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Comparative Growth Rates of *Treculia africana* Decne: Embryo in Varied Strengths of Murashige and Skoog Basal Medium

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Abstract**—**This study provides a regeneration protocol for *Treculia africana* Decne (an endangered plant) through embryo culture. Mature zygotic embryos of *T. africana* were excised from the seeds aseptically and cultured on varied strengths (full, half and quarter) of Murashige and Skoog (MS) basal medium supplemented. All treatments experienced 100±0.00 percent sprouting except for half and quarter strengths. Plantlets in MS full strength had the highest fresh weight, leaf area, and longest shoot length when compared to other treatments. All explants in full, half, quarter strengths and control had the same number of leaves and sprout rate. Between the treatments, there was a significant difference ($P>0.05$) in their effect on the length of shoot and root, number of adventitious root, leaf area, and fresh weight. Full strength had the highest mean value in all the above-mentioned parameters and differed significantly ($P>0.05$) from others except in shoot length, number of adventitious roots, and root length where it did not differ ($P<0.05$) from half strength. The result of this study indicates that full strength MS basal medium offers a better option for the optimum growth for *Treculia africana* regeneration *in vitro*.

Keywords**—**Medium strengths, Murashige and Skoog, T*reculia africana*, zygotic embryos.

I. Introduction

TRECULIA africana Decne, commonly called African breadfruit (also known as "Ukwa" by the Igbo tribe), is a member of the taxonomic family Moraceae, genus Treculia and a multipurpose tree crop in Nigeria and Africa as a whole [1]. Propagation is by seed, but it can also be propagated vegetatively through cuttings and shield grafting. The seeds are highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbohydrates, and fats [2]. Despite its importance, little or no effort has been made to propagate the species as this valuable plant is being lost because of deforestation and urbanization, and nothing is being done about replanting the species. This, however, results in the plant declining at an alarming rate, and thus, needs priority conservation. This decline is due to non-improvement and non-cultivation of the species since it takes ten years or more to fruit. Hence, the need for mass propagation is necessary [3].

Plant tissue culture, precisely embryo culture, has been found to be the best means of mass propagating for the declining plants. Zygotic embryo culture is a technique which involves isolating and culturing of immature or mature zygotic embryos on a nutrient media under aseptic conditions. This technique helps in understanding the concepts related to nutrient requirements of the growing zygotic embryo. Murashige and

Skoog (MS) medium is a plant basal medium mainly used in the laboratory for the cultivation of plant cell *in vitro.* It is the most widely used culture medium because most plant cell cultures react to it favorably. It is classified as a high salt medium in comparison to other formulations with high level of nitrogen, potassium, and some of the micro nutrients, particularly boron and manganese [4].

Different studies have been conducted on growth response of *T. Africana* seeds to the factors such as organic nutrient (poultry manure) [5], storage method [6], [7], but there is no literature as regards *in vitro* (seed or embryo) growth of the plant.

Thus, the objective of this study is to establish an *in vitro* regeneration protocol from the mature zygotic embryo explant of *Treculia africana* seeds in varied strengths of MS (Murashige and Skoog) basal medium.

II. Materials and Methods

A. Site of Experiment

This study was conducted at the Tissue Culture and Molecular Biology Laboratory of the National Biotechnology Development Agency (NABDA) located at University of Nigeria, Nsukka.

B. Source of Explant

Mature fruits (Fig. 2) of *Treculia africana* Decne. were collected from a tree (Fig. 3) in Umunkpume, Orba in Udenu Local Government Area of Enugu state. They were identified in the Herbarium of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The seeds (Fig. 4) were manually separated from fruits and were soaked in water for 24h then were dissected to excise the embryos which served as the explants for the study. Embryo excision was done under a dissecting microscope. Embryo explants were measured between 1.1 and 1.5 cm.

