BIOLOGIA PLANTARUM 35 (2): 209-215, 1993

Effect of auxins and cytokinins on plant regeneration from hypocotyls and cotyledons in niger (*Guizotia abyssinica* Cass.)

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Abstract

Tissue cultures were established from hypocotyl and cotyledonary leaf segments of *Guizotia abyssinica* Cass. on MS medium supplemented with various concentrations of auxins (IAA, NAA, IBA or 2,4-D) and cytokinins (KN or BA). Explants cultured on media with cytokinins or in combination with auxins produced shoot buds. Maximum number of shoot buds (20 - 25 per culture) were differentiated from cotyledonary leaf segments on medium with 2 mg l^{-1} each of KN and IBA. Rooting of regenerated shoot buds was acheived on medium with NAA. The obtained plantlets were successfully transferred to soil.

Introduction

Guizotia abyssinica Cass., commonly known as niger, is an important oil seed crop in Indian subcontinent and East Africa. India and Ethiopia are the principal niger producing countries. Tissue culture studies in niger showed multiple shoot bud formation in shoot tip culture (Ahmed and Pandey 1988 a) and a report on histology of shoot bud formation in callus cultures (Ahmed and Pandey 1988 b). In the present paper, a method has been described for the regeneration of plantlets from hypocotyl and cotyledonary leaf.

Material and methods

Seeds of *Guizotia abyssinica* Cass. cv. No. 71 obtained from Karnataka Agro Seeds Corporation, Dharwad, were surface sterilised with saturated chlorine water for

Received 22 April 1992, *accepted* 9 November 1992.
Acknowledgements: Authors thank Dr. P.S. Rao, Head, Plant Biotechnology Section, B.A.R.C., Bombay, for critical appraisal of the manuscript. Financial assistance from M/S Kirloskar Consultants Ltd., Pune, to the research project "Vegetative propagation of economically important plants through tissue culture" is gratefully acknowledged.

30 min. Following repeated washing with sterile distilled water, the seeds were germinated on Murashige and Skoog's (1962) agar nutrient medium with 2% sucrose (MS). From 2-week old seedlings, the segments (0.8 cm long) of hypocotyl and cotyledonary leaf were excised and cultured on MS medium alone and on MS supplemented with IAA, NAA, IBA, 2,4-D, KN and BA individually or in combinations. The pH of the medium was adjusted to 5.8 by 1 M NaOH or HCl before autoclaving (15 min).

24 cultures were raised for each treatment. The percentage and the average number of shoot buds differentiated from bud forming explants were taken and the standard error was calculated. All cultures were maintained at 25 ± 2 °C under daily light (10 h), and 50 - 60 % relative humidity. For histological studies, the cultured materials were fixed in formalin-acetic-alcohol. After dehydration and embedding in paraffin, they were sectioned at 10 - 13 μm thick and stained with safranin-fast green. Squash preparations of the callus were stained with acetocarmine.

Results

Hypocotyl segments produced callus and roots directly on MS medium containing IAA, NAA, or IBA (0.5 - 5 mg l^{-1}), whereas on MS basal medium the explant did not show any response. Explants cultured on various concentrations of 2,4-D alone showed scanty callus formation, similar to those cultured on MS with 0.5 mg l^{-1} of KN. When KN concentration was raised to 1 mg l^{-1}, 3 to 5 shoot buds originated directly (Table 1). The shoot buds which originated near the cut end developed further and had 2 or 3 pairs of leaves in four weeks (Fig. 1A). BA alone induced only callusing of the explant and no differentiation of shoots was observed, however explants on MS + KN (0.5 mg l^{-1}) + NAA (1 mg l^{-1}) produced light yellow soft callus and many shoot buds with a greater frequency compared to MS + KN (0.5 mg l^{-1}) + NAA (2 mg l^{-1}), MS + KN (1 mg l^{-1}) + NAA (0.5 mg l^{-1}) and MS + KN (1 mg l^{-1}) + NAA (1 mg l^{-1}). Likewise combinations of KN + IBA also induced callus and shoot buds (Table 1). The frequency of shoot bud formation was more on MS with KN (0.5 mg l^{-1}) + IBA (1 mg l^{-1}). Among the BA + IAA combinations tested, BA (1 and 2 mg l^{-1}) + IAA (1,2 and 5 mg l^{-1}) favoured profuse callusing and shoot bud differentiation with 50 - 100 % frequency. Maximum number of shoot buds (9 per culture) were differentiated (Fig. 1B) at BA (2 mg l^{-1}) + IAA (5 mg l^{-1}) (Table 1). Histological preparation revealed that the shoot buds originated directly from the explants. On media with BA and NAA, light green, soft callus followed by a few incipient shoot buds were observed.

