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Effect of plant growth regulator on micropropagtion of ginger (*Zingiber officinale* Rosc.) cv- Suprava and Suruchi

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An efficient, simple micropropagation method was developed for *Zingiber officinale* Rosc.) cv, Suprava and Suruchi using fresh rhizome sprouting bud in semisolid culture media. Explants were cultured on to Murashige and Skoog's (MS) medium supplemented with different concentrations and combinations of BAP (6-benzyl- amino-purine) and NAA (α- Naphthalene acetic acid) for shoot and root induction. Explants cultured on MS basal medium supplemented with 2.0 mg/l BAP + 0.5gm/l NAA showed the highest rate of shoot multiplication. *In vitro* shootlets were rooted on to the half strength MS basal media supplemented with 2.0 mg/l NAA and rooting was better growth. Rooted shoots were transplanted in the green house for hardening and their survival was 95% in the field condition.

Key words: *Zingiber officinale*, micropropagation, rhizome sprouting bud, growth regulators, tissue culture

Introduction

Zingiber officinale Rosc. (Ginger) of the family *Zingiberaceae* is an important tropical horticultural plant, values all over the world as an important spices for its medicinal properties. The *Zinggiberaceae* is an herbaceous moderate sized family of relatively advanced monocotyledonous plant of the order Zingiberales. Zingiberaceous plants are rhizomatous, perennial and aromatic herbs often of large size, bearing flowers either terminally on aerial leaf shoots or from ground level. These are plants of tropical and subtropical regions distributed mainly in East Asia. Several authors have quoted different figures for the total number of genera and species but it is probably appropriate to quote the world record at least 51 genera and 1500 species (Newman, 2001). The Indo-Malayan region is reportedly the centre of diversity for *Zingiberaceae* with at

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least 20 genera species occurring in Borneo, while Holttum (1950) recorded 23 genera and 200 species. It is rich in secondary metabolite such as Oleoresin (Sakamura *et al*., 1986). Breeding of ginger is seriously handicapped by poor flowering and seed set. It is propagated vegetatively through rhizome. The germplasm collections in clonal repositories are also seriously affected by fungal diseases. Moreover since pathogenic fungi, bacteria or viruses are readily transmitted through traditional practices, it was deemed important to develop a micro propagation technique and to make available for commercial use to the pathogen free ginger germplasm. *In vitro* regeneration of auxiliary and adventitious shoots from shoot tips has already been attempted by callus culture (Hosoki and Sagawa, 1977) and clonal multiplication methods through meristem tip culture have also been reported by (Pillai and Kumar, 1982; Smith and Hamill, 1996). However in these methods, the propagation rate was not shown high enough to obtain disease free quality micro plantlets for commercial use, and the acclimatization of the plantlet was very slow and unsatisfactory. Slow multiplication rate limited availability of high yielding genotypes extensive field maintenance of planting material, high susceptibility to rot diseases that necessitates application of tissue culture techniques as a solution to these problems (Nayak and Naik, 2006). *In vitro* propagation has long been recognized as an efficient means for rapid clonal multiplication and conservation of important taxa. However *in vitro* culture is the best method as a continuous source of supply of disease free planting material for commercial utilization. The utility, the various method of propagation includes efficient cost, effective method of *in vitro* multiplication is essential for improvement of ginger. The most important role of *in vitro* propagation is to conserve the genetic variation and evolutionary process in viable populations of ecologically and commercially viable varieties/ genotypes in order to prevent their potential extinction. The high yielding varieties (*Zingiber officinale* Rosc. cv- Suprava and Suruchi) of ginger were collected from High Altitude Research Station, Pattangi, Orissa University of Agriculture and Technology, Koraput, Orissa (Hand Book of Hort, 2003). The rhizomes were planted in the nursery bed for sprouting. The young fresh buds of sprouting rhizomes were used for *in vitro* propagation. In present study reports a rapid micropropagation of the two elite cultivar (cv-Suprava and Suruchi) of *Zingiber officinale* using fresh rhizome sprouting bud as an explant which is not included in the earlier studies. As these two cultivar has high potential demand with good market value. The purposed of the study was to develop a technique for more rapid and more convenient clonal propagation of ginger in a cost effective manner for obtaining large scale diseases free planting material for off season and year round cultivation for the benefit of the farmers.

