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Effect of Mother Tree Size on Salt Tolerance of *Melia azedarach* Seeds

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Abstract: The size of mother tree is an important factor affecting seed quality. In this study, the seeds of 18-year-old neem mother trees with different DBH without obvious disease in Taishan District, Tai'an City, Shandong Province were collected, the tree height and DBH were measured, the seed size characteristics were compared, and the seed germination law under different salt concentrations was studied under different salt concentrations. The results showed that the size of mother trees had significant effects on the maximum length, maximum width and 1000-seed weight of seeds, and the above indexes of small and medium diameter classes mother trees were significantly greater than those of large diameter class mother trees. With the increase of salt concentration, the germination rate and germination index decreased significantly, and the dormancy rate increased significantly. The medium diameter class mother trees had the highest germination rate and the lowest dormancy rate among different diameter classes. Therefore, the seed quality of medium diameter class mother tree for *M. Azedarach* is the best and has the strongest salt tolerance. This study revealed that, on the whole, the seed quality of the middle DBH mother tree was the best, with a large 1000 seed weight, the highest germination rate and the lowest dormancy rate, as well as the strongest salt tolerance.

Keywords: *Melia azedarach*, Mother Tree Size, Salt Stress, 1000-Seed Weight, Seed Germination

1. Introduction

Neem (*Melia azedarach*) is an important afforestation tree in China and an excellent urban street tree [1]. It has strong salt tolerance and is widely used in coastal saline alkali areas [2]. The size of the mother tree has an important impact on the seed quality, and the summary of several tree species shows that the impact law is inconsistent [3]. The research shows that the seeds of Melia azedarach have certain salt tolerance, but are affected by the growth environment of the mother tree and the seed source [4-6]. At present, the main seedling raising method of Melia azedarach is field sowing, so the guarantee of seed quality is very important [7-9]. In order to provide theoretical support for the selection of high-quality seeds, this paper compared the germination ability of Melia azedarach mother trees with different sizes under salt stress and discussed the effect of mother tree size on the salt resistance of seeds.

2. Material and Method

2.1. Overview of the Study Area

Tai'an City, Shandong province belongs to temperate

continental semi humid monsoon climate zone, with an annual average temperature of 12.8°C. The highest temperature in July is 25.3°C, and the lowest temperature in January is 2.6°C. The annual average sunshine duration is 2622.9h, and the interannual variation is 2356.3 ~ 3421.7h. The annual average precipitation in this area is 684mm. The average frost free period is 196 days, with a maximum of 242 days and a minimum of 162 days.

2.2. Seed Collection and Treatment

In April 29, 2019, 18-year-old neem trees without obvious disease were selected in Taishan District, Tai'an City, Shandong Province, and the tree height and DBH were measured. According to the measurement results, the mother tree is divided into three diameter classes according to the DBH. Select 5 mother trees for each diameter class to collect seeds. See Table 1 for the size characteristics of mother trees. Neem seeds are drupe fruits with hard endocarp. When taking seeds, first fix them with pliers, then gently smash them longitudinally with a hammer, take out the seeds, and select full seeds for the test. Select full seeds, measure the 1000 grain weight of each diameter class, and randomly select 15 seeds to measure their maximum length and width.

Table 1. *Size characteristics of mother tree of neem seed collection.*

	DBH (cm)	Tree height (m)
I	19.9 – 23.4	50.2
II	29.8 – 31.9	51.5
III	38.5 – 41.1	52.4

2.3. Test Method

Seed germination test began in late April 2019. In this experiment, the seeds of each mother tree diameter step were germinated at NaCl concentrations of 0 (CK), 1‰, 3‰, 5‰, and 7‰. Each treatment was repeated for 5 times, with a total of 75 culture dishes. Clean the culture dish and sponge and put them into the autoclave for disinfection together with the cut filter paper. Soak the seeds of different mother trees in 5‰ potassium permanganate solution for 20min, wash the seeds with deionized water until they are colorless, soak the seeds with deionized water under normal temperature for 24h, and then put them on a tray. Place 40 seeds in each culture dish. The culture dish was placed in a constant temperature light incubator (temperature 25°C±0.5°C) for 21 days. During the germination process, it was observed once every 24 hours, and the germination number of each plate of seeds was recorded. Regularly check the water condition in the Petri dish and replenish water once every 1 day. The standard of seed germination was 1 / 2 of the seed length after the radicle broke the seed coat and began to record. At the end of 21 days, the seed germination number was counted and the germination rate was calculated. Germination index (Gi), and average germination time (MGT) were calculated.

