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Original Article

In Vitro Shoot Induction and Micropropagation of Niger (*Guizotia abyssinica*)

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Abstract

Niger (Guizotia abyssinica), which is one of the major oilseed crops that are largely grown in Ethiopia and India, is used for the extraction of oil, soil erosion check and land reclamation. The objective of the current research was to optimize an In Vitro shoot regeneration protocol for Niger from seed explants by assessing the impact of different concentrations of auxin Indole-Butyric-Acid (IBA) on Murashige and Skoog (MS) medium. Seed explants were grown on MS medium containing 0.1 to 0.5 mg/l concentrations of IBA to determine shoot initiation and elongation. The results indicated that 0.4 mg/l IBA significantly increased shoot induction percentage and elongation compared to the other treatments. This enhanced hormone formulation enabled reliable shoot multiplication and provided a uniform process for mass propagation of genetically identical plantlets. The research highlights the ability of micropropagation to overcome limitations of conventional Niger cultivation, including limited seed availability and vulnerability to environmental stress. Through facilitating mass production of superior quality planting material, the protocol can conserve genetic resources, increase agricultural productivity, and facilitate sustainable agriculture. In addition, the research highlights the importance of auxin regulation in plant tissue culture, especially during organogenesis. Successful application of seed explants sets a platform for further investigation into genetic improvement and clonal multiplication of enhanced Niger varieties. This is crucial to countries that are reliant on Niger agriculture for food security and economic stability in terms of enhanced crop productivity. The research incorporates biotechnology and sustainable agriculture, which offers the possibility of embracing tissue culture methods in developing countries.

Keywords: *Guizotia abyssinica, Micropropagation, In Vitro shoot induction, Indole-Butyric-Acid (IBA), MS medium.*

Introduction:

Niger is an important oilseed crop. It is mainly grown in Ethiopia and India, accounting for around 50% and 33% of world oilseed production of this crop, respectively. In Ethiopia, niger grows mainly on waterlogged soils on which many crops and some oilseeds will not grow well. This characteristic makes niger an important crop in soil conservation and land rehabilitation, especially on marginal lands. There are six species in the genus Guizotia and of them, these five, niger, are native to the highland of Ethiopia. It is an herb dicotyledonous. *Guizotia abyssinica* grows to as high as 2 meters in moderate to fairly branching habit. The seed of this plant has around 40% oil in its composition and contains linoleic acid around 75-80%, the others are palmitic and stearic acids (7-8%), oleic acids 5-8%. This information is gotten from Getinet and Teklewold, 1995. The Indian varieties of niger oil contain 25% oleic acid and 55% linoleic acid in the fatty acid composition (Nasirullah *et al.*, 1982).

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The meal remaining after oil extraction is free from toxic materials but contains a higher proportion of crude fiber compared to most other oilseed meals. Niger has traditionally been cultivated in Ethiopia in rotation with cereals and pulses, and its genetic pools in Africa and India have diverged into different types. Collections of this germplasm from both continents are highly extensive, with the primary collections preserved and archived at the Biodiversity Institute of Ethiopia and the Indian National Bureau of Plant Genetic Resources. Ethiopian germplasm is mainly collected from farmers' fields with no breeding lines.

Although it is an oilseed crop with nutritious oil and low-input tolerance, the large-scale multiplication and breeding of *Guizotia abyssinica* are limited by traditional breeding challenges. Seed-based propagation suffers from poor germination, susceptibility to both biotic and abiotic stresses, and significant variability in yield. Micropropagation is viewed as a potential solution to these challenges through the generation of disease-free, genetically uniform planting material. Currently available in vitro protocols for niger are far from being optimally developed. Main bottlenecks include low shoot induction efficiency, undefined growth regulator regimes (for example, ratios of auxin and cytokinin), high contamination rates of explants, and inconsistent rhizogenesis during acclimatization. Moreover, very limited studies have been undertaken to determine the effect of the type of explant, the conditions of the culture, or the scalability of regeneration systems. Thus, many critical gaps still remain in developing reproducible protocols.

