International Journal of Agriculture and Forestry 2016, 6(2): 99-102
DOI: 10.5923/j.ijaf.20160602.07

The Effect of Dormancy Breaking Treatments among Four Provenances on Twin Seedling Emergences of *Zizizphus spina-christi* (L.) Wild Kernels

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Abstract This study was conducted at the National Tree Seed Centre of the Forestry Research Centre at Soba-Khartoum, Sudan, 2009, to investigate the importance of the morphological abnormalities in seedling as polyembryony of *Ziziphus spina-christi* (L.) Wild, as an important indigenous and multipurpose species in Sudan. The study revealed the strategy of the species of producing large kernels that contain 2-seeded to guarantee the production of twin seedlings for survival purposes. This phenomenon was studied at provenance level after subjecting the species kernels to many dormancy breaking treatments to show their variation in twin seedling characteristics and the response of the twin seedling emergences to the dormancy breaking treatments. The results showed a very high significant response of twin seedlings' emergence to the dormancy breaking treatments, and twin seedlings' emergence showed a very high significant response to provenance.

Keywords Twins, Polymorphism, Dormancy, Provenance

1. Introduction

The seed is a fundamental unit for tree propagation, accordingly, reforestation and aforestation programmes require seeds of high quality [1]. Seed production and quality are affected by many factors, for instance, the impact of provenance ecosystems on seeds is of great importance. Therefore, climatic factors can affect phenology of flowering, and thus, indirectly seed production [2]. Of all the problems limiting seed germination, none is important than seed dormancy. The strength of dormancy has been observed to vary according to latitude and provenance [2].

However, the study of species that exhibit a good establishment, adaptation and tolerance to the drought factors has to be the most desired objectives. Among these species, *Ziziphus spina-christi* (L.) Wild, is considered an endangered species being removed from vast areas of its habitat [3], and as a tropical species, the rapid rate of forest loss and degradation across the tropics have continued to increase risk of species' extinction [4].

This species is a multipurpose tree, in Sudan, *Ziziphus spina-christi* has to be well tested for more effective use in agroforestry, shelterbelt, fodder, apiculture, timber, shade, pesticide, and hedges...etc. And it has a kind of mechanical dormancy slows its germination speed.

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Published online at http://journal.sapub.org/ijaf

Ziziphus spina-christi was described by [5-8] and many other authors.

It is a shrub, var. *microphyla* A. Rich, or a tree, var. *spina-christi* (L.) Willd.

Which was the subject of this research. Family is *Rhamnaceae*.

Tree height reaches more than 5 m. long, the shrub is less in height than 5 m. long. Bark is pale grey, fissured. Branches are intertwisted, branchlets are pale or nearly white, glabrous. Leaves are less than 3 cm. long in var. *microphyla*, whereas var. *spina-christi* leaves are larger, (4-6) cm. long, they are ovate-lanceolate, with acute or obtuse apex (1-3.5) cm. broad, with slightly crenulated margin and three nerves from the base, lateral nerves obscure, glabrous below, about 20 mm. in diameter. Thorns in pairs are obviously seen, one straight (8 mm.) and the other recurved and shorter. Flowers are small (10-25) in heads beside leaves, yellow green stalk and calyx hairy white. Flowering emergence (Aug.-Dec.). Fruits are round, varied in color, mostly brown, pale-brown, yellowish-brown or reddish-yellow (1-2) cm. in diameter, with edible flesh, and (1-2) seeds, rarely 3 seeds may be found. Trees and shrubs are evergreen in wet sites, and shed their leaves in long dry seasons.

The species is found over the whole Sahelian area, from Senegal to Sudan, also found in Arabia [8]. It is found in flooded riverbanks and at edges of cultivation areas [9]. It is found on light silty soils in the short grass savanna, also along the Nile tributaries [10-7]. It is often allowed to remain within or on the vicinity of settlements because of its edible fruits [8]. It is approximately wide spread throughout many

zones of Sudan.

Ziziphus spina-christi requires rainfall ranges between (50-300) mm. and prefers light silty soils, but can grow in clays with available water, altitude (0-600) m. It tolerates high temperatures and propagates (600-2,500) fruits/Kg [8]. The species also tolerates saline and limestone soils [6].

Morphological abnormalities in seedling as polyembryony, double embryo, twin and triple seedlings, albino and chlorophyll mutant seedlings are widely reported. Such abnormalities are due to several factors such as developmental error during development of ovary, during fertilization, genetic factors or mutation [11] *Ziziphus spina-christi* (L.) Wild, is one of these species, having the ability of combating the adverse conditions by producing many big kernels that contain twins.

This study was carried out to show whether the dormancy breaking treatments accelerate twin seedling emergence, and to reveal the effect of provenance on their emergence variation.

2. Materials and Method

Fruits of *Ziziphus spina-christi* (L.) Wild var. *spina-christi* were collected from four provenances. These provenances were selected to represent the variation of the ecological and climatic factors affecting the species characteristics and growth status. The four provenances were:

Provenance	Rainfall	Latitude	Longitude
Damazin	600 mm	11° 53' N	34° 19' E
Sinar.	450-600 mm	13° 33' N	33° 35' E
Khartoum,	150 mm	15° 40' N	32° 32' E
ElFashir	75-150 mm	13° 37' N	25° 21' E

Before collection, sheets of plastic sacks were spread under the tree crowns, seeds were collected manually by picking the fruits from the branchlets or by hitting the crowns gently by a woody stick. Sample from each provenance was kept in cotton sacks, the four samples were labeled and carried to The National Tree Seed Centre at Soba, where they were stored and processed. Sampling was done randomly and the species fruits for each provenance were collected from (10-15) trees.