$$Gr = G1/N \times 100$$

where G1 is the number of germination; N is the total number of tested seeds.

$$MGT = \Sigma(D \times n_i)/ \Sigma n_i$$

where: n_i is the number of normal germinated seeds on the corresponding day, and D is the number of days since the seeds were placed in the bed.

$$Gi = \Sigma (n_i / D)$$

where: D is the corresponding germination day.

After the seed germination test, the viability of the non germinated seeds in the culture dish was measured with red tetrazolium (TTC) at a concentration of 0.1%. Cut the non germinated seeds crosswise, put half of them into a clean Petri dish, and the other half of the seeds for standby. Add TTC solution to the culture dish, and it is appropriate to immerse the seeds, and then put them into a 30°C incubator for 3 hours. After the color development time, pour out TTC solution and rinse it with tap water for 2-3 times to observe the coloring of embryos and judge the viability of seeds according to whether they turn red. The percentage of dormant seeds was calculated.

2.4. Data Analysis

All data were analyzed with SPSS17.0 software (SPSS, Chicago, IL, USA). The maximum length, maximum width and 1000 grain weight of seeds were compared by one-way ANOVA. The germination rate, germination index, average germination time and dormancy rate were compared by two-way ANOVA. Since the germination rate of seeds of I and III under 7‰ salt concentration was 0, the difference of average germination time from CK to 5‰ salt concentration was compared by two-way ANOVA. If the difference is significant, the least significant difference method (LSD) was used for multiple comparisons. The significance difference test level was $p < 0.05$.

3. Results and Analysis

3.1. Seed Size Characteristics of Mother Trees with Different DBH

With the increase of the diameter class of the mother tree, the maximum length, maximum width and 1000 grain weight of the seeds increased first and then decreased (Figure 1). The results of variance analysis showed that the size of mother tree had significant effects on the maximum seed length ($F = 39.89$, $P < 0.01$), the maximum seed width ($F = 22.17$, $P < 0.01$) and the 1000 grain weight ($F = 22.71$, $P < 0.01$). The maximum seed length was I < III < II ($P < 0.01$); The maximum seed width and 1000 grain weight of I and II were not significantly different ($P > 0.05$), but they were significantly greater than III ($P < 0.01$).

