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Genetic Variation among *Terminalia laxiflora* (Engl and Diels) Trees within One Population in the Blue Nile - Sudan Trees

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ABSTRACT

Terminalia laxiflora (Engl and Diels) is one of the most important tree species in Sudan. The germination of this species is highly variable ranging from 0 - 70% under the same condition using the same pretreatment within a seed lot. Therefore, the objective of this study is to determine the genetic variation among *Terminalia laxiflora* as reflected on its seed germination in order to select the individuals that can have a high rate of germination for a forestation and conservation purposes. The study was carried out on trees that were systematically selected from one population from El Nour forest in the Blue Nile State, Sudan. The genetic variation was examined using RAPD marker method. The study results revealed high genetic variation among the trees. The linkage distance ranged from 0.0 to 0.86 indicating a rich genetic diversity that might influence the germination of this species. The variation in germination percentage among the evaluated trees was highly significant (P= 0.002).

Key Words: Genetic variation, engl, diels.

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INTRODUCTION

The genetic profile of whole populations varies from place to place across a species range. These differences may arise as result of chance occurrences, such as the genetic composition of dispersing individuals that create a new population (founder effect), or changes in allele frequencies that result from chance mating in very small populations (genetic drift) *(Primack and Kang, 1989 and Templeton, 1991)*. Differences among populations can also arise systematically, especially if the environment in various places exposes individuals to different optima for survival and reproduction (fitness). Populations often diverge from one another in their genetic composition. Such divergence is especially strong and rapid when there is little gene flow between populations. Populations are defined as much (or more) by patterns of mating and gene flow as by the physical distribution of individuals, although the two are often closely related *(Hartl and Clark, 1997)*. Gene flow can be obstructed by physical barriers as well as disturbance *(Levin, 1981 and Slatkin, 1987)*. If the variation within populations is low, there may be a chance for considerable variability among populations. A variety of measures are used to quantify the distribution of genetic variation among individuals within populations and among populations. These measures are the basis for describing how genetic variation is partitioned within the species. Differences among populations are commonly quantified by the use of one of several statistics, including Wright's inbreeding coefficient (F_{ST}) and Nei's coefficient of gene variation (G_{ST}). These indices are functions of how heterozygosity is partitioned within and among populations, based on differences in allele frequencies *(Wright, 1969; Nei, 1975 and Chai, 1976)*.

A major component of the observed genetic variation for pre-harvest sprouting in wheat (*Triticum aestivum L.*) appears to be the level of seed dormancy. Group 3 chromosomes have received attention as carrying the R genes for seed-coat colour and the taVp1 genes which encode a dormancy-related transcription factor. Hence it was concluded that the high dormancy associated with chromosome 3A of Zen is ascribable to QPhs.ocs-1 on the short arm *(Miura et al., 2002)*.

Terminalia laxiflora is a common indigenous tree species in woodland and semi humid Savannah of the Sudan. It is a multipurpose species with a high potential of timber production, medicinal uses etc. From previous studies at the National Tree Seed Centre *(Mahgoub, 2002)* the poor germination of seeds is an obstacle for establishing plantations of this species. Poor germination was found to be partly due to the combined (chemical, mechanical and indigenous) dormancy and partly genetically due to crossing hybrid between species which may create species that are sterile or produce seeds that are difficult to germinate. The aim of this study is to determine the genetic variation among *Terminalia laxiflora* trees as reflected on its seed germination in order to select the individuals that can have a high rate of germination for a forestation and conservation purposes investigate the relationship between genetic variation and germination in one population of *Terminalia laxiflora* trees.

MATRIALS AND METHODS

Seed material used in the study:

Terminalia laxiflora seeds were collected from El Nour forest

(11° 50\ N and 34° 29\ E) in the Blue Nile State in Sudan. Ten (10) trees were systematically selected. The average distance between each tree and the other was about 100 metres.

DNA extraction protocol:

Due to the difficulty of germination of the seeds of this species the DNA seed extraction protocol as described by *Kang, et al. (1998)* was adopted. The seeds were cut in two halves and the half seeds containing the storage tissue (Endosperm or cotyledon parts) were used in DNA extraction. The seed coats were removed and the seeds were cut in half. The half seed was placed in 1.5 ml micro centrifuge tube. 400 µ of extraction buffer (200 mM Tris-HCl (pH 8.0), 200 mM NaCl, 25 mM EDTA, 0.5% SDS) were added containing proteinase K (50 g) and incubated at 37°C for 1 hour. The seed was grind the buffer with a glass rod. 400 µ of a 2% CTAB solution (2% CTAB (w/v), 100 mM Tris-HCl (PH 8.0), 20 mM EDTA (PH 8.0), 1.4 M NaCl, 1% PVP (polyvinylpyrrolidone) Mr 40,000) were added and gently extracted using chloroform: Isoamyl alcohol (24:1) with 5% phenol. The supernatant was centrifuged at 12,000 rpm in micro centrifuge at 4°C for 10 min and the supernatant was transferred to new tubes. Isopropanol 2/3 volume was added and the tubes were incubated at room temperature for 10 min to precipitate DNA. The tubes were then centrifuged again at 12,000 rpm for 5 min, after which the supernatant was discarded and the DNA pellet was washed with 70% Ethanol, air dried and resuspended in 50 µl of TE buffer. RNA was removed by adding 1 µ of RNase (10 mg/ml).