The cotyledonary leaf segments on MS alone did not show any response, whereas upon addition of IAA, NAA or IBA (0.5 - 5 mg l^{-1}), the explants differentiated roots directly and also proliferated into a soft callus. The explants on MS with 0.5 mg l^{-1} KN remained green upto 4 weeks and gradually turned brown, but at 1 mg l^{-1} these explants produced a scanty callus from the cut end differentiating a few shoot buds in 10 % of the cultures (Table 2). The shoot buds thus formed developed one or two

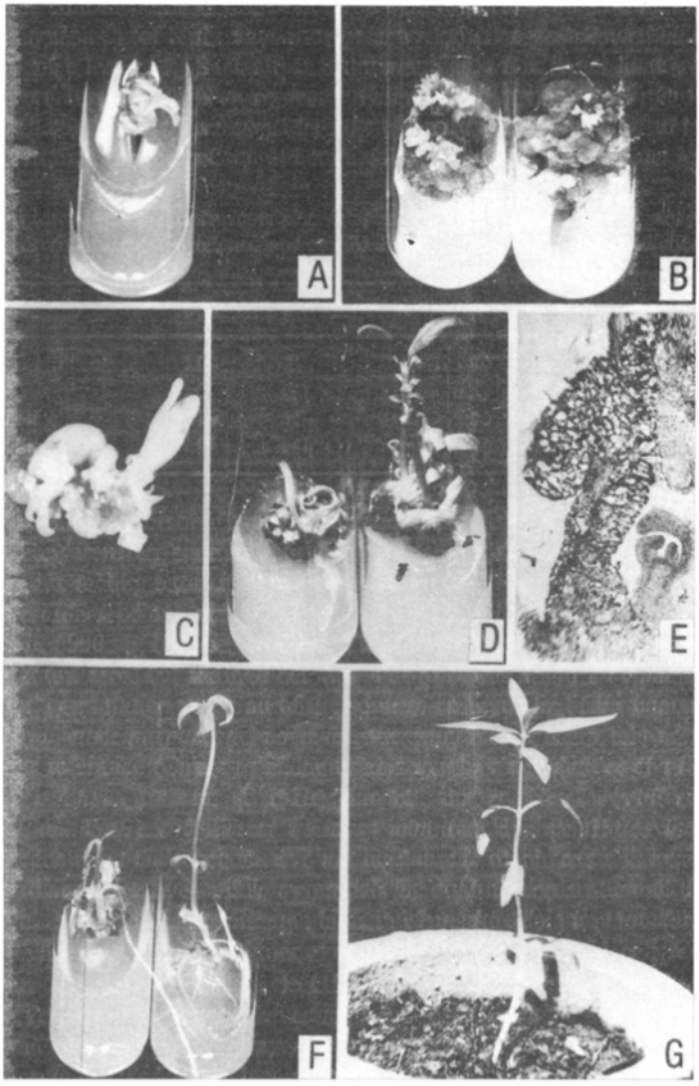

Fig. 1. Hypocotyl and cotyledonary leaf cultures. *A*- development of shoot buds in four-week-old cultures of hypocotyl on MS + KN (1 mg l^{-1}); *B* - profuse callus development and shoot bud formation in hypocotyl segments after four weeks on MS + BA (2 mg l^{-1}) + IAA (5 mg l^{-1}); *C* - shoot buds and callus from cotyledonary leaf cultures on MS + KN (5 mg l^{-1}) + IAA (0.5 mg l^{-1}); *D* - well developed shoot buds, callus and a few roots from cotyledonary leaf segments after four weeks on MS + KN (0.5 mg l^{-1}) + IBA (2 mg l^{-1}); *E* - histological preparation of a portion of cotyledonary leaf segments from MS + BA (0.5 mg l^{-1}); note the differentiation of shoot buds from sub-epidermal tissue; *F* - shoots rooted on MS+NA (1 mg l^{-1}) after three weeks; *G* - transplanted plantlet growing in pot (four-week-old).

pairs of leaves in 4 weeks. Similarly on MS with 0.5 mg l^{-1} BA, explants formed a scanty callus and differentiated 4 - 8 shoot buds from sub-epidermal tissues in 33 % of the cultures.

