 5 and 15 ppt salinity respectively, but no germination was observed for higher salinity levels (25 to 35 ppt). Germination Initiation Time (GIT) of seeds varied significantly ($p<0.05$) among salinity levels. GIT was the lowest (5 days) at 0 ppt and highest (10 days) at 20 ppt salinity level. Mean Germination Time (MGT) of seeds didn't vary significantly ($p>0.05$) among salinity levels. MGT was the lowest (9 days) at 0 ppt and highest (12 days) at 20 ppt salinity level. Germination Index of seeds varied significantly ($p<0.05$) among salinity levels. GI was the highest (2.60 seed/day) at 0 ppt and lowest (0.12 seed/day) at 20 ppt salinity level. Germination value (GV) of seeds varied significantly ($p<0.05$) among salinity levels. GV was the highest (30.90%/day^2) at 0 ppt and lowest (0.15%/day^2) at 20 ppt salinity level. Therefore, we concluded that the salinity is a critical factor for germination of seed and it reduces germination success with increasing salinity either by inhibiting water absorption or by creating ion toxicity on seed.

Keywords: Germination, mangroves, *Millettia pinnata*, salinity, Sundarbans.

1. INTRODUCTION

The Sundarbans is the single largest tract of mangrove forest in the world [1, 2] and supports a wide diversity of flora and fauna. It is highly productive and complex ecosystem in the tropical and subtropical coastal zones [3] which serves as a link between terrestrial and estuarine aquatic ecosystems [4]. It plays an important socio-economic and ecological role [5, 6]. Generally, mangroves are categorized as true mangrove species that are limited to the mangrove habitat and mangrove associates that are mainly distributed in a terrestrial habitat but also occur in mangrove ecosystem [7, 8]. Species composition and their diversity in the Sundarbans mangrove forest are determined by several factors but salt is an outstanding environmental feature in this ecosystem [9]. Water salinity not only regulates the distribution of the species [10] but also influences the morphology, physiology and biochemical processes of mangroves and mangrove associates [11]. Seed viability and germination of mangrove species depend on the level of salinity [9, 12-15]. Soil salinity affects seed germination through preventing water uptake to germinating seed or by toxic effects of Na^+ and Cl^- ions [16].

Recently, salinity is increasing in the Sundarbans mangrove forest and in the coastal areas of Bangladesh due to the climate change consequences (increased temperature enhances evaporation, less rainfall, sea level rise); reduce fresh water flow from upstream etc. [17, 18]. Environmental degradation in coastal areas has stimulated an increased interest in establishing plantations on degraded coast areas [19].

Millettia pinnata is an associate mangrove tree species and found to grow with *Heritiera fomes*, *Excoecaria agallocha*, *Hibiscus tiliaceus*, *Brownlowia tersa*, and with *Clerodendrum inerme* and *Dalbergia spinosa* in the less saline and moderate saline zones of the Sundarbans [20, 21]. This species is capable of occurring in both littoral and terrestrial habitats [22-24]. It has capacity of nitrogen fixing, good coppicing ability, control soil erosion for its dense network of lateral root system, and a potential source of biodiesel [24-28]. Moreover, this species can withstand in waterlogged areas [29]. Considering these characteristics, *M. pinnata* has appeared as an important species for the Sundarbans mangrove ecosystem and saline influenced coastal areas for the protection of embankment and riverbank areas.

Seed germination is one of the most important and critical stage of the life cycle of a plant [11]. Knowledge about salinity influences on germination of this species is quite important to understand the dynamics of seed

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germination of this species which control the natural occurrence in the Sundarbans and coastal areas. Therefore, present study was design to explore the implications of salinity on germination traits of *Millettia pinnata*, which may help us to predict the fate of this species in accordance to change in salinity in the Sundarbans and coastal areas of Bangladesh.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The Sundarbans is located between 21° 30' and 22° 30' N latitudes and between 89° 00' and 89° 55' E longitudes. It is a unique habitat for a wide diversity of flora and fauna [30] and has been declared as world's heritage site by UNESCO in 1997 [31]. Based on the level of soil salinity, Sundarbans is divided into less saline (LS), moderate saline (MS) and strong saline (SS) zones having salinity <2dS/m, 2-4dS/m and >4dS/m respectively [32]. Rainfall is strongly seasonal (from May to October) with 87% of the mean annual rainfall (1500mm). Temperature ranges from 18.50 to 35.20°C in summer and from12.20 to 28.80°C in winter. Soil is silty to sandy clay loam, and bulk density, particle density and porosity vary from 1.18 to1.27g/cc, 2.31 to 2.52g/cc and 46–52%, respectively. Soil pH is 7.8 [32].