Primers, markers and amplification conditions:

Four primers (10 mer) (University of British Columbia, Canada) were used to screen all the individuals (Table 1) by RAPD-PCR and band sharing analysis. Amplification reaction requires 25µl reaction mixture which contains 15 µl ddH_2O, 2.5 µl 10X PCR buffer, 1.5 µl of 50 mM $MgCl_2$, 0.5 µl of Taq polymerase (5U), 2.5 µl (2 mM) of DNTPs mixtures, 2 µl (10 pmol) primer and about 50 ng of DNA. The amplification process was performed in a Biometra (Thermo Personal) beginning first with denaturation at 94°C for 5 minutes. This was followed by 40 cycles of 1 minute denaturation at 94°C,1 minute annealing at 33°C and ended with 1 minute extension at 72°C. Further extension time at 72°C for 7 minutes was also included. The PCR product was checked on agarose gel 2% stained with ethidium bromide (10 mg/ml) 1 µl for 100 ml. For each sample, a mix of 10µl PCR of product and 2 µl of 6X loading dye were loaded on the gel. A marker (1Kb DNA ladder) was run together with the samples. The electrophoresis was run for 2.5 hours at 70 V. The product was then visualized using Bio-Rad gel documentation system.

Table 1: RAPD primers and primer sequences (5′ to 3′) used for the detection of polymorphism in *T. laxiflora* trees.

NO.	Primer	Sequence (5′ to 3′)
1	UBC 101	GCGGCTGGAG
2	UBC 104	GGGCAATGAT
3	UBC 155	CTGGCGGCTG
4	UBC 122	GTAGACGAGC

RAPD products have a large number of DNA bands of various sizes from each of the different samples. These bands migrate according to size during electrophoresis. The total number of unique bands was counted for each primer used. Then, the presence or absence of each individual band was recorded for each plant sample. The presence of a band was recorded as a one (1) and the absence as a zero.

Cutting Test:

Two hundred seeds were taken at random from the working samples for the 10 trees. Seeds were divided into 2 replicates of 100 seeds each. The seeds were cut transversely with the aid of a pruning shear. The cut seeds were visualized by naked eye and a hand lens to identify the different types as follows:

- Sound seeds (normal, firm, fresh and full size).
- Empty seed (empty seed coats, with no embryos).
- Dead seeds (fragile, darkly, colored and decayed).

Pre-treatment:

For each tree, 100 seeds were used and divided into 4 replicates of 25 seeds each. The seeds were sown immediately after treatment using sulphuric acid (97%) for 45 min. The seeds were sown in round aluminum dishes filled with moist sand. The dishes were watered daily with a fine shower. Seed germination was carried out in a controlled germination room at the National Tree Seed Centre – Soba at 30°C, under light for 12 hours from fluorescent lamps. Germination counts were made at 7 days interval and for a period of 6 weeks.

CBRD were used with four reps. The means compare with Tukey – Krammer. JMP Statistical package was used (program improved by SAS corporation). STATISTICA program was used to draw the dendogarm.

RESULTS AND DISCUSSION

The results showed significant differences between *T. laxiflora* trees in their seeds character. Tree No 2 has the longest seeds and the longest seed wings and high germination percentage and genetically far away from other trees (the germination test done for the sound seeds only, this may explain the differences between seed viability and germination, this step was done to eliminate the damaged seeds so the result focus on the seed ability to germinate, also the inhibitors effect was eliminate with the most proper method). Tree No 1 has closely the same result high germination percentage, hasn't significant differ on its size from No 2. Tree No 10 has the widest seeds and shortest seed wings, it has the lowest germination percentage with tree No 7, 8 although they were significantly different in their size. Trees No 3, 6, 9 were closely related and in the same time their germination percentage were not significantly different (Table 2) (Figure 1). This variation may have an effect on germination as recorded by *Austin and Longden (1967)*, who reported that within any one properly matured sample of carrot seeds there was a distinct advantage of large seeds both in germination percentage and more particularly in seedling emergence. This may lead to the differences in germination percentage between the ten trees of *T. laxiflora* (Table 2) and may explain the differences in germination in the same bulk or between single trees. These differences may be attributed to genetically differences among trees. In addition there were differences in seed viability between the ten trees. This may be due to different chemical components of seeds that made some seeds more favorable to insects (Table 2).

Table 2: Seeds characterization among *Terminalia laxiflora* trees.

