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Mass Propagation and Ex vitro Survival of Banana cv. 'Basrai' Through Tissue Culture

In vitro Massenvermehrung und ex vitro Überlebensrate der Banane 'Basrai'

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Summary

A protocol is described for large-scale multiplication and ex vitro survival of banana var. 'Basrai' through tissue culture. Multiple shoot cultures from shoot-tip explants of 'Basrai' were established on modified Murashige and Skoog (MS) medium. Maximum 28 shoots could be achieved on MS medium containing KH_2PO_4 (170 mg/l), Ascorbic acid (50 mg/l), Adenine hemisulphate (100 mg/l), Benzyladenine (100 mg/l), Kinetin (3.0 mg/l) and Indole acetic acid (0.5mg/l). Extending subculture cycle 8 or 9 showed variants in culture. Maximum 90 % rooting were achieved in well-developed shoots on MS medium supplemented with Indolebutyric acid (1.0 mg/l). More than 25,000 plantlets produced through tissue culture were transferred to field with 90 % survival rate. The in-house protocol developed is reproducible and shows potential for commercial scale production of banana plants of var. 'Basrai'.

Zusammenfassung

Es wird ein Protokoll beschrieben zur Massenvermehrung in vitro und Überführung ex vitro der Bananensorte 'Basrai'. An Sproßspitzen-Eyplantaten wurde multiple Sproßbildung auf einem modifizierten MS-Medium induziert. Ein Maximum von 28 Sprossen pro Explantat wurde auf Medium mit 170 mg/l KH_2PO_4, 50 mg/l Ascorbinsäure, 100 mg/l Adeninsulfat, 100 mg/l Benzyladenin, 3,0 mg/l Kinetin und 0,5 mg/l Idolessigsäure erzielt. Beginnend mit der 8. bzw. 9. Subkultur wurden Vitrifikationen und morphologische Veränderungen beobachtet. Maximal 90 % Bewurzelung von gut entwickelten Sprossen wurden mit 1,0 mg/l erzielt. Mehr als 25.000 Pflanzen wurden ins Freiland mit einer Überlebensrate von 90 % überführt. Das vorgestellte Verfahren besitzt wirtschaftliche Bedeutung für die Massenproduktion von Pflanzend er Sorte 'Basrai'.

Introduction

Bananas (*Musa* sp.) being the 4th important food crop in terms of gross value in the world and most significant fruit crop after mango in India. 'Basrai' (Dwarf Cavendish) is the popular cultivar of commercial growers in states of Maharashtra, Andhrapradesh and few parts in Tamilnadu, Kerela and West Bengal. Conventional method of propagation of banana is through daughter suckers arise at the base of the main pseudostem, however, the planting material is almost always in short supply. Tissue culture micropropagation of banana is advantageous in several ways (CRONAUER and KRIKORIAN, 1986a; DORESWAMY and LEELA SAHIJRAM, 1991; KODYM and ZAPATA-ARIAS, 1999; KRIKORIAN, 1989; MA and SHII, 1974; SWENNEN et al., 1991; ZIMMERMAN, 1986). Extensive basic work has been done on the in vitro propagation in different cultivars of banana throughout the world (BANERJEE and DE LANGHE 1985; CRONAUER and KRIKORIAN 1986; DORESWAMY and LEELA SAHIJRAM, 1991; JARRET et al., 1985; KRIKORIAN and CRONAUER 1984; NOVAK et al. 1989; WONG et al. 1986). The objective of present investigation was to develop an in-house micropropagation system for large-scale production of banana in cultivar 'Basrai'.

Materials and Methods

Explant preparation and culture conditions:

Vegetative shoot-tips from sword suckers were used for establishment of shoot cultures in cultivar 'Basrai'. The explants were collected from true-to-type, high yielding, disease-free plants through Banana Research Station, Jalgaon (Fig. 1 A). Explants washed thoroughly in tap water and Tween-20 (1 %). Traces of detergent were removed by repeated washings in running tap water. Leaves and extraneous rhizome tissue were chopped with a stainless steel knife. Trimmed suckers soaked in a solution of Bavistin (0.5 %) and Tetracyclin (0.05 %) for 2 to 3 hrs. Shoot-tips containing several sheathing leaf bases enclosing the axillary buds with subjacent rhizome tissue and measuring 2.5 to 3.5 cm in length were isolated. These shoot-tips were surface sterilized with sodium hypochlorite 10 % v/v) for ? hr. Traces of chlorine were removed washing several times with sterile distilled water. From the sterilized shoot-tips, explants were readied using sterilized stainless steel scalpels. Cut surfaces of the rhizomatous tissue and leaf bases were further trimmed so that shoot-tips finally contain at least six to eight overlapping leaf bases enclosing axillary buds. Murashige and Skoog basal (BMS) medium (MURASHIGE and SKOOG, 1962) was used with modifications. The level of phosphate was raised using KH_2PO_4 at 170 mg l. Thiamine concentration was increased from 0.1 mg/l to 1.0 mg/l and sucrose at 3 % was used. The medium was supplemented with various combinations of growth regulators e.g. Adenine hemisulfate (ADS), Benzyl adenine (BA), Kinetin, 1-Naph-

thalene acetic acid (NAA), Indole 3-acetic acid (IAA) and Indolebutyric acid (IBA) at different concentrations. The gelling agent was Phytagel (0.25 %, Sigma Co., St. Louis) and pH of the medium was adjusted to 5.8 before autoclaving at 121 °C. Cultures were incubated under 16-h photoperiod (60 uEm2s1) at 25 ± 1 °C using fluorescent and incandescent lamps. Data were taken completion of each subculture after 4 weeks and experiments were repeated twice.