C. Stock Solutions

Stock solutions (macro elements-stock A, $CaCl_2$- stock B, Na_2EDTA-stock C, micro element- stock D, Myo-inositol-stock E, Vitamins- stock F) of [4] were used to formulate MS medium (full strength), while the composition of various stock solutions A-F was divided into two and four for ½ and ¼ MS

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medium, respectively. Appropriate volumes of stock solutions were measured and transferred to a beaker containing 700 ml of distilled water. Sucrose (3% w/v) was added to the media. The pH of the medium was adjusted to 5.8 with 1M NaOH or HCL, and each medium was solidified by using Fluka agar at 7.0 gl^{-1} prior to autoclaving by steam sterilization at 103 kN M^{-2} pressure and 121 °C for 15 mins. The embryos were cultured singly in Pyrex test tubes at 27±2 °C under 16h light/ 8h dark photoperiod at a photon flux density of 60 µmol $m^{-2}S^{-1}$ provided by cool white fluorescent tubes. All operations starting from the preparation of explants to establishment of cultures were carried out in a Laminar flow hood previously kept sterile by exposure to ultraviolet light for 30 min. The cultured embryos were left to grow for three weeks after which they were scored for the requisite growth parameters. Ten replicate tubes containing an embryo each were randomly selected under each treatment and scored for the following: percent sprouting (%), sprout rate (sprout rate is obtained as the reciprocal of the number of days to 50% sprouting), mean length of shoots and roots, mean fresh weight of sprouts produced, number of roots and area of leaves produced in culture.

D. Experimental Design and Statistical Analysis

This study was carried out in a completely randomized design that involves four treatments (full MS, ½ MS, ¼ MS and control) with ten cultures per treatment for each of the basal medium. Embryos were cultured one per vessel. One-way analysis of variance (ANOVA) was used to analyze data collected from the germination studies. Gen-Statistical package was used for the data computation. Treatment means were tested for significance ($P<0.05$) by using least significant difference (LSD). Error bars were represented at 5% value.

III. Results and Discussion

Sprouting of the zygotic embryos started within 2-3 days of inoculation. The embryo (Fig. 5), which appeared white in colour from the onset, enlarged and began to turn green leading to the emergence of radicle from the radicular end and plumule from the plumular end within 3 days in culture. The radicle and plumule finally resulted in the root and new shoot, respectively. The addition of sucrose to the cultures was necessary for autotrophy as seen in this study. Since embryonic axis turned from white to green about 3 days from the time of inoculation, this, however, indicates that photosynthesis may have taken place. This is a clear manifestation of a transition from semiautotrophy to full autotrophy, which is a characteristic of *in vitro* systems. In mature plants (*in vivo*), photosynthesis takes place in the green leaves under favourable conditions of light intensity, temperature, water, and carbon (IV) oxide. Green plants are self-dependent because they can synthesize their own food materials. Leaves generally appear green because the wavelengths of light from the red and blue regions of the visible spectrum excite the chloroplast electrons, and unused green light is reflected [8].

The results obtained in the germination studies showed different MS salt strengths (full, half and quarter) (Figs. 6-8) had significant effect on the growth and development of *Treculia africana* cultured *in vitro*. Varied responses in terms of embryo sprouting and plantlet development were observed in the three treatments including the control (Fig. 9). For percent sprouting, explants in both full strength and control treatment had 100±0.00, while half and quarter strengths had 94±0.32 and 87±0.42, respectively. The observation where all treatments had above 50% sprouting is in agreement with the findings made by [9]. The healthy seeds do not really need supplementary nutrients to germinate as they can do well with the nutrient materials from their endosperm and sometimes cotyledon. In addition, supplementing the basal medium with carbon source (sucrose) at 3% in all the media may have also caused the explants to sprout including the one devoid of mineral nutrient (control). Full strength was found to have the longest root (5.00±0.54 cm), average number of adventitious roots 18.30±1.44 as well as maximum fresh weight of 0.10± 0.00 g. In addition, it had the highest leaf area 1.11±0.17 cm^2 and longest shoot length 2.60±0.30 cm when compared to the explants cultured on half strength, quarter strength, and the control (Table I**)**. Also, it was obvious from the data that quarter strength MS medium gave the lowest results in root length (3.20±0.41), leaf area (0.67±0.93 cm^2), number of adventitious root (12.80±1.95), fresh weight (0.89±0.01), and shoot length (1.91±0.14). The explants in all the treatment had the same number of leaves 2.00±0.00 and the same sprout rate of 0.33±0.00. Generally, the obtained results indicate that full strength MS medium improved in most growth parameters investigated. This is in line with the work of [10] which reported that among the different strengths of media, the highest growth (shoot height and number, leaf area and number, root length and number) was recorded in apple rootstocks (MM106 and B9) shoots grown in full strength MS medium for 30-60 days, while the above mentioned parameters were lowest when the shoots of both rootstocks were grown on quarter strength for the same duration. Moreover, the obtained results also agreed with [11] on *Cymbidium aloifolium* (L.) Sw. and [12] on *Prunus ameniaca.* However, in contrast to these findings, [13] reported that reduction of major salts to half strength improved the germination percentage of zygotic embryos in *Givotia rottleriformis*.