Figure 1: Flowers (Left) and Seeds (Middle and right) of *Millettia pinnata.*

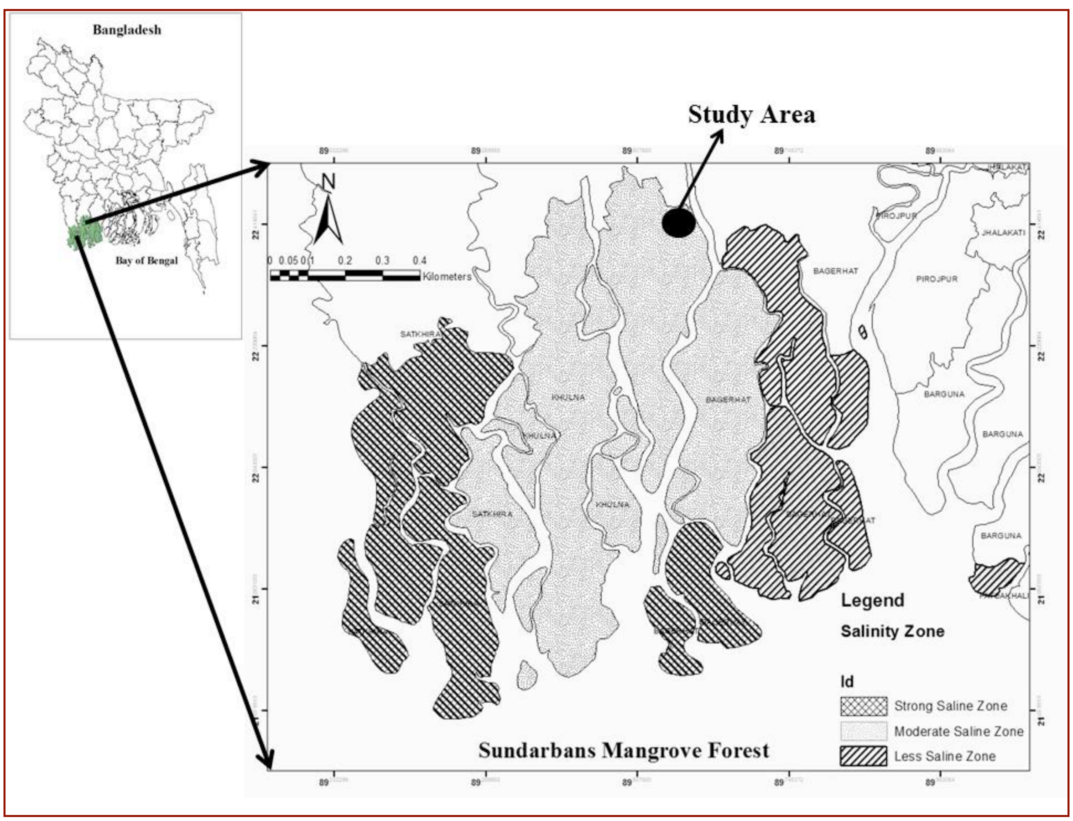

Figure 2: Map of Study area in the Sundarbans Mangrove Forest in Bangladesh.

2.2. Seed Collection

Mature fruits (Figure **1**) of *M. pinnata* were collected from the moderate saline zone of the Sundarbans (Figure **2**). Seeds were sorted and defective ones were discarded.

2.3. Experimental Setup

Forty eight germination trays (75cm×75cm×6cm) were prepared. The trays were filled with 3cm thick layer of coarse sand. Each tray contained 30 seeds. Crude sea salt was used for preparing saline treatments. Eight levels of saline treatments (0, 5, 10, 15, 20, 25, 30 and 35 ppt) with six replications were applied randomly to the germination trays. Salinity level in each treatment was checked and corrected at every 24 hours interval. Mean temperature and relative humidity during the experimental period were recorded as 36.50°C and 70.50% respectively. Initiation of root was considered as germination. Number of germinated seeds were counted and recorded at 24 hours interval.