Materials and methods

Explant source

Healthy sprouts with active buds were collected from the rhizome of *Zingiber officinale* Rosc. cv-Suprava and Suruchi maintained in the nursery bed experimental garden of P.G. Department of Botany, Utkal University. The healthy sprouts were cut in to 1.5 to 2.0 cm length with active buds intact. These rhizome sprouting with active buds were washed with 5% (v/v) detergent solution Teepol (Qualigen, Mumbai, India) for 10 minute and rinsed several times with running tap water. These rhizome sprouting bud cuttings were surface sterilized with bavistin 0.3% followed by streptomycin 0.2 % for 10 minute and then washed with sterile distilled water and transferred to laminar air flow cabinet .In the laminar chamber the sprouting bud cuttings were again dipped with 70% alcohol for 30 second to one minutes followed by another treatment in 0.1% (w/v) mercuric chloride ($HgCl_2$) for another 5 minutes. Finally, the sprouting bud cuttings were washed 3 to 4 times throughly with sterile distilled water and soaked with sterile blotted paper and used as explants for *In vitro* cultures before the inoculation in sterilized nutrient agar media pre-packed in culture tubes (Smith and Hamill, 1996).

Culture medium and condition

Sterilized blotted explants were implanted on to the Murashige and Skoog's (Murashige and Skoog's, 1962) agar-gelled medium fortified with various concentrations and combinations of growth hormones. For shoot induction, the medium was supplemented as follows 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l BAP (6-benzyl- amino-purine) and 0.25 and 0.5 mg/l NAA (α-naphthalene acetic acid) either individually or in combination. For root induction in vitro raised shoots measured about 4-5 cm grown in multiplication medium were cultured on half-strength MS medium supplemented with either NAA (α-Naphthalene acetic acid) or IBA (Indole 3-butyricacid) in concentration of 0.25, 0.5, 1.0,1.5, 2.0,2.5 and 3.0 mg/l .The pH of the medium was adjusted to 5.8 before autoclaving at 1.04 kg/cm^2 pressure and 121°C temperature for 15 - 20 minute .Molten medium of 20 ml was dispensed into the culture tube and plugged with non absorbent cotton wrapped in one layer of cheese cloth. All cultures were incubated in 16 h light/8 h dark photoperiod (cool, white fluorescent light -30µ mol $m^{-2}S^{-1}$). The cultures were incubated at 25 ± 3°C in diffused light under 60 - 70% relative humidity in the culture room. Each treatment had 20 culture tubes and the experiment was

repeated thrice. The cultures were maintained by regular subcultures at 4 week intervals on fresh medium with the same medium compositions.

Acclimatization

Rooted micro-propagules were removed from the culture tube and the roots were washed under running tap water to removed agar .Then the plantlets were transferred to sterile poly pots (small plastic cups) containing pre-soaked vermiculite (TAMIN, India) and maintained inside growth chamber set at temperature 28^0C and 70-80% relative humidity. After three weeks were transplanted to earthen pots containing mixture of soil + sand + manure (FYM) in 1:1:1 ratio and kept under shade house for a period of three weeks for acclimatization.

Observation of cultures and presentation of results

Twenty cultures were used per treatment and each experiment was repeated at least three times. The data pertaining to mean percentage of cultures showing response, number of shoots/culture and mean percentage of rooting were statistically analysed by the Post- Hoc Multiple Comparison test at the $P < 0.05$ level of significance (Marascuilo and McSweency ,1977).

Results and discussion

The response of *Zingiber officinale* Rosc. cv-Suprava and Suruchi rhizome with slightly initiating buds as explants cultured on different shoot proliferation media over a period of six weeks were presented in Table 1. Culture medium devoid of growth regulators (control) failed to stimulate the bud break response in the explants even when the cultures were maintained beyond the normal observation period of four weeks. MS medium with growth regulator supplements produced better results in terms of percentage explants response, shoots /explant, average shoot length and average number of nodes produced per shoot. In such media combinations bud break was noticed within 6-8 days of culture (Fig. 1A, Table 1**).** Of the combination tested MS+BAP (2.0 mg/l) + NAA (0.5mg/l) elicited optimal response in which an average of 7.5 ± 0.45, shoot lets (Fig. 1A, Table 1) with a mean shoot length of 6.2 ± 0.37 cm per explant was recoded the second best shoot multiplication 4.5 ± 0.41 was obtained in the medium MS+BAP (1.5 mg/l)+NAA (0.5 mg/l) with a mean shoot length of 5.2 ± 0.43 cm .Higher concentration of BAP (2.5,3.0 mg/l) with NAA (0.5 mg/l) showed callusing of the explants with fewer number of shoots.

