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Effect of BAP and 2-ip on Indirect Regeneration from Vegetative Bud Derived Callus of Turmeric (*Curcuma longa* L.) Variety Suguna

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Abstract *Curcuma longa* L. (Turmeric) is one of the oldest and most popular spices known for its distinct flavor and medicinal properties. This vital spice is infested by various insects and pests, and none of the cultivars are resistant. It's now imminent to conserve turmeric genepool. We in this study have attempted to induce callus cultures that were obtained from vegetative buds of turmeric on Linsmaier and Skoog's medium (LS) supplemented with various concentrations of growth regulators. The presence of 2, 4-D (2, 4-Dichlorophenoxy acetic acid) in the culture medium at 3 mg/l resulted in the callus growth. Organogenesis and plantlet formation were not reported in the presence of auxin (i.e. 2, 4-D). The friable callus cultures were transferred on the medium supplemented with benzyladenine or benzylaminopurine 4 mg/l^{-1} and 2, 4-D 0.5 mg/l^{-1} to obtain the multiple shoots (1 : 27). When the shoots were transferred to medium devoid of phytohormones, completed roots were obtained. These plants transferred to hardening and they are established in the soil (is about 90%).

Keywords Vegetative bud, Plant regeneration, *Curcuma longa*, 6-Benzylaminopurine, 2, 4-Dichlorophenoxy acetic acid.

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Introduction

Turmeric (*Curcuma longa* L.) belonging to the family Zingiberaceae is known as the "Golden Spice" and "Spice of Life" as well. It has been used in India as a medicinal plant, and held sacred from time immemorial and is reported to be a therapeutic agent for several major human diseases (Hung Hsu and Lii Cheng 2007). The primary biological active constituent of turmeric is the curcumin, a polyphenol that has potent anti-inflammatory and anti-oxidant properties which is known for its anti-inflammatory, anti-HIV, anti-oxidant and aromatic stimulant properties (Ammon and Wahl 1991). Lack of seed setting and vegetative propagation has made turmeric genetically stable with less or no variation. Hence, the present investigation we have initiated turmeric callus cultures and regeneration resulting in the development of useful somaclonal variants, which are not available by conventional methods.

Rapid clonal multiplication and micropropagation in turmeric are quite common though (Nadagauda et al. 1980, Sugaya 1992, Yasuda 1988, Balachandran et al. 1990, Salvi et al. 2001) but research on indirect regeneration from callus cultures (except leaf base callus) of turmericis lacking. Compared to

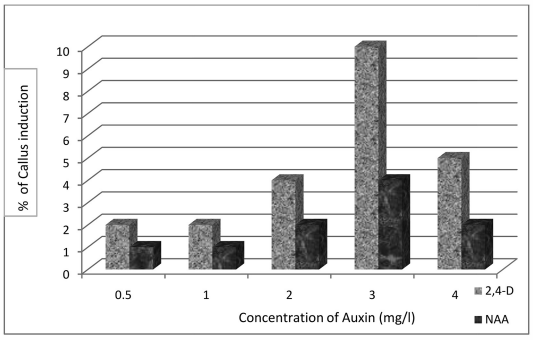

Fig. 1. Effect of different concentrations of auxins such as 2, 4-D and NAA for callus induction from vegetative bud explant of turmeric variety Suguna (*Curcuma longa* L.) on LSBM cultured for 40 days at 3 mg/l of 2, 4-D.

micropropagation from isolated shoot tips, somatic embryogenesis or shoot formation through organogenesis leading to genetic alteration, the introduction of valuable variation through somaclonal variation may help in programs designed to improve the characteristics of the crop. In the present investigation, the initiation of callus from *in vivo* vegetative bud and subsequent regeneration of turmeric plant has been studied.

Materials and Methods

Rhizomes of turmeric (*Curcuma longa* L.) collected from healthy and disease plants at harvest time (Feb–Jun i.e. early summer) were cleaned thoroughly by repeated washing of young buds measuring 10–20 mm in length with small portions of attached rhizomes were used as explants.