This study aims at overcoming the aforementioned limitations by creating a strong and efficient in vitro shoot induction and micropropagation system for *G. abyssinica*, which will permit the rapid multiplication of elite genotypes, thus conserving the genetic resources of the crop to meet the burgeoning demand for niger oil both in food and industrial sectors.

Niger oil has various applications in the kitchen and industries. It is basically used for cooking, lighting, anointing, painting, and cleaning machines. Niger oil can be used as a substitute for sesame oil in medicines and can also be utilized in soap production. Niger seed is also an important source of food. Seeds are typically heated in a kettle over a hearth fire, pounded in a mortar, and blended with pounded pulse seed. The mixture combined with roasted cereals makes a preferred food amongst the young boys in Ethiopia. Niger oil also has medicinal benefits like a promoter of heart health due to its high levels of omega-3 and linoleic acid, a sleep aid, anti-inflammatory, reliever of gastrointestinal issues, and pain reliever. It is used for rheumatism, as a birth control agent, and for syphilis and coughs.

Plant Tissue Culture (PTC) of *Guizotia abyssinica*:

Plant tissue culture (PTC) has proven to be an essential tool for the mass propagation of crops through micropropagation. Micropropagation is a newer technique adopted for vegetative propagation of stock plant material for large-scale production of genetically similar progeny plants. This technique has become increasingly important for niger, particularly in the context of addressing the challenges faced by traditional breeding methods. The main role of micropropagation is reducing the spread of diseases, pests, and pathogens by regenerating plants in a sterile environment which is vital for avoiding viral infection in plant stock. The work on the PTC use of regenerating niger using a variety of explants including hypocotyl, cotyledons, and anther callus has been observed in the new studies. In addition to that, micropropagation is crucial for rapid multiplication of elite genotypes and therefore conserves the *G. abyssinica* germplasm. While possessing great potential, in vitro regeneration protocols for niger are far from being optimally optimized. This paper standardizes techniques in the area of *In Vitro* shoot induction and micropropagation of niger and is expected to significantly enhance scalability and efficiency in regeneration systems in order to help overcome current shortcomings.

Micropropagation of Niger:

The German botanist Haberlandt, (1902) who first introduced the concept of plant tissue culture. He had already successfully managed to culture the cells from leaves of *Lamium purpureum*. The first viable callus culture was reported by White in tobacco and Gautheiet in carrot in 1939. Following these developments, it was realised that plant cells have the extraordinary ability to

regenerate a complete plant from any cell, which is known as cellular totipotency. It has great utility for virus-free stock production and rapid propagation (Moral and Martin, 1952; Moral, 1960; Murashige, 1972). Guha and Maheshwari (1966) utilized embryo culture to produce haploid and homozygous lines. Protocols for the preparation of somatic hybrid through the protoplast method were given by Carlson *et al.* (1972). The regeneration frequency is genotype dependent in tissue culture-induced plant and the response varies among genotypes (Steward *et al.*, 1975; Dunwell *et al.*, 1981). Murashige (1974-1978) demonstrated clonal propagation of horticulturally important species by using shoot tips and apical meristems. For niger, Simmonds and Keller (1986) have established regeneration protocols based on explants derived from leaf tissue, whereas Sarvesh *et al.* (1993a, 1994b) reported the protocols for the regeneration of plants from hypocotyl and cotyledon tissues. Moreover, diploid niger plants have been obtained through anther culture (Sarvesh *et al.*, 1994a).