In such cultures shoots were stunted with a mean shoot length of 2.5 ± 0.43 cm The dependence of cultured explants on bud break response and shoot multiplication has already been established and extensively discussed (Babu *et al.*, 1992). Recently, it is reported the case of micro propagation of other *Zingiberaceae* like *Curcuma longa* (Balachandran *et al*., 1990), *Zingiber officinale* (Bhat *et al*., 1994, Hosoki and Sagawa, 1977;Sunitibala *et al*., 2001). In present study, sprouting rhizome bud of *Zingiber officinale* Rosc. cv-Suprava and Suruchi showed significantly higher response in the medium with the combination of BAP(2.0 mg/l) + NAA (0.5mg/l). The quality of shoots and the over all growth response in terms of average shoot length was shown better in this growth regulator combination. A comparatively lower response was recoded when BAP was added alone in the medium. Indicates that the addition of either NAA or IBA or IAA in the culture medium improved the response in a number of species in terms of shoot growth. It is reported that *Spathiphyllum floribundam* when cultured on media with BA supplement alone, a limited proliferation of explants with a maximum of average of 1.8 shoots per cultured explants was observed, while addition of IAA produced an average number of 11.6 shoots per explant (Malamug *et al*., 1991; Ramierez-Magon *et al*., 2001). Similar observation was reported in *Hovenia dulcis* nodal culture (Echeverrigaray *et al*., 1998). Rahman *et al*., (2004) reported that MS medium supplemented with 2.0 mg/l BAP is the best hormonal concentration for multiple shoot production in *Cucuma longa*. Similarly Panda *et al*., (2007) reported that, MS medium supplemented with 3.0 mg/l BAP is the best hormonal concentration for multiple shoot production in *Cucuma longa* (cv-Roma). This study observed the addition of NAA 0.5 mg/l with BAP (2.0mg/l) shown to improve response over BAP alone and also suggested that the combination of BAP and NAA were needed for producing more number of multiple shoots on *Zingiber officinale* (Hashim *et al*., 1998; Hoque *et al*., 1977 ; Noguchi and Yamakawa., 1998).

Rooting and establishment of plants in soil

A well developed of the elongated shoots were measured about 4-5 cm in length that were excised from shoot clump and transferred to half strength MS medium containing NAA or IBA. The rooting responses of excised shoots on different media, which includes rooting percentage, days required for root initiation mean number of roots/shoot and mean root growth over a period of four weeks were different (Fig. 1B and 1C, Table 2). There was no rooting in case of shoot planted on auxin free basal medium (control). Similarly, at lower level of NAA (0.25 mg/l) treatments, there was hardly grown on any rooting in the cultured shoots within the 4 weeks of observation period. However, higher

concentrations of NAA (1.5 and 2.0 mg/l) and IBA at all concentrations tested response well. Rooting was shown better in culture which had a combination of 1/2 MS+2.0 mg/l NAA about 95% cultures responded with an average number of 8.5 ± 0.33 roots per plantlet and an average root length 3.5 ± 0.38 cm was recorded (Fig. 1B and 1C, Table 2) .The second highest response (70%) was recorded at 1.5 mg/l of NAA with an average number of 6.0 ± 0.18 roots per plantlet and an average root length 3.0 ± 0.09 cm, which was similar with the findings of Pillai and Kumar (1982); Sugaya (1991) Zakaria and Ibrahim (1986). In present study observed that root primordial emerged from the shoot base starting from 6 to 8 days after shoot inoculation and soon after that the root growth was rapid. NAA has more effective than IBA in induction of rooting as days required to rooting was only 6-8 days as against 10 to15 days required for similar response in case of IBA. Production of plantlets with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil (Sharma and Singh, 1994; 1995). The auxins NAA and IBA were used singly to induce rooting from in vitro raised shoot lets. A range of different concentrations of auxin (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) were tried for development of root system. In this study 1/2 strength MS basal medium supplemented with different concentration of NAA and IBA exhibited optimum growth pattern. The maximum results on rooting were obtained on half strength with NAA (2.0 mg/l) than IBA (2.0 mg/l). Rahman *et al.* (2004) reported that ½ strength MS medium supplemented with 0.1-1.0 mg/l of any auxin (NAA, IAA and IBA) was effective for rooting in *Curcuma longa* found in Bangledsh. In this study observations are in accordance with the result of Inden *et al.,* (1998) in Zingiber officinale.