The vegetative buds were washed thoroughly with tap water using labolene solution (till the remove of labolene) followed by washing with distilled water. In laminar airflow cabinet, the buds were rinsed with 70% ethyl alcohol for 45 seconds then treated with 0.1% $HgCl_2$ solution for 5–6 minutes (with frequent shaking). The buds were washed 3 times in double distilled water (5 min, 5 min, 7 min) and 0.1% streptomycin sulfate for 1 min. The buds were transferred to sterile petri plate dissected with a sharp scalpel to remove the outer leafy scales and inoculated on Linsmaier and Skoog 1965 basal medium (3% sucrose and 0.8% agar) fortified with different concentrations of NAA and 2, 4-D ranging from 0.5–4 mg/l for callus induction (Fig. 1). All the growth hormones and reagents used in the experiments were procured from HiMedia Laboratories, Mumbai, India.

The callus initiated from vegetative bud explants was further subcultured on the same medium to harvest profusely formed callus. Further, initiation of shoot primordia was obtained when the profuse callus was transferred to LSBM fortified with different concentrations and combinations of 2-ip, BAP and 2, 4-D viz 1-5mg/l. *In vitro*, grown plantlets were carefully taken out from the culture tubes and washed with water to remove the traces of agar. They were later transferred to plastic pots containing different combinations of a potting mixture consisting of peat, perlite, and sand (v/v). Among the different potting mixture composition studied, we found that Peat : Sand : Perlite in the ratio of 50 : 25 : 25 (v/v) to be the best for hardening of tissue culture raised plants of turmeric variety Suguna. About 92% of the plants were hardened and transferred to the pots. The potted plants were kept in the greenhouse conditions, covered with polythene bags to maintain high humidity. After 21 days, the covers were removed in phases to expose the plants to less humid conditions. After 7 weeks of hardening period, the *in vitro* raised and rooted plants were transferred to the field. The data obtained from the results of all the experiments carried out were analyzed statistically by One-Way Analysis of Variance (ANOVA) to determine the variation between the treatments using the SPSS9 software.

Results and Discussion

The vegetative bud explants measuring 0.5–1 cm was inoculated on LS basal medium supplemented with 0.5 to 4 mgl^{-1} of 2, 4-D (Fig. 2). After 25 days of inoculation on LS basal medium supplemented with 2, 4-D the explants callused and a large amount of compact, creamish white callus formed from vegetative bud after 45 days (Fig. 3E), 60 days and 80 days of culture were kept for observation. We found that LS basal medium supplemented with 3 mgl^{-1} of 2, 4-D alone was found to be the best medium for