2.4. Germination Traits

Cumulative Germination Percentage (CGP), Final Germination Percentage (FGP), Germination Initiation Time (GIT), Mean Germination Time (MGT), Germination Index (GI) and Germination Value (GV) were calculated separately for seeds. The formulas of each category are as follows:

$$GP\ (\%) = \frac{\text{Number of germinated seeds at a particular day}}{\text{Total number of sown seeds}} \times 100 \quad (1)$$

Here, GP is the germination percentage. Then from GP (%) Cumulative Germination Percentage (CGP) were calculated.

$$FGP\ (\%) = \frac{Number\ of\ germinated\ seeds\ at\ the\ end\ of\ the\ test}{Total\ number\ of\ sown\ seeds} \times 100 \quad (2)$$

$$GIT\ (day) = Day\ of\ first\ count - Day\ of\ seed\ sowing \quad (3)$$

$$MGT\,(day) = \frac{\sum n_i d_i}{\sum n_i} \quad (4)$$

Where, n_i = number of germinated seeds in d_i; d_i = number of days after seed sowing;

$$GI\ (seed/day) = \sum \frac{n}{d} \quad (5)$$

Where, n= number of seedling emerging on day d; d= day after seed sowing to find out vigorous seed and higher value of it indicates good quality;

$$GV\ (\%^2/day^2) = (Final)\ MDG \times PV - [35, 36] \quad (6)$$

Where, MDG is the Mean Daily Germination, PV is the maximum mean daily germination reached at any time during the test period [37].

2.5. Statistical Analysis

FGP and GV values were transformed to Arcsine. FGP, GIT, MGT, GI and GV values at different salinity levels were compared by One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) and correlation using MS Excel and SAS 6.12 statistical software.

3. RESULTS

3.1. Cumulative Germination Percentage (CGP)

Cumulative Germination Percentage of *M. pinnata* seed was the highest at 0 ppt salinity and lowest at 20 ppt salinity. Throughout the experiment CGP varied from 73.89% to 27.78% among 0 to 10 ppt and from 17.22% to 4.44% between 15 to 20 ppt salinity levels within 16 days of seed sowing. No germination was recorded at salinity 25 to 35 ppt (Figure **3a**).

3.2. Final Germination Percentage (FGP)

Final Germination Percentage of seed varied significantly ($p<0.05$) among salinity levels. Highest germination (73.89%) was found at 0 ppt and the lowest (4.44%) was at 20 ppt salinity level. Strong negative correlation ($r = -0.88$) was observed between salinity levels and FGP (Figure **3b**).

3.3. Germination Initiation Time (GIT)

Germination Initiation Time of seeds varied significantly ($p<0.05$) among salinity levels. GIT was the lowest (5 days) at 0 ppt and highest (10 days) at 20 ppt salinity level. Positive correlation ($r = 0.78$) was observed between salinity levels and GIT (Figure **3c**).

3.4. Mean Germination Time (MGT)

Mean Germination Time of seeds didn't vary significantly ($p>0.05$) among salinity levels. MGT was the lowest (9 days) at 0 ppt and highest (12 days) at 20 ppt salinity level. No correlation ($r = 0.16$) was found between salinity levels and MGT (Figure **3d**).

3.5. Germination Index (GI)

Germination Index of seeds varied significantly ($p<0.05$) among salinity levels. GI was the highest