Ziziphus spina-christi kernels, according to the variation of their sizes, [10] graded them into two categories, large and small kernels. Large kernels always have the likelihood to be sound 2-seeded and the small contain one seeded or have 2-seeded but the other seed may be abortive. The large kernels diameters were (1-1.5) cm and the smaller were the smaller than 1 cm in diameter. Accordingly large kernels of (1-1.5) cm diameters were used for the experiments.

Germination was carried out in a controlled germination room. Temperature at ±30°C, light for 12 hours a day from fluorescent lamps.

The kernels from each provenance were divided into 4 replicates of 25 kernels each, for all dormancy breaking treatments and the control kernels which were sown without treatment.

3. Dormancy Breaking Treatments

3.1. Hot Water Treatment

Water was heated on a hot plate for a period before reaching the boiling point, (40-60°C) then it was poured on 100 kernels of the species which were randomly drawn from each provenance sample and left soaked to cool off for 24 hours and then were sown.

3.2. Boiled Water Treatment

One hundred of the species kernels were taken randomly from each provenance sample, they were soaked in water, and the water was boiled for ten minutes (100°C). The soaked kernels were left to cool off for 24 hours, and then planted in germination trays.

3.3. Sulphuric Acid Treatment

One hundred of the species kernels were taken randomly from each provenance sample, they were soaked in concentrated H_2SO_4 for 30 minutes and for one hour. The kernels were cleaned thoroughly with tap water, and then sown in germination trays.

3.4. Oven Treatment

Replicated 8 samples of 100 kernels were taken randomly from each provenance sample, each provenance kernels were replicated into 8 samples. They were put in an oven at (50°C) for 3 hours, and then they were sown in germination trays after getting them out the oven.

3.5. Seed Extraction Treatment

Kernels of *Ziziphus spina-christi* were drawn randomly from each provenance sample, each of them was 100 kernels. The kernels were soaked in water for 6 hours to get rid of their woody coats, the woody coats were then hit gently with a woody peace to avoid seed damage inside the kernels. The extracted seeds were sown as twins (in a one bed) for the 2-seeded kernels and as single seeds for the one-seeded kernels in germination trays separately.

JMP statistic software was used to analyze the results, analysis of variance (ANOVA) was used to determine the significance of the variation of provenance twin seedling emergence that were affected by the dormancy breaking treatments and the effect of the dormancy breaking treatments on these twin seedling emergence. Means comparison *we*re made using Tukey-Kramer's analysis procedure. All experiments were completely randomized design.

Germination counts were done at 7 days interval and for 5 weeks.

4. Results and Discussion

The results showed a very high significant response of twin emergence to the dormancy breaking treatments (P=0.0001), and seed extraction was the best treatment followed by sulphuric acid for one hour and for thirty minutes, absolutely the treatment by sulphuric acid for 30 minutes is recommended.

[12] found that treating one-year-old *Corchorus tridens* seeds with concentrated sulphuric acid for 10, 20, and 30 min significantly broke the seed dormancy and promoted the germination of the seeds compared to control seeds.

Oven treatment was the lower value of germination, and this may be due to the decreasing of the seed moisture caused by the dry heating, [3] observed the decreasing of this species seeds when treated by dry heat, although [14] obtained high value of germination when three tropical species seeds exposed to 80°C, this may explain the differences between species according to their physiological and/ or physical characteristics. [4] stated that, boiling water and hot water for 10 minutes, the effect of hot water on some tropical species.

Twins in *Ziziphus* spp. are always correlated with the big seeds, the large seeds are 2-seeded and produce twin seedlings for survival purposes [10]. Hence, the stress of the ecological factors on the species to produce 2-seeded kernels is a one of the many methods of the species adaptability.

Table 1. Effect of dormancy breaking treatments on twin seedlings' emergence for the four provenance sources

Treatment	Mean of twin seedling emergence%
H_2So_4 for on hour	18 ab
H_2So_4 for 30 minutes	14 b
Boiled water	13 b
Hot water for 10 minutes	7 bc
Dry heat at 50°C for 3 hours	3 d
Seed extraction	21 a
Control	9 c
	P=0.0001 S.E.±0.79

a. Effect of provenance on twin emergence

Twin emergence showed a very high significant response to provenance (P=0.0001), and Damazin source was the best, followed by Sinar source, while Khartoum and Elfashir sources were the same. These results indicated that Damazin and Sinar were the richest in soil nutrients and rainfall amounts. Accordingly. Good species genotypes are expected to be found within these provenances. Based in differences in ecological conditions, environmental factors and geographic locations, it would be expected that natural selection could generate considerable genetic differentiation and diversity between populations [13-10], also pointed out from results that the phenomenon of polymorphism of *Ziziphus spina-christi* species is related to environmental conditions in that the plant produces seeds with low or no dormancy to germinate readily when the conditions are favorable for seedling survival.

Reporting of such variations is most important for future genetic improvement and conservation programmes [11].

Table 2. Effect of provenance on twin seedlings' emergence

Provenance	Mean of twin seedling emergence%
Damazin	14 a
Sinar	13 ab
Khartoum	11 b
Elfashir	11 b
	P=002 S.E.±0.60

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