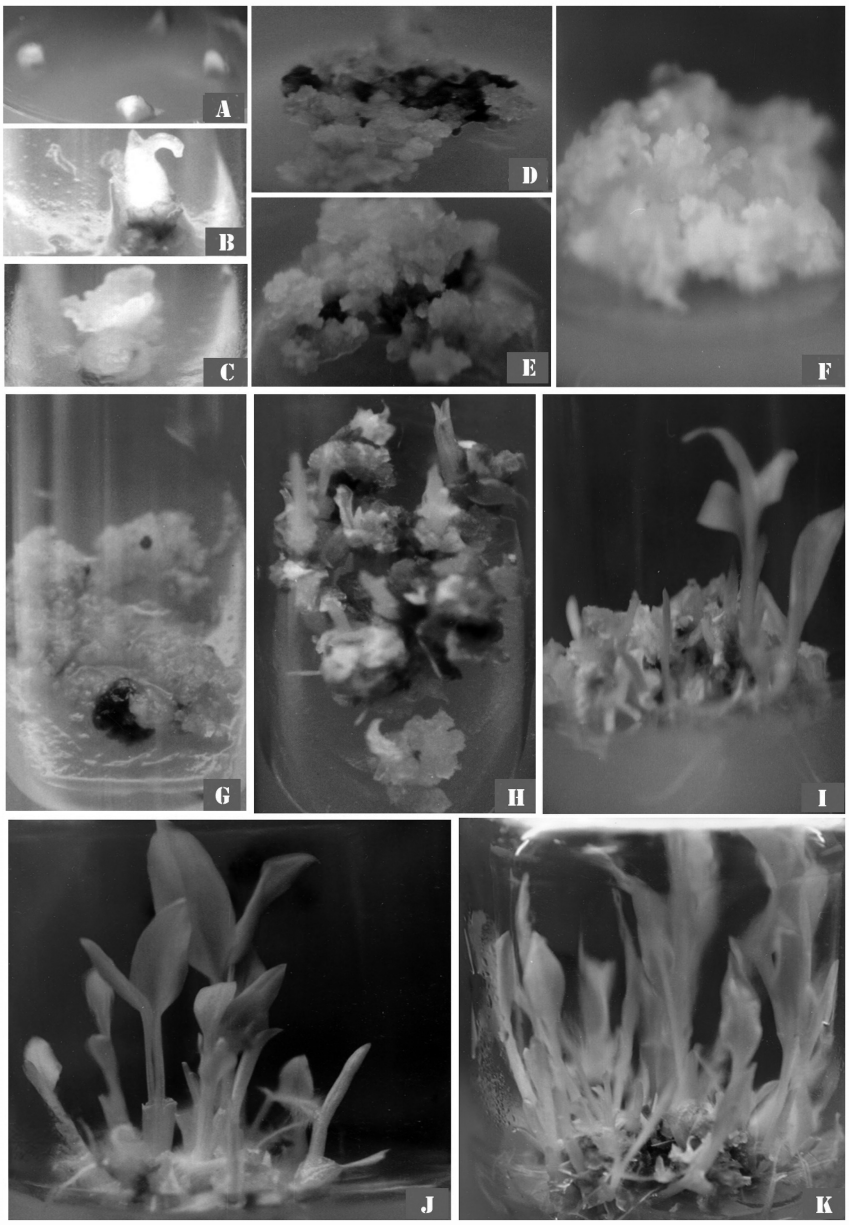

Fig. 2. *Curcuma longa* L. variety Suguna indirect regeneration ; (2, A-F) vegetative bud explant on LSBM + 2, 4-D 3.0 mgl^{-1} ; (A) explant at culture ; (B) 25 days old culture showing initiation of callus ; Fig. (C-D) 45 days old culture showing callus formation ; (E) compact and creamish white callus after 45 days of culture ; (F) further profuse callusing after 60 days of culture. (2yG-K) shoot differentiation and regeneration on LSBM + BAP 4.0 mgl^{-1} + 2, 4-D 0.5 mgl^{-1} ; (G) subcultured callus at culture ; (H) initiation of shoot primordial after 21 days of culture from callus ; (I) development of shoots and initiation of roots, after 40 days of culture ; (J) multiple shoot formation after 75 days of culture ; (K) elongation of multiple shoots (25–30) with roots after 96 days of culture.

vegetative bud callus induction in the turmeric variety Suguna (Fig. 1). An optimum concentration of auxin (3.0 mg/L) is essential to raise callus, which was subcultured on the LSMB to get profusely formed compact and creamish white callus. It was observed that 3 mg/L of 2, 4-D was found to be the best auxin

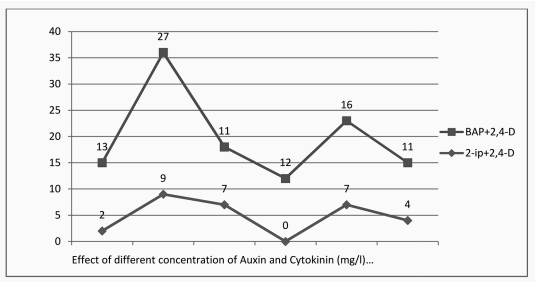

Fig. 3. Effect of different concentrations od auxin and cytokinin for the regeneration from vegetative bud derived callus.

for the effective induction of callus when compared to NAA from *in vitro* derived pseudostem of *Curcuma longa* (variety Suguna) (Banerjee and Nigam 1978, Bhavani Sankar and Srinivasamurthy. 1974, Gomathy et al. 2014). The observation corroborates with the findings of Babu et al. (1992) and Malmug et al. (1991) in ginger and Chellappan Soundar et al. (2015) in turmeric. However, Ventura et al. (2016) reported that callus induced from the pseudostem derived explants on MSBM supplemented with 2, 4-D 1.5 mg^{-1} and BAP 0.2 mgl^{-1}.