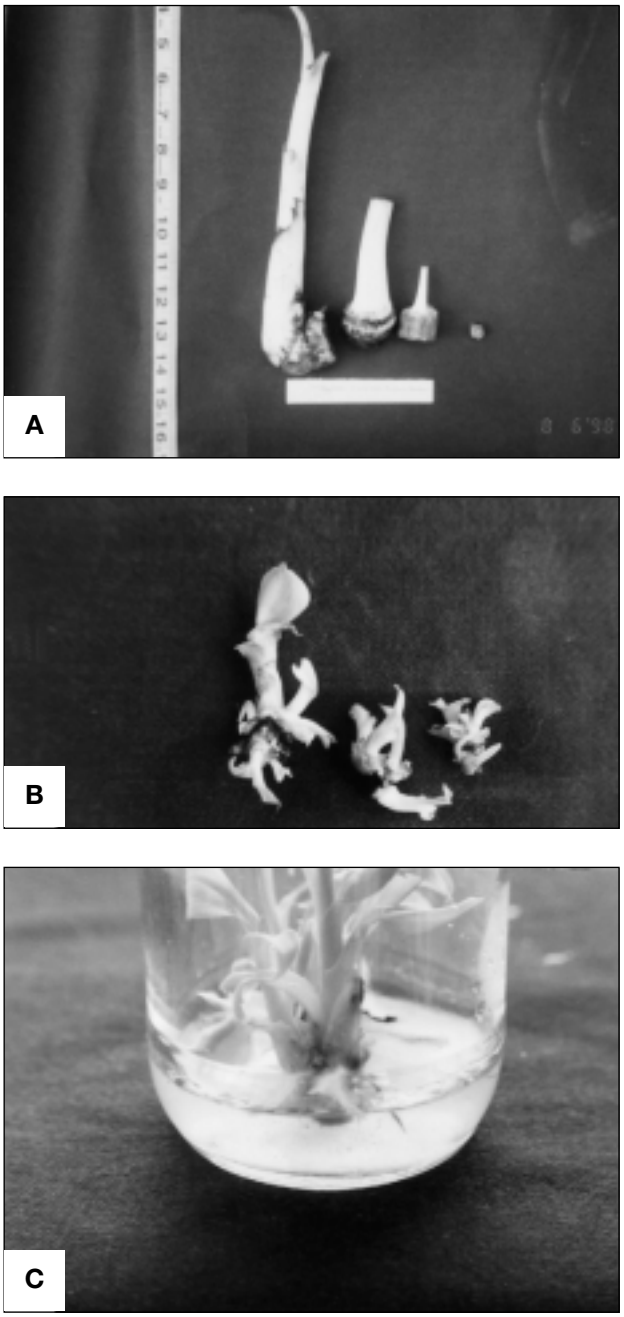

Fig. 1. Mass propagation in Banana var. 'Basrai'.
Explants (sword suckers) (A), Induction of multiple shoots from shoot-tip explants on MS medium containing KH_2PO_4 (170 mg/l), ADS (100 mg.l), IAA (0.5 mg/l), BA (2.5 mg/l) and Kinetin (2.5 mg/l) (B), and Rooting in regenerated shoots on MS medium supplemented with IBA (1.0 mg/l) and activated charcoal (2 gm/l) (C)
Massenvermehrung von Banane 'Basrai'.
(A) Esplantate, (B) Induktion multipler Sproßbildung auf MS-Medium mit 170 mg/l KH_2PO_4, 100 mg/l ADS, 0,5 mg/l IAA, 2,5 mg/l BA und 2,5 mg/l Kinetin und Bewurzelung von Sprossen auf MS-Medium mit 1,0 mg/l IBA und 2 mg/l Aktivkohle

Rooting and Transplantation of plants to field:

At the end of the multiple shoot production cycle individual shootlets were excised and transferred to semi-solid BMS containing IBA (1.0 mg/l). Well-developed single plantlets removed from the culture vessel washed carefully in running tap water to remove the adhered agar in roots. A dip (1–2hrs.) in Bavistin (0.5 %) solution follows and finally plantlets transferred to pots containing sterilized promix and soil (1:1) covered with polybags for hardening. Transferred plantlets were lightly irrigated and maintained under high humidity for a fortnight. Established plantlets were transplanted in polybags (9" x 5") containing the same potting mix and weaned in greenhouse. Normal irrigation was carried until plantlets were distributed to farmers.

Results and Discussion

The shoot-tip explants were initially cultured on stationary liquid (BMS) medium supplemented with ADS (100 mg/l), BA (2.5 mg/l), Kinetin (2.5 mg/l) and IAA (0.5 mg/l). In a week, explants swelled a little, outer leaf sheaths and surface of explants turned brownish. Addition of activated charcoal (2.0 gm/l) was found suitable to overcome the problem of browning and phenolics. The curved superficial and overlapping leaf sheaths carefully excised under a microscope to expose apical meristem (shoot apex). The apical meristem measuring 5mm in diameter was excised and transferred to semi-solid BMS of the original composition. About four weeks later, the explant swells up, turns green showing morphogenetic activity. Initially tiny, cream-greenish protuberances begin to appear which eventually developed in large shoot clusters with leafy structures in eight weeks (Fig. 1 B). No appreciable difference was observed in morphogenetic response of shoot-tip explants of three cultivars of banana var. 'Basrai', 'Ardhapuri' and 'Shreemanthi' (data not shown in later two cultivars). Induction of multiple shoots in var. 'Basrai' was observed on various combinations of growth regulators (Table 1). Maximum 28 shoots could be achieved on BMS containing IAA (0.5 mg/l), BA (2.5 mg/l), Kinetin (2.5 mg/l) and ADS (100 mg/l). Multiple shoots were subculture on fresh medium generated high number of populations in subsequent cycles of four weeks each. Extending eight or nine subcultures showed vitrification and morphological variation in shoots. Spontaneous rooting was observed in regenerated shoots. However, MS medium supplemented with IBA (1.0 mg/l) was found optimal for root formation (Fig. 1 C). Rooting was induced within a week and nearly 90 % rooting achieved in 'Basrai'. In-vitro produced plantlets performed well in field conditions after hardening. A self-sustaining tissue culture micropropagation system for var. 'Basrai' is shown in Flow-chart (Fig. 2). It is clear from the presentation that one shoot-tip explant could produced approx. 829 plantlets in a year. Following the procedure as shown in flow-chart

Table 1. Effect of growth regulators on multiple shoot regeneration from shoot-tip explants of banana (var. 'Basrai'). At least 12 explants were used in each treatment and data were scored after 4 weeks of growth.
Einfluß von Wachstumsregulatoren auf die multiple Sproßbildung an Sproßspitzen-Explantaten der Banensorte 'Basrai'. Wenigstens zwölf Explantate pro Versuchsglied wurden kultiviert und nach vier Wochen Kulturdauer bonitiert.