A well rooted plants were transferred to sterile poly pots (small plastic cups) containing pre-soaked vermiculite (TAMIN, India) and maintained inside growth chamber set at temperature of 280^{0} C and 70-80% relative humidity. After three weeks there were transplanted to earthen pots containing mixture of soil + sand + manure (FYM) in 1:1:1 ratio and kept under shade house for three weeks for acclimatization. (Fig. 1D).Upon transferred to shade house, the plants started to produce fresh shoots after two week of transplanting. Later, there were transferred to the field condition and the survival rate was 95%. The efficient micro-propagation technique described may be appropriated as a highly useful for raising disease free quality planting propagules of *Zingiber officinale* for commercial and off season cultivation not only help the socioeconomic development of the farmers but also fulfill the spicies value and market demand (Kuruviashetty, *et al.,* 1982; Noguchi and Yamakawa, 1998).

Table 2. Influence of different levels of NAA and IBA on rooting response of *invitro* generated shoot lets of *Zingiber officinale* Rosc. (cv-Suprava and Suruchi) (20replicates/treatment, data scored after 4 weeks).

Different treatments	Growth regulators augmented with1/2 strength MS basal medium(mg/l)		% of Explant Response	Days to root initiation	Mean root numbers ±S.E.	Mean root length (cm) ±S.E.
	NAA	IBA				
T1	0	0	-	-	-	-
T2	0.25	0	20	12-15	1.0 ±0.30+	1.0 ± 0.14+
T3	0.5	0	40	10-12	2.0 ± 0.24+	2.0 ± 0.12+
T4	1.0	0	50	10-12	2.5 ± 0.04+	2.2 ± 0.16+
T5	1.5	0	70	6-8	6.0 ± 0.18	3.0 ± 0.09
T6	2.0	0	95	6-8	8.5 ± 0.33	3.5 ± 0.38
T7	2.5	0	50	10-12	2.2 ±0.32+	2.3 ±0.14+
T8	3.0	0	40	10-12	1.4 ±0.24+	1.7 ± 0.28+
T9	0	0.25	30	12-15	1.0 ± 0.14+	1.5 ± 0.12+
T10	0	0.50	40	12-15	2.2 ± 0.16+	2.4 ± 0.16+
T11	0	1.0	60	10-12	2.5 ± 0.04+	2.5 ± 0.12+
T12	0	1.5	55	10-12	2.6 ± 0.04	2.2 ± 0.08
T13	0	2.0	70	10-12	2.8 ± 0.12	2.6 ± 0.04
T14	0	2.5	50	10-15	1.8 ±0.38+	2.2 ±0.14+
T15	0	3.0	40	10-15	1.6 ±0.14+	1.5 ±0.14+

(20 replicate per treatment; repeated thrice. Means are calculated by Post-Hoc Multiple Comparisons tests at $P < 0.05$ level of significance ,+ callusing at the\ basal end , S.E.:- Standard error of mean)

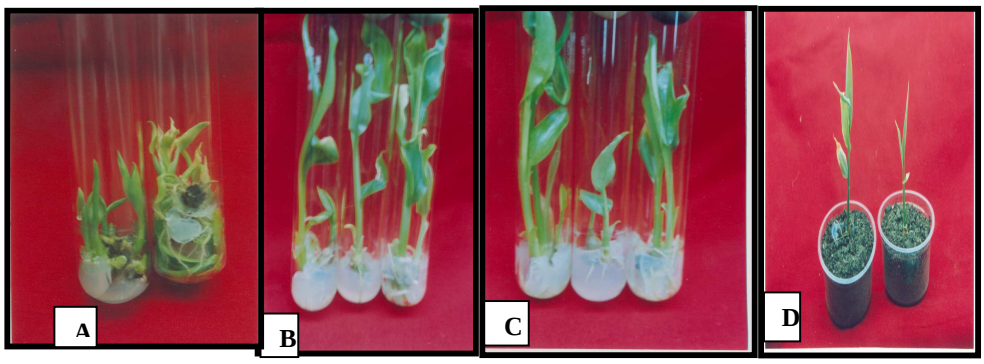

Fig.1. (A-D) *Invitro* regeneration and plant establishment of *Zingiber officinale* Rosc.(cv-Suprava and Suruchi (A) Multiple shoot emergence in MS+2.0mg/lBAP+0.5mg/l NAA (B) Subculturing of the micro shoots in fresh shooting media (C) Microshoots rooted in 1/2MS+NAA (2.0 mg/l). (D) Hardening of rooted micro-plantlets in plastic pots.