IV. Conclusion

T. Africana, an important forest tree species from both economical and ecological perspectives has great potentials of enhancing rural livelihoods and national food security. Therefore, contentious effort made in this study to ascertain the basal medium necessary for its growth ushers the preliminary steps in developing appropriate conservation measures for this highly valuable plant. These results as described in this study have the potentials for the mass production and shortening the germination time required to obtain *Treculia africana* plantlets. The plantlets, after hardening, would be raised *ex vitro* for ensuring a steady

supply of seeds important for food and other products derivable from it.

TABLE I
LEAF AREA, SHOOT LENGTH, NUMBER OF ADVENTITIOUS ROOT, ROOT LENGTH AND FRESH WEIGHT OF *TRECULIA AFRICANA* EMBRYO EXPLANTS IN VARIED STRENGTHS OF MS (1962) MEDIUM

Treatments	Number of Leaves	Leaf Area (cm^2)	Number of Root	Root Length (cm)	Fresh Weight (g)	Shoot Length (CM)
Full MS medium	2.00 ± 0.00^{a}	1.11 ± 0.17^{c}	22.40 ± 3.11^{b}	5.24 ± 0.54^{b}	0.22 ± 0.08^{b}	2.60 ± 0.30^{c}
½ MS medium	2.00 ± 0.00^{a}	0.77 ± 0.13^{b}	$18.30 \pm 1.44^{a,b}$	5.00 ± 0.54^{b}	0.10 ± 0.01^{a}	$2.37 \pm 0.12^{b,c}$
¼ MS medium	2.00 ± 0.00^{a}	0.67 ± 0.93^{b}	12.80 ± 1.95^{a}	3.20 ± 0.41^{a}	0.89 ± 0.01^{a}	1.91 ± 0.14^{b}
Control	2.00 ± 0.00^{a}	0.02 ± 0.00^{a}	12.70 ± 1.27^{a}	2.61 ± 0.35^{a}	0.53 ± 0.00^{a}	0.68 ± 0.15^{a}

Different superscript lowercase letters within column indicate significant difference among treatments (Duncan's new multiple range test P≤0.05).

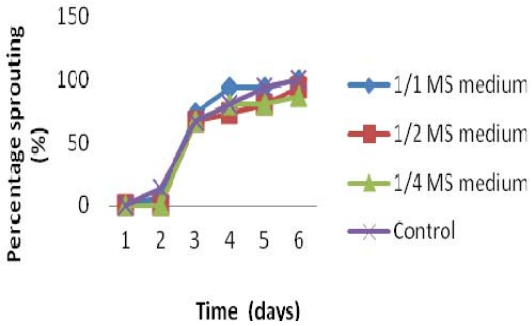

Fig. 1 Time course in percent sprouting of *Treculia africana* plantlets in varied strengths of Murashige and Skoog basal medium

Fig. 2 Fruits of *Treculia africana* Decne (0.2)

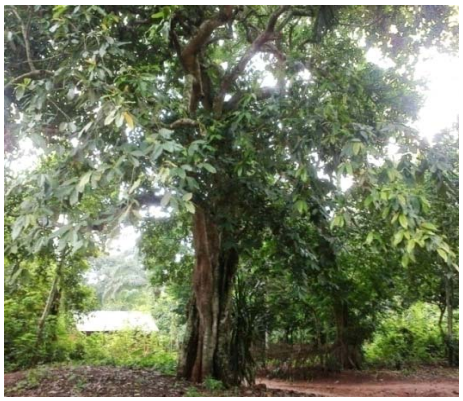

Fig. 3 *Treculia africana* Decne tree (0.002)

Fig. 4 Seeds of *Treculia africana* Decne (0.2)

Fig. 5 Embryos of *Treculia africana* Decne (0.2)

Fig. 6 21 day-old plantlets of *Treculia africana* in full strength MS medium

Fig. 7 21 day-old plantlets of *Treculia africana* in half strength MS medium

Fig. 8 21 day-old plantlets of *Treculia africana* in quarter strength MS medium

Fig. 9 21 day-old plantlets of *Treculia africana* in control treatment

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