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Regeneration of niger (*Guizotia abyssinica* Cass.) CV Sahyadri from seedling explants

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Abstract

Root, hypocotyl and cotyledonary explants of niger (*Guizotia abyssinica* Cass) CV. Sahyadri were aseptically cultured on Murashige and Skoog's basal medium (MS) containing BAP and kinetin. Multiple shoot regeneration was induced from hypocotyl and cotyledonary explants while root explants produced only callus on MS medium supplemented with BAP. BAP (1 mg l^{-1}) was optimum for shoot regeneration. Regenerated shoots were transferred to MS medium without auxins, with auxins and with increasing concentrations of sucrose for rooting. Complete plantlets were obtained in all cases; however, 0.5 mg l^{-1} NAA was the best for induction of roots. Ninety-seven per cent of the plantlets survived and completed their life cycle when transferred to natural conditions.

Abbreviations: BAP – 6-benzylamino purine, NAA – α-naphthaleneacetic acid, IAA – indole-3-acetic acid, IBA – indole-3-butyric acid

Introduction

Niger (*Guizotia abyssinica* Cass.) is an important rainfed oil seed crop, belonging to family compositae, cultivated in hilly regions of India. Seeds of niger are a source of edible oil and are used for making chutney and condiments. Some superior cultivars of niger have been developed by Mahatma Phule Agriculture University, Rahuri, India and a considerable amount of breeding work has being carried out with the objective of improving the crop. Multiple shoot formation has been obtained from shoot tips cultured on Murashige and Skoog's (MS) medium containing BAP (Ahmad & Pande 1988a). Callus was obtained from hypocotyl-produced shoots (Ahmad & Pande 1988b). However, there is no information available on the development of complete plantlets from either explants or callus. This paper reports *in vitro* multiplication of niger CV Sahyadri.

Materials and methods

Certified seeds of cultivar Sahyadri of niger were obtained from the Niger Research Project, Dindori, Nashik, Maharashtra, India. Seeds were surface sterilized by treatment of 2 ml Teepol (neutral liquid soap, Glaxo India Ltd.) in 98 ml sterilised distilled water for 2 min, followed by rinsing two times with sterile distilled water, followed by stirring in 0.1% mercuric chloride for 6 to 8 min. After rinsing seven times with sterile distilled water, the seeds were germinated on a sucrose (1%) and agar (0.8%) medium. The MS basal medium consisted of MS salts and vitamins (Murashige & Skoog 1962) sup-

plemented with 3% sucrose and solidified with 0.8% agar. The growth regulators, BAP, kinetin and NAA added in the medium before autoclaving while IAA and IBA were filter sterilized with a 0.22 μm filter and added under aseptic condition to the autoclaved medium after cooling to 60 ± 4°C [MS salts, sucrose and agar (extra pure grade) were obtained from Glaxo India Ltd. Vitamins and growth regulators were from Sigma Chem. USA]. The pH of the medium was adjusted to 5.7 with 0.5 N NaOH before autoclaving at 121°C for 20 min. A volume of 20 ml nutrient medium was dispensed into 150 × 25 mm corning glass test tubes that were plugged with non-absorbent cotton.

Complete cotyledons (18 mm^2), segments of hypocotyl (8 to 10 mm) and roots (8 to 10 mm) from one week old seedlings were excised aseptically and were incubated on the MS basal medium supplemented with BAP or kinetin at concentrations ranging from 0.2 to 2 mg l^{-1}. Well developed shoots were carefully isolated from the explant after a four-week culture and were transferred either to MS basal medium lacking growth regulators or containing IAA or NAA or IBA at concentrations ranging from 0.2 to 2 mg l^{-1} and with increasing concentrations (4 to 8%) of sucrose. The cultures were incubated under 10 h fluorescent light/day (4.49 Watt/m^2), at 25 ± 2°C and a relative humidity of 50 to 60%.