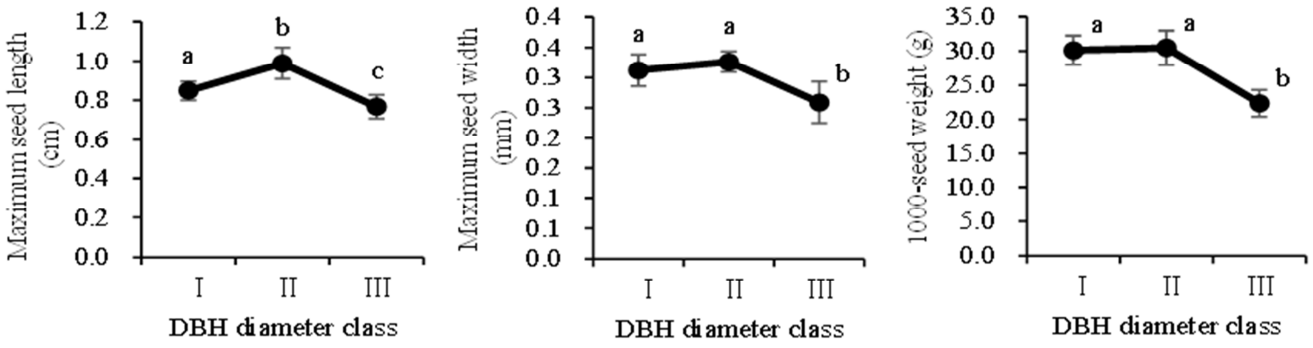

Figure 1. *Characteristics of seed size with increasing DBH of parent tree.*

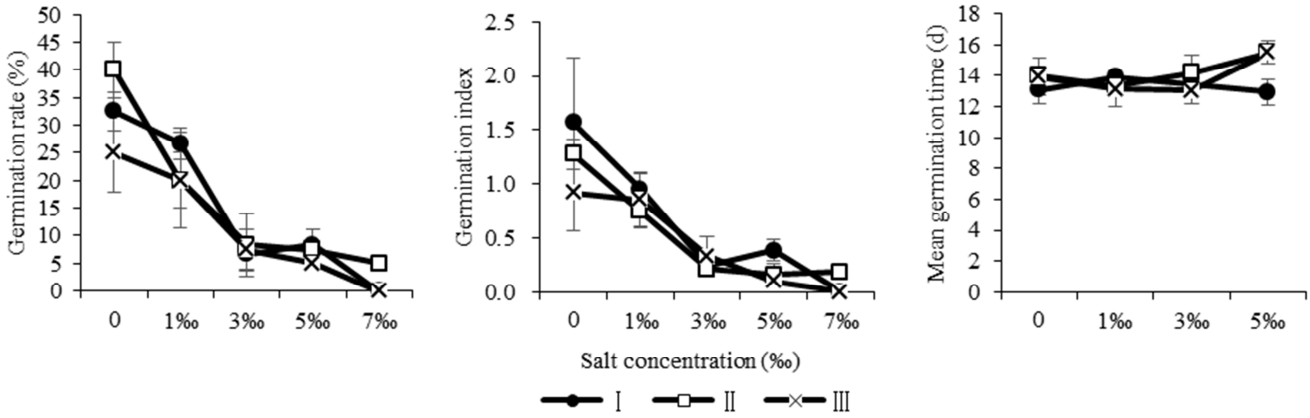

Figure 2. *With the increase of salt concentration, the germination indexes of different size mother tree seeds changed.*

3.2. Effects of Mother Tree Size and Salt Stress on Seed Germination

The results of variance analysis showed that the size of the mother tree had a significant impact on the germination rate, and the salt concentration had a very significant impact on the germination rate and germination index, but the size of the mother tree and the salt concentration had no significant impact on the average germination time (Table 2). Among different sizes of mother trees, DBH diameter class II was significantly larger than diameter classes I and III, but there was no significant difference between I and III ($P > 0.05$, Figure 2). With the increase of salt concentration, the germination rate and germination index of seeds with different DBH classes decreased significantly, and the average germination time fluctuated (Figure 2). The germination rate decreased sharply when the salt concentration was 3‰. When the salt concentration was 7‰, the diameter classes I and III were 0, and the diameter class II was close to 0.

3.3. Effects of Mother Tree Size and Salt Stress on Seed Dormancy

The results of variance analysis showed that the size of the mother tree had a significant impact on the seed dormancy rate, and the salt stress had a very significant impact on the seed dormancy rate, and the two had a very significant interaction (Table 2). The dormancy rate of different mother trees was I > III > II ($P < 0.05$). With the increase of salt concentration, the seed dormancy rate increased significantly, and increased sharply at the salt concentration of 3‰ (Figure 3). In terms of interaction, the higher the salt concentration, the closer the seed dormancy rate of different mother tree sizes (Figure 3).