Table 1. Effect of different concentrations of cytokinins (KN, BA) and auxins (IAA, NAA, IBA) in MS medium on shoot bud differentiation from hypocotyl segments of *G. abyssinica* cv. No. 71 (n = 24).

Phytohormones [mg l^{-1}]	Frequency of shoot buds [%]	Number of shoot buds per culture
KN (1)	16	4.0 ± 0.70
KN (0.5) + NAA (1)	50	4.5 ± 2.47
KN (0.5) + NAA (2)	33	2.51 ± 06
KN (1) + NAA (0.5)	10	1.5 ± 0.33
KN (1) + NAA (1)	33	1.5 ± 0.33
KN (0.5) + IBA (1)	33	4.0 ± 1.41
KN (2) + IBA (0.5)	10	4.5 ± 0.35
BA (0.5) + IAA (1)	10	4.5 ± 0.35
BA (1) + IAA (0.5)	10	5.5 ± 0.35
BA (1) + IAA (2)	50	5.0 ± 0.70
BA (2) + IAA (1)	100	5.5 ± 3.18
BA (2) + IAA (5)	100	9.0 ± 0.70
BA (0.5) + NAA (0.5)	10	4.5 ± 0.35
BA (0.5) + IBA (0.5)	10	5.5 ± 0.35
BA (0.5) + IBA (2)	10	2.5 ± 0.35
BA (5) + IBA (1)	10	2.5 ± 0.35

On MS media with 0.5 mg l^{-1} each of KN and IAA, a whitish scanty callus developed from the cut end of the explants. But on MS + KN (0.5 mg l^{-1})+IAA (1 mg l^{-1}), in addition to whitish soft callus, 1 - 3 shoot buds differentiated in 25 % of the cultures (Table 2). Almost similar responses were noted on MS + KN (0.5 mg l^{-1}) + IAA (5 mg l^{-1}). Higher level of KN (5 mg l^{-1}) along with IAA (0.5, 1, 2 or 5 mg l^{-1}) promoted callusing and shoot bud differentiation and the highest number of buds was at 5 mg l^{-1} KN with 0.5 mg l^{-1} IAA (Fig. 1C). The combinations of KN and NAA (0.5 mg l^{-1}) produced a light green, soft callus and shoot buds(1 - 7 per culture) in 66 % of the cultures and a few roots in 33 % of cultures. Similar results were observed on MS + KN (0.5 mg l^{-1}) + NAA (1 or 2 mg l^{-1}). But the explants on MS + KN (0.5 mg l^{-1}) + NAA (5 mg l^{-1}) produced a yellowish, soft, subculturable callus and roots (2 - 5 per culture) in 33 % of the cultures. Among the combinations of KN-IBA tried, more number of shoot buds differentiated along with a few roots at 0.5 mg l^{-1} KN and 2 mg l^{-1} of IBA (Fig. 1D). Histological studies revealed that the shoot buds had their origin from the sub-epidermal tissues (Fig. 1E).

The explants implanted on 0.5 mg l^{-1} each of BA and IAA yielded a scanty callus at the cut end along with the differentiation of few shoot buds. The higher number of differentiated shoot buds was at 2 mg l^{-1} BA with 1 mg l^{-1} IAA (an average of 10 per culture). Among BA - NAA combinations tried, BA (0.5 mg l^{-1} and NAA (0.5 or 1

differentiated shoot buds was at 2 mg l^{-1} BA with 1 mg l^{-1} IAA (an average of 10 per culture). Among BA - NAA combinations tried, BA (0.5 mg l^{-1} and NAA (0.5 or 1 mg l^{-1}) induced shoot bud formation and callusing of the explants. Similarly shoot bud differentiation was noted on medium with BA (0.5 mg l^{-1}) and IBA (0.5, 1 and 2 mg l^{-1}).

Table 2. Effect of different concentrations of cytokinins (KN, BA) and auxins (IAA, NAA, IBA) in MS medium on shoot bud differentiation from cotyledonary leaf segments of *G. abyssinica* cv. No. 71 ($n = 24$).