Well developed plantlets were transferred to earthen pots containing soil and were kept under shade in a glass chamber (light maximum 8.97 Watt/m^2, temperature 25 ± 2°C, humidity 85 to 90%), the lid of glass chamber was opened frequently. After every 24 h, 10 plantlets were transferred from the glass chamber directly to natural conditions (light maximum 550 Watt/m^2, temperature 28 ± 7°C, humidity 70 to 80%) and 10 plantlets to the shade outside the glass chamber (light maximum 14.96 Watt/m^2, temperature 25 ± 4°C, humidity 80 to 85%) and then transferred to natural conditions.

Results and discussion

Regeneration of shoots

Root explants showed no response on the MS basal medium without growth regulators (Table 1). Rooting was generally observed on hypocotyl

Table 1. Effect of BAP on shoot regeneration.

Explant	MS medium with BAP mg l^{-1}	No. of explant cultured	Explants producing only roots %	Explants producing callus and roots %	Explants producing only shoots %	Explants producing callus and shoots %	No. of shoots per explant	Mean
Cotyledon	0.0	30	60	–	–	–	–	–
	0.2	30	60	–	56.66	–	2–3	2.7 ± .06
	0.5	30	60	–	80.00	–	5–8	6.57 ± .18
	1.0	30	60	–	93.33	–	9–13	11.07 ± .09
	1.5	30	60	–	–	66.66	2–6	4.29 ± .07
	2.0	30	–	–	–	56.66	2–3	2.21 ± .06
Hypocotyl	0.0	30	50	–	–	–	–	–
	0.2	30	–	–	26.66	–	2–5	3.5 ± .15
	0.5	30	–	–	50.00	–	2–6	4.2 ± .06
	1.0	30	–	–	63.33	–	8–11	9.6 ± .15
	1.5	30	–	–	–	56.66	7–9	7.67 ± .12
	2.0	30	–	–	–	20.00	4–7	5.5 ± .15
Root	0.0	30	–	–	–	–	–	–
	0.2	30	–	100	–	–	–	–
	0.5	30	–	100	–	–	–	–
	1.0	30	–	100	–	–	–	–
	1.5	30	–	100	–	–	–	–
	2.0	30	–	100	–	–	–	–

Results are mean of three replicates (30 × 3) ± = SD

and cotyledon explants on MS basal medium (Table 1). Addition of BAP at 0.2 mg l^{-1} to the MS medium induced shoot regeneration with 2 weeks in cultures from hypocotyl and cotyledon explants. Usually 2 to 3 shoots developed per explant without callusing at the cut end (Table