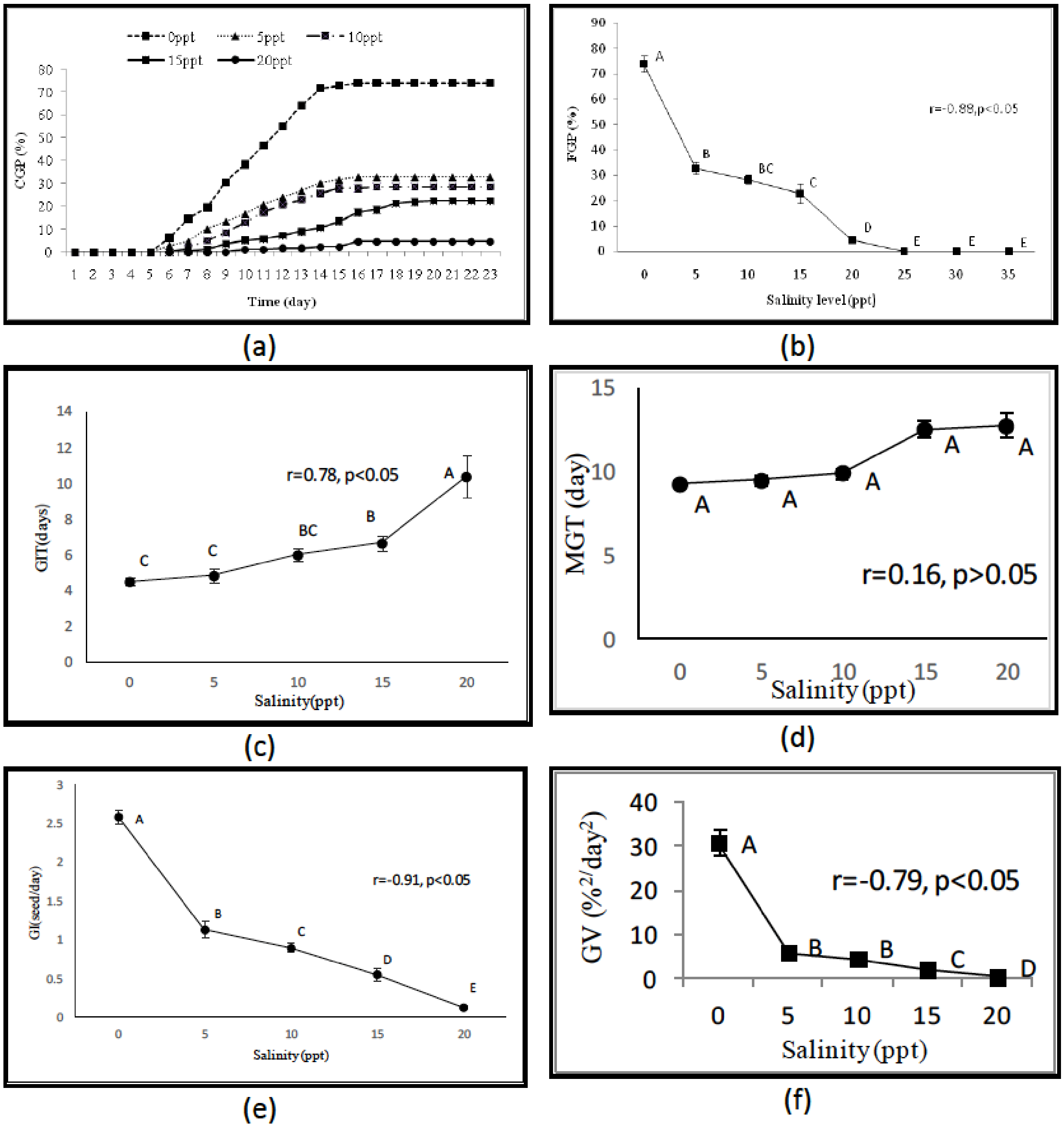

Figure 3: Germination traits of *Milletta pinnata* (a) CGP (%) (b) FGP (%) (c) GIT (day) (d) MGT (Day), (e) GI (seed/day), (f) GV ($\%^2/day^2$) at different level of salinity. Means with the same letter are not significantly different (DMRT). Vertical bar shows standard error. Value of r shows correlation.

(2.60 seed/day) at 0 ppt and lowest (0.12 seed/day) at 20 ppt salinity level. Strong negative correlation (r = -0.91) was found between salinity levels and GI (Figure **3e**).

3.6. Germination Value (GV)

Germination value of seeds varied significantly ($p<0.05$) among salinity levels. GV was the highest (30.90$\%^2/day^2$) at 0 ppt and lowest (0.15$\%^2/day^2$) at 20 ppt salinity level. Negative correlation (r = -0.79) was found between salinity levels and GV (Figure **3f**).

4. DISCUSSION

Germination is the most critical period for a species in saline affected areas [11]. The precise salinity level causes delay and reduction in the proportion of seed germination which also depends on the salt tolerance capacity of a species [38]. The initiation of germination is delayed due to increased salinity which creates high osmotic potential in the germination medium that affects imbibitions; induce Na^+ toxicity on seeds [39, 40, 16]. Salinity reduces water availability or preventing water uptake for germination which influences enzyme activity and cell division [41]. Salinity has inhibitory effect on seed germination and increased salinity causes increased GIT and MGT as well as decreased GI and GV which is finally responsible for decreasing patterns in CGP and FGP of *M. pinnata* seeds. Similar observation was also reported by [9] with different mangrove species (*H. fomes*, *Xylocarpus mekongensis*, *X. granatuma* and *A. cucullatta*). Increased salinity reduces the germination of both halophyte and glycophyte seeds [39]. In case of glycophyte, germination of *Acacia tortilis* and *Acacia oerfota* decreased by increasing salinity [42]. Similar observation also found for germination percentages under various NaCl concentrations which were decreased in both lentil cultivars after 7 days of treatment [43].

CONCLUSION

Salinity inhibits seed germination by reducing germination vigor and speed of germination. In conclusion, this species can successfully germinate upto 15 ppt salinity, but 0 to 5 ppt salinity appeared to be a favorable range of salinity for seed germination.

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