Micropropagation Studies on Niger:

Substantial numbers of micropropagation studies had been carried out to identify the best condition for shoot induction and further multiplication in niger. For instance, Tesfaye Disasa (2003) induced multiple shoots from cotyledon explants on MS medium supplemented with 0.1 mg/lit Naphthalene-Acetic-Acid (NAA) in combination with 1 mg/lit Benzyl-Amino-Purine (BAP). Tesfaye Disasa (2003) later later found that 3 mg/lit Indole-Acetic-Acid (IAA) in combination with 1 mg/lit BAP does have any effect. Ganapathi *et al.* (1992) were successful in achieving multiple shoot induction from hypocotyls and cotyledons on MS media supported with 1 mg/lit BAP and 0.1 mg/lit NAA. Others, such as Sarvesh *et al.* (1994), achieved multiple shoots from cotyledons and epicotyls in MS media supported with BAP (0.1 mg/lit) and NAA (0.1 mg/lit). Dwivedi *et al.* (1995) and Nikam *et al.* (1997) have contributed to the shoot induction protocols of niger, and recent work by Kivadasannavar *et al.* (2007) and Blakrishanan *et al.* (2011) explored growth regulators like IAA and GA3 for shoot regeneration improvement of niger and thus demonstrated optimization of culture conditions to obtain high success rates in micropropagation. In all, these innovations in micropropagation will better overcome the limits that exist while significantly improving in vitro regeneration potential of *G. abyssinica*, where the crop remains capable of taking up the niger oil growth demand while increasing genetic diversity without losing valuable germplasm.

Materials and methods:

The present investigation entitled "*In Vitro* Shoot Induction and Micropropagation of Niger (*Guizotia abyssinica*)" was conducted in the Department of Microbiology and Biotechnology, B.N.N. College, Bhiwandi. The experimental material comprised Niger. The seeds of *Gulzotia abyssinica* were obtained from a nearby local market.

Materials:

Glassware:

All the glassware is made of good-quality borosilicate glass (resistance to heat), such as test tubes (25 mm x 150 mm) called tissue culture test tubes, flasks (250 ml, 1000 ml), measuring cylinders, pipettes, volumetric flasks, petri plates, and beakers (250 ml, 100 ml).

Equipment:

Surgical scalpel with a supply of removable blades (straight sharp, tipped), fine forceps, tipped dissecting needles, and other used tissue culture kits.

Washing and sterilization of glassware:

All the glassware needed was initially cleaned with tap water. Soak this glassware in a detergent solution for 2 hours. Again, wash with tap water and soak them for 24 hours in dilute nitric acid. Next day, they were thoroughly washed with tap water to remove all traces of nitric acid and washed with double distilled water.

Dry in oven at 80°C for 2 hours. Sterilization of glassware was advised by autoclaving. Dry beakers and Petri plates were all covered with brown paper. Also, forceps blades and scissors were wrapped in brown paper. All these were first autoclaved at 121oC for 15 minutes/15 lb. pressure. During inoculation, forceps and blades were again sterilized by dipping in absolute alcohol and holding on the flame alternatively.

Media preparation:

Double distilled water was available in the media as per given composition as per requirement. The composition is given in table no. 1.

Table no. 1 Composition of Suitable medium

	Constituents	Amount (mg/lit)
Stock solution I	$MgSO_4.7H_2O$	370
	KH_2PO_4	170
	KNO_3	1900
	NH_4NO_3	1650
	$CaCl_2H_2O$	440
Stock solution II	H_3BO_3	6.2
	$MnSO_4HO$	22.3
	$ZnSO_4.7H_2O$	8.6
	$Na_2MoO_{4.2}H_2O$	0.25
	$CuSO_4.5H_2O$	0.025
	$CaCl_2H_2O$	0.025
Stock solution III	$FeSO_4.7HO$	27.8
	Na EDTA.$2H_2O$	37.3
Stock solution IV	Thiamine HCL	4
	Pyridoxine HCL	0.5
	Nicotine acid	0.5
	Glycine	2
	Yeast extract	500
Equipment	Sucrose	30
	Agar	25
	pH	5.8

Note: To make 1 liter of media, take 50 ml of stock solution 1 and 5 ml of stock solutions 2-4. As required, suitable amounts of growth hormones were added. The media was dissolved in double distilled water. Growth hormone of different concentrations with 30% sucrose. The pH of the medium was neutralized to 5.8. Agar was supplemented at a concentration of 20%, and the medium was then steamed to dissolve the agar powder. The medium was distributed, 20 ml per tube. The tubes were autoclaved at 121°C for 15 min/15 lb pressure. The test tubes were then placed in a tray after autoclaving to support the butts in place. Check the contamination for 24 hours, and then a proper explant was inoculated.