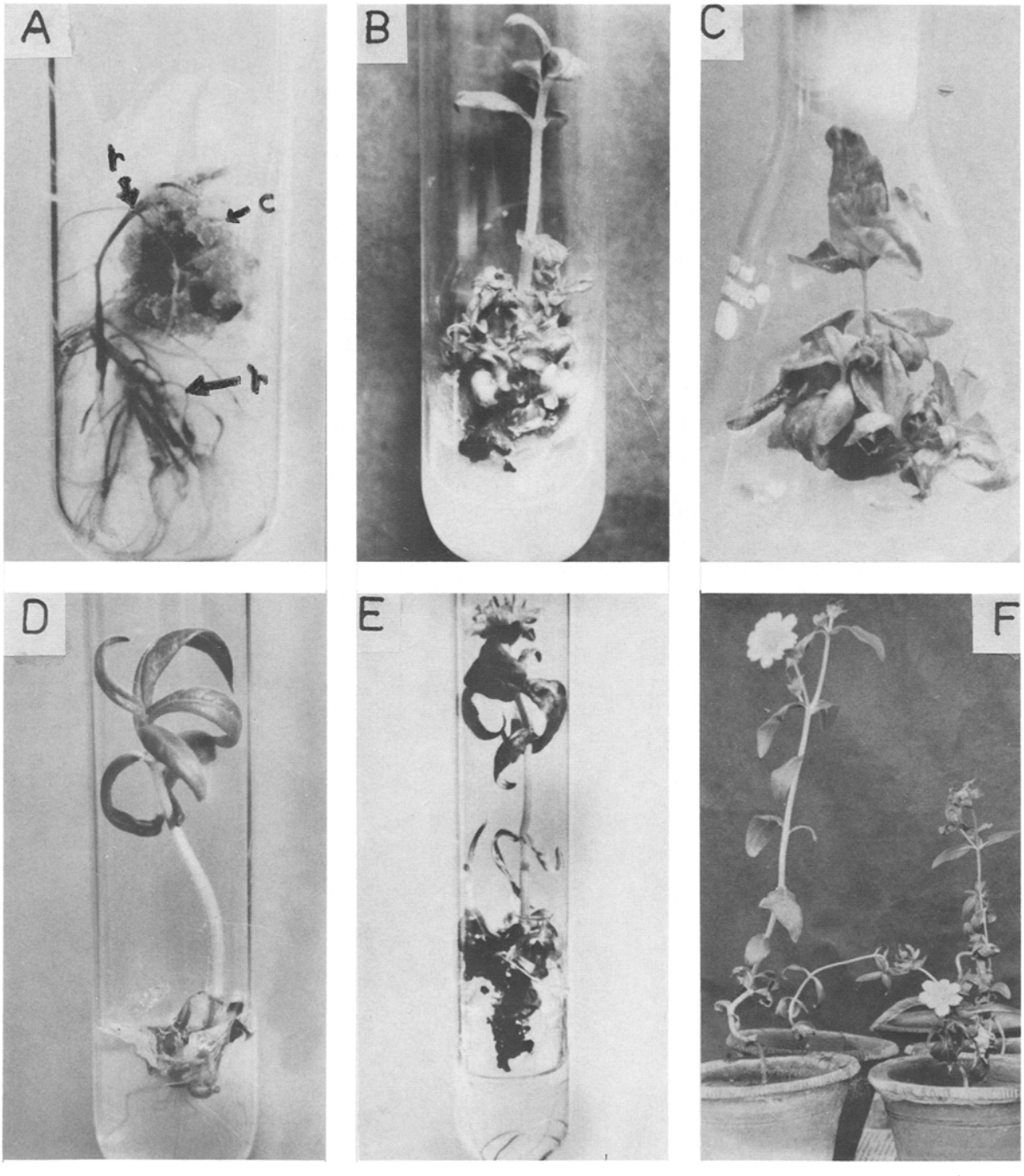

Fig. 1. Guizotia abyssinica Cass cv Sahyadri (*A*) root explant produced callus and roots on the MS medium with 1 mg l^{-1} BAP (c. callus, r. roots). (*B*) shoots from hypocotyl on the MS medium with 1 mg l^{-1} BAP. (*C*) shoots from cotyledon on the MS medium with 1 mg l^{-1} BAP. (*D*) rooted shoot on the MS medium with 0.5 mg l^{-1} NAA. (*E*) rooted shoot flowered *in vitro* on the hormone free MS medium (*F*) hardened plants under natural conditions.

1), while root explants formed callus only (Table 1).

Raising the level of BAP (0.5 to 2.0 mg l^{-1}) resulted in an increase in the percentage of hypocotyl and cotyledon explants producing shoots (Table 1). The maximum number of shoots on hypocotyl explants (8 to 11) and on cotyledon explants (9 to 13) were observed at 1 mg l^{-1} BAP (Table 1, Fig. 1B,C). But at higher levels (1.5 and 2.0 mg l^{-1}) of BAP, callus formation takes place at cut ends and a reduction in number of shoots per explant was (Table 1) observed. Shoot multiplication was obtained from shoot apices cultured on MS medium with 1 to 3 mg l^{-1} BAP in niger (Ahmad & Pande 1988a), which suggested that a wide range of BAP was favourable for shoot regeneration. However, our results revealed that 1 mg l^{-1} BAP was the optimum concentration thereby indicating that the BAP requirement of shoot apices, hypocotyl and cotyledonary explants in this genus is slightly different.