Trees	Seed Wing Length (cm)	Seed Width (cm)	Seed length (cm)	Number of Wings per seed	Sound seeds %	Mean %
1	0.84 bc	1.49 c	7.55b	2	2	65a
2	0.96a	1.63 b	8.19a	2	36	71a
3	0.90abc	1.66b	7.28bc	2	14	14.7b
4	0.84 c	1.57bc	7.27bc	2	10	15.1b
5	0.78 c	1.45cd	6.11e	2	30	/
6	0.81 c	1.43cd	7.05cd	2	58	18.9b
7	0.8 c	1.14e	8.46a	2	54	4.2c
8	0.94 ab	1.19e	6.05e	2	74	0c
9	0.70e	1.34d	6.27e	2	70	14.7b
10	0.71e	1.92a	6.74d	2	24	4.2c
Probability	≤0.001	≤0.001	≤0.001	1		≤ 0.002
SE ±	0.02	0.03	0.09	0		4.1
CV %	20	18	14	0		79

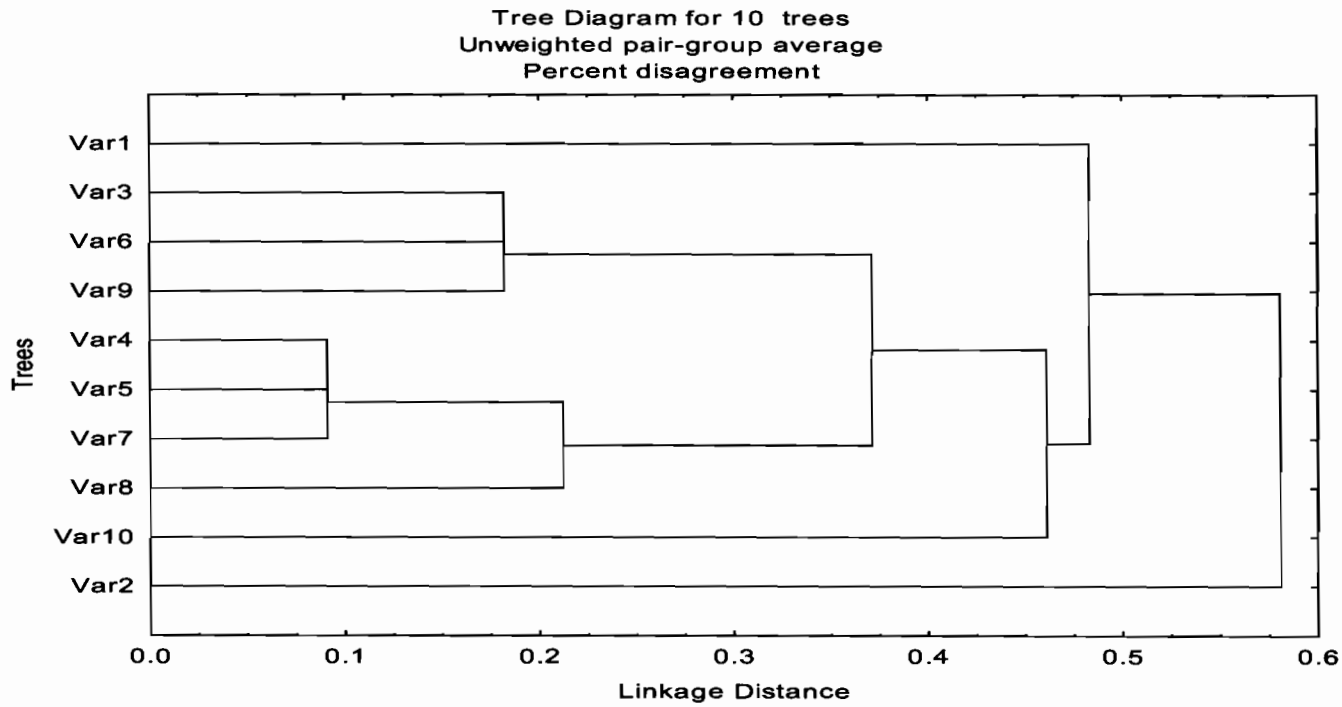

Figure 1: Combined cluster analysis derived from RAPD analysis of 10 *T. laxiflora* trees using four primers.

Total of 22 amplified fragments were distinguished across the selected primers and the statistical analysis showed 4(18.1%) monomorphic band and 18 (81.9) polymorphic bands. Nei's genetic diversity detected values of diversity ranging from 0.0 to 0.86 (Figure 1).

The results of genetic variation among the ten trees of *T. laxiflira* showed that trees No 3, 6, 9 were closely related which mean that they are genetically similar. Similarly trees No 4, 5, 7 were closely related. Tree No 1 and 2 were genetically different and there were far distances between them and the two clusters (3, 6, 9 and 4, 5, 7). Tree No 8 was close to 3, 6, 9 cluster (Figure 1). These findings suggest that there were genetic differences between *T. laxiflora* individuals within the same population (El Nour forest).

Furthermore, this variation is a sign of rich genetic diversity within this species. It is known that the high variability is a positive mark of protecting a genus or species against a biotic and biotic stress. Some individuals of the population can survive due to their heterogeneity. More investigation in this side is needed.

CONCLUSION

There were seed polymorphism between *T. laxiflora* trees but it isn't associated with differences in germination percentage. The genetic variation seems to be associated with germination percentage. These suggest selecting trees that produce seed which germinate in high percentages for a forestation programmes which reduce the efforts and cost of planting this species.

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