Methods:

Preparation and Inoculation of Explants:

All the aseptic operations were performed in a laminar flow cabinet.

A. Explant preparation and surface sterilization in 3 stages

Stage 1: Seeds (niger) were washed thoroughly with double distilled water.

Stage 2: Seeds were washed with 70% alcohol for 5-7 seconds, followed by washing with double distilled water.

Stage 3: Seeds were surface sterilized with 0.1% mercuric chloride solution for 1-2 minutes and washed well in sterile distilled water 3-4 times for 5-10 minutes each.

B. Inoculation

After the explant (seed) sterilization. They were inoculated in MS medium with different concentrations of IAA under aseptic conditions. The culture was grown in light using a 16-hour light photoperiod at 25+27°C with a relative humidity of 60%.

C. Induction of shoot in Niger, *Gulzotia abyssinica*

For the induction of shoots in Niger, the composition of the basal medium is given in Table No. 1. The procedure for sterilization of glassware and media was described earlier; the seed was cultured. On MS basal medium, supplement with different concentrations of IAA.

D. Different concentrations of IBA

The concentration of IBA is given in Table No. 2. The culture was incubated at 25±2°C under 16 hours of light. After 5 days, initiation of the shoot was formed, and after 8 days, elongated shoots were developed.

Result and discussion:

The current research was conducted using Niger (*Gulzotia abyssinica*) in an attempt to standardize the explant and medium of *Gulzotia abyssinica* for shoot induction in Gulzotia abyssinica. Seeds were pre-treated with asepsis and inoculated on MS medium spiked with different concentrations of IBA for shoot development. Shoot development has been observed and documented from the 4th to the 10th day after inoculation. Table no. 2 depicts the different concentrations of auxin on MS medium. It can be seen that IBA (0.4 mg/lit) was the most favorable treatment, as it exhibited shoots, that is, the maximum among all the treatments.

Table No. 2: Production of shoot in MS medium supplemented with IBA

Name of auxin	Concentration (mg/lit)	Observation	
		5 Days	8 Days
MS+IBA	0	-	-
MS+IBA	0.2	-	-
MS+IBA	0.4	Initiation of shoot	Elongation of shoot

The initial treatment lacked IBA, which is unable to induce shoots. The second treatment was IBA (0.2 mg/lit), and it too was unable to induce shoots. When the concentration of IBA was raised (0.4 mg/lit), shoot induction was noted after 5 days, and shoot elongation was noted after 8 days. This tells us that with the increase in concentration of auxin (IBA) from 0.2 to 0.4 mg/lit, both elongation and shoot initiation were noticed. From this table, one may infer that the optimal concentration of IBA would be (0.4 mg/lit) in MS basal media. From this current work, one may infer that for shoot elongation and initiation, MS media should be fortified with IBA (0.4 mg/lit).

Initiation of Shoot in MS Medium + IBA at [0.4 mg/lit] after 5 days

Initiation of Shoot in MS Medium + IBA at [0.4 mg/lit] after 8 days

Summary:

The present project in vitro induction of shoot in Niger (*Gulzotia abyssinica*) was carried out with explants, seed of *Guizotia abyssinica*.

Explants were tested against different concentrations of IBA on MS media. Observation was recorded after 8 days in terms of elongation of the shoot from *Gulzotia abyssinica*.

The effect of different concentrations of IBA was examined for the development of shoots from seeds; IBA at 0.4 mg/l was found to be the best treatment for the development (elongation) of shoots from seeds.

From this work I would like to suggest the optimum concentration for shoot induction: one should use 0.4 mg/lit IBA along with MS media.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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