MS medium + Growth regulators (mg/l)			Explants differentiated (%)	No. of shoots/ explant (±S.D.)	Shoot length (±S.D.)
MS control (HF*)			29	1±0.89	1.2±0.83
NAA	Kinetin	BA			
0.1	1.0	-	33	2±0.51	2.1±0.51
	2.5	-	37	3±0.76	3.7±0.76
	5.0	-	50	3±1.2	4.2±0.69
0.5	1.0	-	42	5±1.82	3.6±1.2
	2.5	-	46	6±1.69	3.9±0.95
	5.0	-	58	5±1.82	4.4±1.32
IAA					
0.1	-	1.0	42	3±1.13	2.1±0.56
	-	2.5	46	7±1.82	3.1±0.87
	-	5.0	54	10±2.08	3.6±1.2
0.5	-	1.0	46	4±1.77	2.3±1.32
	-	2.5	58	9±2.02	3.8±0.76
	-	5.0	66	14±2.17	4.7±1.1
IAA					
0.1	1.0	2.5	42	4±1.81	3.1±0.87
	2.5	2.5	46	7±1.77	3.4±1.35
	5.0	2.5	58	9±2.02	3.9±0.95
0.5	1.0	2.5	54	11±2.17	2.9±1.1
	2.5	2.5	66	19±2.39	3.4±1.35
	5.0	2.5	58	28±2.7	4.2±0.69
IBA					
0.1	1.0	-	37	3±1.69	1.2±0.95
	2.5	-	42	5±1.82	2.4±0.38
	5.0	-	42	5±1.82	3.8±1.2
0.5	1.0	-	33	7±1.77	1.8±0.78
	2.5	-	46	8±2.48	2.1±0.56
	5.0	-	58	7±1.77	3.4±1.35

* HF- Hormone-Free

more than 25,000 plants of 'Basrai' have been produced.

Micropropagation of various cultivars of banana through shoo-tip explants is well documented (DREW et al., 1989; KO et al., 1991; KOTECHA and KADAM, 1998; PANDEY et al., 1993; SUDHAVANI and REDDY, 1997). In almost all cases, different combinations of cytokinin and auxin in various concentrations were reported for multiple shoot regeneration. The physiological state of explants, seasonal and cultivar difference are the reasons, perhaps that different workers have reported different media for plantlets regeneration in banana. Different liquid medium and culture methods for meristem propagation of bananas have been compared with gelled culture medium (ALVARD et al., 1993). It is not desirable to subject most banana genotypes to more than 8 or 9 subcultures in the production cycles due to increased incidence of somaclonal variants in culture (REUVENI et al., 1985). Rooting in regenerated shoots has been achieved on modified medium

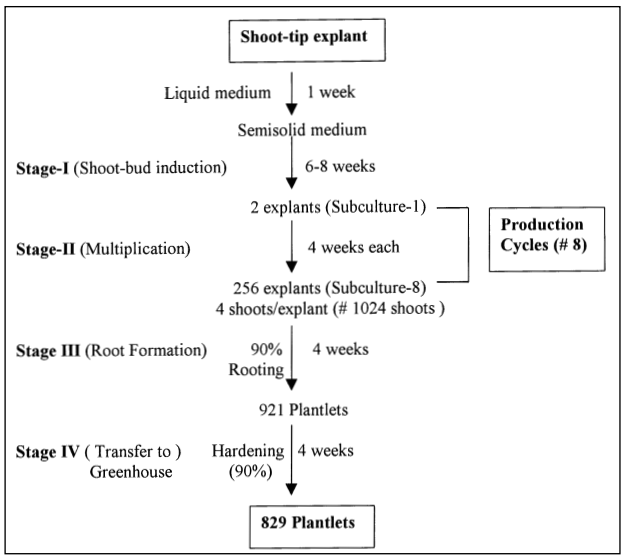

Fig. 2. Flow-Chart: In vitro Plantlet Regeneration in Banana cv. 'Basrai'
Schema zur in vitro Pflanzenregeneration von Banane 'Basrai'

supplemented with or without an auxin. In present case, spontaneous root formation in shoots was observed, however, BMS containing IBA (1.0 mg/l) was found suitable for rooting. Sometimes, shoots were directly transferred to soil (BHAGYALAKSHAMI and SINGH, 1995). Modern cultivated bananas are prone to several diseases, such as bunchy top, cucumber mosaic virus and Panama wilt (SIMMONDS, 1966). Vegetative apical meristem culture in consort with thermotherapy can be used for eradicating the cucumber mosaic virus (BERG and BUSTAMANTE, 1974). The technique outlined in this communication can be gainfully employed for pathogen-free and large-scale production of banana plants in var. 'Basrai', 'Ardhapuri' and 'Shreemanthi'.

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