Root explants of this niger cultivar did not respond to any of the concentrations (0.2 to 2.0 mg l^{-1}) of BAP (Table 1) and kinetin for shoot regeneration. They showed only callusing which produced roots (Fig. 1A).

The effect of kinetin on shoot regeneration from root, hypocotyl and cotyledon explants revealed that none of the concentrations (0.2 to 2.0 mg l^{-1}) used were able to induce shoots, but produced green coloured callus from which roots developed in all cases.

Rooting

Rooting of four week old shoots was obtained in about 80% of cultures within 12 to 17 days on MS basal medium lacking growth hormones (Table 2). For shoots which did not root, after 28 days, the basal part of shoots was removed and shoots were transferred to fresh MS basal medium. Root formation was observed. Higher concentrations (5 to 8%) of sucrose were found to increase roots to 100% within 7 to 12 days (Table 2).

Table 2. Response of shoot to different levels of IAA, IBA, NAA and sucrose for root induction.

Compounds	MS medium with compound mg l^{-1}	No. of shoots	No. of days required for induction of roots	Mean	Shoot produce only roots %	Shoot produce callus and roots %
IAA	0.00	15	12–17	14.5 ± .17	80.0	–
	0.2	15	7–10	8.5 ± .14	100.0	–
	0.5	15	7–10	8.5 ± .16	100.0	–
	1.0	15	12–15	13.5 ± .14	–	100
	2.0	15	12–15	13.5 ± .17	–	100
IBA	0.2	15	7–10	8.5 ± .11	100.0	–
	0.5	15	7–10	8.5 ± .13	100.0	–
	1.0	15	12–15	13.5 ± .09	–	100
	2.0	15	12–15	13.5 ± .05	–	100
	2.0	15	12–15	13.5 ± .17	–	100
NAA	0.2	15	5–7	6.0 ± .07	100.0	–
	0.5	15	3–5	4.0 ± .10	100.0	–
	1.0	15	6–9	7.5 ± .13	–	100
	2.0	15	6–9	7.5 ± .11	–	100
Sucrose Percentage						
	4.0	15	12–17	14.5 ± .16	80.0	–
	5.0	15	7–12	9.6 ± .13	100.0	–
	6.0	15	7–12	9.5 ± .10	100.0	–
	7.0	15	7–12	9.5 ± .08	100.0	–
	8.0	15	7–12	9.5 ± .07	100.0	–

Results are mean of three replicates (15 × 3) ± = SD.

The effect of auxins IAA, IBA and NAA at concentrations 0.2 to 2.0 mg l^{-1} showed that 0.5 mg l^{-1} NAA was the best for inducing root formation in this cultivar of niger (Table 2, Fig. 1D). IAA and IBA at the concentrations 0.2 and 0.5 mg l^{-1} induced roots within 7 to 10 days while NAA induced within 3 to 5 days. Higher concentrations (1.0, 2.0 mg l^{-1}) of NAA, IAA and IBA produced callus at the base of the shoot (Table 2). Thus rooting in niger is good as found with other members of compositae investigated *in vitro* such as *Gerbera* (Pierik et al. 1975) and *Chrysanthemum* (Earle & Langhans 1974 a, b). Twenty per cent of the rooted as well as non-rooted plantlets developed on MS basal medium produced flowers *in vitro* within 56 days after inoculation of explants (Fig. 1E).

Hardening

Plantlets of this cultivar of niger were very sensitive to dehydration when transferred from *in vitro* to natural conditions and died within three days of transfer. Plantlets which were transferred to pots and kept for 1 to 6 days under shade conditions in a glass chamber and then exposed to natural conditions also died after five days of exposure. Plantlets removed from glass chamber after 6 days and maintained 7 to 9 days under shade conditions then transferred to natural conditions survived (80%) and grew well under natural conditions. Plantlets maintained as above under shade for 10 to 15 days and transferred to natural conditions, survived at 97% (Fig. 1F) indicating requirement of shade during initial period of transferring. The plantlets grown to a height of 20 to 50 cm and were flowered and completed the life cycle within 70 to 90 days.

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