Table 2. *Two factor variance analysis of the effect of the size of the mother tree and salt concentration on germination index of the neem seeds.*

Index	Mother tree size		Salt stress		Mother tree size × Salt stress	
	F	P	F	P	F	P
Germination percentage	3.73	<0.05	65.27	<0.01	2.02	0.08
Germination index	3.09	0.06	51.37	<0.01	1.81	0.12
Average germination time	2.69	0.09	2.61	0.08	2.12	0.08
Dormancy rate	4.28	<0.05	318.55	<0.01	7.09	<0.01

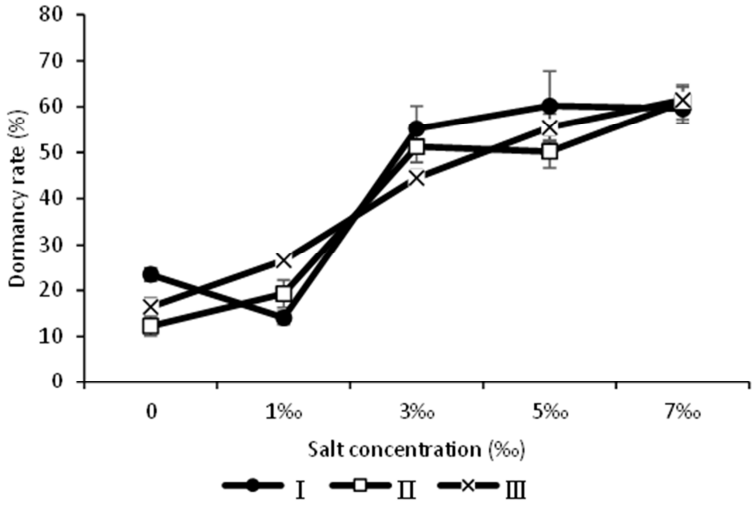

Figure 3. *With the increase of salt concentration, the dormancy rate of different size mother tree seeds changed.*

4. The Change of Inheritance Mechanism of Wood Carving Industry in the Process of Urbanization

It was found that the size of mother tree had a significant effect on the quality of neem seeds. With the increase of DBH of the mother tree, the seeds of diameter class I and II have larger maximum length, maximum width and 1000 grain weight, and the seeds of diameter class III are the lowest. It is found that the seed (fruit) size increases first and then decreases with the increase of the DBH of the parent tree [10], Sihemu [11] and Taibai Sequoia [12]. Wang Xiaoan et al. [12] believe that the reduction of 1000 seed weight is related to the reduction of reproductive input of the mother tree. In terms of germination rate, the middle DBH II of Melia azedarach is the highest, which is consistent with the studies of isatidis [10], pangdahai [13], Pinus echinata [14] and Sorbus torminalis [15]. Under salt stress, with the increase of salt concentration, the germination rate and germination index of Melia azedarach seeds significantly decreased, which is consistent with the research results of Wu Liyun, Cao Banghua [3], Zhang Zihan and others [4]. As for the germination rate, it also increased significantly with the increase of salt concentration, which was related to the inhibition of the germination process, but in the dormancy rate, DBH II was the lowest. Therefore, on the whole, DBH II has the best seed quality, which is reflected in the larger 1000 grain weight, the highest germination rate and the lowest dormancy rate, and the strongest salt tolerance.

5. Conclusion

In this study, the seeds of 18-year-old neem mother trees with different DBH without obvious disease in Taishan District, Tai'an City, Shandong Province were collected, the tree height and DBH were measured, the seed size characteristics were compared, and the seed germination law under different salt concentrations was studied.

The results showed that the size of the mother tree had a significant effect on the maximum length, maximum width and 1000 grain weight of the seed. The maximum length, maximum width and 1000-seed weight of the seed increased first and then decreased. The above indexes of the mother tree with breast height diameter of 19.9 - 23.4cm and 29.8 - 31.9cm were significantly greater than those of the mother tree with breast height diameter of 38.5 - 41.1cm. The size of the mother tree has a significant effect on the germination rate, the salt concentration has a very significant effect on the germination rate and the germination index, the size of the mother tree has a significant effect on the seed dormancy rate, and the salt stress has a very significant effect on the seed dormancy rate. With the increase of salt concentration, the germination rate and germination index decreased significantly, and the dormancy rate increased significantly. 29.8 - 31.9cm mother trees had the highest germination rate and the lowest dormancy rate. Therefore, this study found that, on the whole, the seed quality of the middle DBH mother tree was the best, with a large 1000 seed weight, the highest germination rate and the lowest dormancy rate, and the strongest salt tolerance.

Author Contributions

The Manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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