Conclusions

This experiment concluded that fresh rhizome sprouting explants in both the cultivated variety Suprava and Suruchi responded well and was the appropriate explants for the formation of multiple shoots in *Zingiber officinale* Rosc. cv-Suprava and Suruchi. MS medium supplemented with BAP (2.0 mg/l) + NAA (0.5 mg/l) were expressed the ideal hormonal concentration for the formation of multiple shoots where as 1/2 MS+2.0 mg/l NAA was the standard growth regulator for profuse rooting in both the cultivated varieties. Both the varieties did not showed any significant variation in the culture medium that perhaps due to close genomic loci among themselves even possessing some morphotypic variation and both the cultivated variety were grown well in high altitude environmental condition.

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Table 1. Shoot formation in rhizome sprouting of *Zingiber officinale* Rosc. (cv- Suprava and Suruchi) cultured on semisolid MS medium supplemented with various concentration of BAP and NAA (20 explants per treatment, data scored after 6 weeks).

Different treatments	Hormonal Supplements (mg/l)		% of Explant response	Days to bud break	Mean No of shoot/explant ± S.E.	Mean shoot length(cm) ± S.E.	Mean No of nodes/shoot ±S.E.
	BAP	NAA					
T1	0	0	-	-	-	-	-
T2	0.25	0	20	12-15	1.6 ± 0.30+	1.0 ± 0.09+	1.0 ± 0.12+
T3	0.5	0	30	12-15	2.5 ± 0.12+	1.5 ± 0.04+	1.5 ± 0.16+
T4	1.0	0	35	10-12	2.5 ± 0.38+	2.0 ± 0.14+	2.0 ± 0.18+
T5	1.5	0	40	10-12	2.6 ± 0.35	2.5 ± 0.26	2.0 ± 0.32
T6	2.0	0	50	8-10	2.8 ± 0.14	2.6 ± 0.23	2.2 ± 0.16
T7	2.5	0	45	8-10	2.0 ± 0.33+	2.8 ± 0.21+	2.2 ± 0.29+
T8	3.0	0	40	8-10	1.8 ± 0.49+	2.2 ± 0.18+	2.0 ± 0.33+
T9	0.25	0.25	30	10-12	2.1 ± 0.12+	2.4 ± 0.16+	1.5 ± 0.12+
T10	0.5	0.25	35	10-12	2.5 ± 0.14+	2.8 ± 0.04+	2.2 ± 0.16+
T11	1.0	0.25	40	7-9	3.0 ± 0.37+	3.0 ± 0.41+	2.0 ± 0.20+
T12	1.5	0.25	60	7-9	3.2 ± 0.25	3.0 ± 0.36	2.5 ± 0.12
T13	2.0	0.25	60	7-9	3.2 ± 0.33+	3.2 ±0.28	2.8 ± 0.09
T14	2.5	0.25	50	10-12	2 .2 ± 0.24+	2.0 ± 0.26+	2.5 ± 0.21+
T15	3.0	0.25	45	10-12	2 .0 ± 0.31+	1.8 ±0.12+	2.3 ±0.16+
T16	0.25	0.5	40	12-15	2.2 ± 0.28+	2.0 ± 0.28+	2.4 ± 0.16
T17	0.5	0.5	50	12-15	2.3 ± 0.04+	2.2± 0.30+	2.5 ± 0.08
T18	1.0	0.5	60	8-10	3.5 ± 0.42+	3.0 ± 0.32+	3.0 ± 0.20+
T19	1.5	0.5	75	6-8	4.5 ± 0.41	5.2 ± 0.43	4.7 ± 0.23
T20	2.0	0.5	90	6-8	7.5 ± 0.45	6.2 ± 0.37	5.6 ± 0.43
T21	2.5	0.5	60	8-10	3.5 ± 0.08+	3.2 ± 0.18+	2.8± 0.43+
T22	3.0	0.5	50	10-12	2.5 ± 0.43+	2.3±0.18+	2.0 ±0.14+

(20 replicate per treatment; repeated thrice. Means are calculated by Post-Hoc Multiple Comparisons tests at $P < 0.05$ level of significance ,+ callusing at the\ basal end , S.E.:-Standard error of mean)

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