The compact and creamish white callus was subcultured (Fig. 3) using different concentration and combinations of 2-ip, BAP and 2, 4-D (Table 1, Fig. 2). It was found that after 21 days of subculture on LS basal medium supplemented with 4.0 mgl^{-1} of BAP and 0.5 mgl^{-1} of 2, 4-D the callus increased in size and tiny greenish-white shoot primordial were differentiated (Fig. 3). Similar results have been reported by Balachandran et al. (1990). However, the present findings are in contrast with the results of Salvi et al. (2000), who has reported the 0.1 mg/L of NAA for effective rooting. More so, Yaping Kou et al. 2013 has reported 0.53 mM and Ghosh et al. 2013 has reported 0.5 mg/L of NAA for rooting as well. Ventura et al. (2016) reported that the multiple shoots are derived from the leaf base callus with IAA

Table 1. Effect of different cytokinins and auxin for regeneration from vegetative bud callus in turmeric variety Suguna (*Curcuma longa* L.). * : Mean of 10 replications, ** : Significant F value @ 5% level.

Basal media	Cytokinin (mgl^{-1})	2, 4-D (mgl^{-1})	Response (%)	No. of shoots/explants $\overline{X}$* ± SD
			2-ip	
LS	2.0	0.5	0	0 ± 0.00
LS	4.0	0.5	60	8.98 ± 1.16
LS	5.0	0.5	58	6.81 ± 1.00
LS	2.0	1.0	89	0 ± 0.00
LS	4.0	1.0	92	7.29 ± 1.11
LS	5.0	1.0	86	4.14 ± 1.36
			BAP (6-Benzylaminopurine)	
LS	2.0	0.5	89	12.73 ± 1.54
LS	4.0	0.5	92	27.33 ± 1.53
LS	5.0	0.5	86	11.34 ± 1.04
LS	2.0	1.0	64	11.99 ± 1.35
LS	4.0	1.0	80	15.83 ± 1.69
LS	5.0	1.0	71	11.43 ± 1.02

Anova table (for no. of shoots/explant).

SV	DF	SS	MSS	F_{cal} ratio	F_{tab} value**	CD
Treatments	11	6020.99	547.3632	256.84	1.88	1.87
Error	108	230.15	2.13			
Total	119	6251.15				

and kinetin. These shoot primordial developed into shoots with the initiation of roots after 40 days of culture. Further, by 75 days of culture, elongation of shoots and development of roots from the callus were observed and after 96 days of culture well-developed shoots (25–30) with roots from the callus was prominent. Multiple shoots obtained from the vegetative bud callus was recorded and analyzed statistically (Table 1). Analysis of the results revealed that there exists a highly significant difference between and within the treaments. The callus regenerated plants were hardened using a potting mixture consisting of Peat : Sand : Perlite (50 : 25 : 25 V/V) at Relative Humidity (RH) of 90–95% and 25 ± 2°C by keeping them covered with polythene bags for 3 weeks and further transferred to the field with 92% success. Most of the plants were morphologically similar at an early stage with 4–6% of the plants showing some variations. The protocol is being used regularly in our laboratory for the production of somaclones. The present studies have made it possible to obtain indirect regeneration from vegetative bud derived callus cultures of turmeric variety Suguna of *Curcuma longa* L.

The highest mean number of multiple shoots derived from vegetative bud callus was found to be (27.33) on LSBM + BAP (4.0 mgl^{-1}) + 2, 4-D (0.5 mgl^{-1}) and lowest mean number (4.14) on LSBM + 2-ip (5.0 mgl^{-1}) + 2, 4-D (1.0 mgl^{-1}) (Table 1, Fig. 3). We found that LSBM +BAP (4.0 mgl^{-1}) + 2,4-D (0.5 mgl^{-1}) was the most suitable medium for initiation and multiplication of shoots from *in vivo* derived vegetative bud callus compared to other combinations. The analysis of the results depicts that this medium was significantly superior when compared to different concentration and combinational of 2-ip tested with respect to multiple shoot formation through indirect regeneration of vegetative bud callus.

Abbreviations: 2-iP: 2 Isopentenyl adenine, BAP : 6-Benzylaminopurine, 2, 4-D : 2, 4-Dichlorophenoxy acetic acid, LSBM : Linsmaier and Skoog Basal Medium.

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