Phytohormones [mg l^{-1}]	Frequency of shoot buds [%]	Number of shoot buds per culture
KN (1)	10	2.5 ± 0.35
KN (0.5) + IAA (1)	25	2.0 ± 0.70
KN (0.5) + IAA (5)	16	2.5 ± 0.35
KN (5) + IAA (0.5)	33	3.5 ± 1.76
KN(5) + IAA (1)	25	1.5 ± 0.35
KN (5) + IAA (5)	33	3.0 ± 0.70
KN (0.5) + NAA (0.5)	66	4.0 ± 2.12
KN (0.5) + NAA (1)	66	12.5 ± 1.76
KN (0.5) + NAA (2)	10	1.5 ± 0.35
KN (1) + NAA (0.5)	33	1.5 ± 0.35
KN (1) + NAA (1)	10	4.5 ± 0.35
KN (1) + NAA (2)	33	2.0 ± 0.70
KN (0.5) + IBA (0.5)	33	1.5 ± 0.35
KN (0.5) + IBA (2)	50	6.5 ± 3.88
KN (0.5) + IBA (5)	10	1.5 ± 0.35
KN (1) + IBA (0.5)	10	1.5 ± 0.35
KN (2) + IBA (0.5)	10	8.5 ± 0.35
KN (2) + IBA (1)	50	3.0 ± 1.41
KN (2) + IBA (2)	10	22.5 ± 1.76
KN (5) + IBA (2)	10	2.5 ± 0.35
BA (0.5)	10	6.0 ± 1.41
BA (0.5) + IAA (0.5)	10	4.5 ± 0.35
BA (1) + IAA (0.5)	66	8.0 ± 1.41
BA (1) + IAA (1)	10	7.0 ± 0.70
BA (2) + IAA (1)	66	10.0 ± 3.53
BA (2) + IAA (2)	10	2.5 ± 0.35
BA (2) + IAA (5)	66	8.5 ± 2.47
BA (0.5) + NAA (0.5)	34	4.0 ± 1.41
BA (0.5) + NAA (1)	10	4.5 ± 0.35
BA (0.5) + IBA (0.5)	50	4.0 ± 0.70
BA (0.5) + IBA (1)	66	4.0 ± 0.70
BA (0.5) + IBA (2)	50	4.5 ± 1.06

The shoot buds differentiated *in vitro* failed to form roots *in situ*, but produced roots three weeks after transfer to MS added with NAA (1 mg l^{-1}). The regenerated

Discussion

It is generally observed that immature tissues and organs especially seedling parts are morphogenetically more plastic than the mature differentiated tissues (*e.g.* Krikorian 1982). In the present investigation also, seedling hypocotyl and cotyledonary leaf segments proved successful choice.

The involvement of cytokinins especially in bud formation has been reported in many plant species. In *Carthamus tinctorius* the cotyledons, produced buds in the presence of BA but the hypocotyls did not respond (George and Rao 1982). Similar phenomenon was also noted earlier in *Brassica juncea*, where cotyledons produced shoot buds in the presence of KN but failed to do so in presence of BA in the medium. On the other hand cotyledonary leaf segments produced shoot buds in the presence of either KN or BA. The combinations of auxins and cytokinins at definite proportions are very critical and found to be essential for the induction of shoot and root in many species (*e.g.* Lazzeri and Dunwell 1986, Banerjee *et al.* 1988. Reddy and Bahadur 1989, Shankar and Mohan Ram 1990, George *et al.* 1987). In *Brassica oleracea* leaf cultures, BA and IBA in combination were more effective in shoot production (Lazzeri and Dunwell 1986). In the present study, though shoot bud differentiation from hypocotyl and cotyledonary leaf segments was obserevd on media with KN or BA, the response was more pronounced on media which contained both auxin (IAA, NAA or IBA) and cytokinin (KN or BA) and the differentiated shoot buds required NAA for rooting.

The initiation of organs *in vitro* is a complex morphogenetic phenomenon, in which extrinsic and intrinsic factors play a role. The capacity to achieve organogenesis through modification of the medium and the culture environment has formed the basis for the application of tissue culture for commercial propagation of plants of economic value (*e.g.* Biondi 1986). In the present investigation, a successful attempt has been made to regenerate complete plantlets from seedling explants of niger by the manipulation of the nutrient media.

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Communicated by J. TUPÝ