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IN VITRO MICRORHIZOME INDUCTION IN THREE HIGH YIELDING CULTIVARS OF *ZINGIBER OFFICINALE* ROSC. AND THEIR PHYTOPATHOLOGICAL ANALYSIS

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ABSTRACT:

Ginger- *Zingiber officinale* Rosc., zingiberaceae, is one of the major medicinal spice crop in India. Microrhizome induction is a novel biotechnological tool to produce disease free planting materials in this rhizomatous crop since other crop improvement programmes are failed due to low seed set. Pathogen free nature and the genetic stability of the resultant plantlets/ rhizomes increase the acceptance in the commercialization of this technique. In the present study disclosed an efficient method for enhanced microrhizome production in three high yielding varieties of ginger (cultivars IISR Mahima, IISR Rejatha and IISR Varada) using MS medium devoid of NH_4NO_3 with various concentrations of externally provided NH_4NO_3 and photoperiod. Pathogen free nature of the microrhizomes was confirmed using disc culture technique in Casamino acid casein hydrolysate medium.

Key words*:* IISR Mahima, IISR Rejatha, IISR Varada, Ginger, *In vitro* pathological screening

INTRODUCTION

Ginger, botanically known as *Zingiber officinale* Rosc., belonging to the family Zingibereaceae is a rhizomatous medicinal spice. It is an herbaceous perennial grown as an annual crop. Commercially it is grown for its aromatic rhizomes, which are used both as a spice and for medical purposes. The major constraints involved in the conservation of ginger germplasm are the two soil borne diseases– rhizome rot caused by *Pythium aphanidermatum* and the bacterial wilt caused by *Ralastonia solanacearum* (*Pseudomonas solanacearum*).

India is the largest producer of ginger, contributing approximately 30 to 40% of the world production. Ginger plays an important and unavoidable role in traditional medicine. The ancient Indians considered ginger as the mahaoushadha (the great medicine). In Ayurvedic system of medicine, both fresh and dry ginger is used. It is believed to be useful in

anorexia, dyspepsia, for the suppression of inflammation *etc.* Ginger is a unique plant– a spice, that is used universally, which contains volatile oil, fixed oil, pungent compounds, resins, starch, protein and minerals. Crop improvement work in ginger is constrained due to the absence of seed set. As a result, clonal selection, mutation breeding, and induction of polyploidy were the crop improvement methods employed [13].

Many workers have reported micropropagation of ginger using various types explants on basal medium supplemented with cytokinins and auxins. *In vitro* induction of rhizomes and their germination in ginger has been reported by various workers [15,14,1]. Bhat *et al.*, [3] reported *in vitro* induction of rhizomes in ginger at higher sucrose concentrations (9 to 12%).

The present study aims at developing an efficient method for enhanced pathogen free microrhizome production in three high yielding varieties of ginger (cultivars Mahima, Rejatha and Varada). Experiments were conducted using MS medium devoid of NH_4NO_3 with various concentrations of externally provided NH_4NO_3 and photoperiod.

MATERIALS AND METHODS

The experiments were conducted using single shoots isolated from the established stock cultures maintained at the Crop Improvement and Biotechnology Facility of CMPR, AVS, Kottakkal.

Microrhizome induction

This experiment was conducted to study the effects of various concentrations of externally provided NH_4NO_3 and photoperiod on microrhizome induction. In the study MS devoid of ammonium nitrate (control) and MS devoid of ammonium nitrate along with different concentrations of externally provided ammonium nitrate (0.4, 0.825, 1.65 and 3.30 gl^{-1}) used as trial media. All these media contained 9% sucrose and 8gl^{-1} agar. The inoculated cultures were kept under 25±2^{0}C and a photoperiod of 2500-3000 lux irradiance or under dark. The observations were carried out at a regular interval of 30 days upto 3 months and data collected on the number of shoots; shoot length, number of leaves and the microrhizome induction.

Phytopathological analysis

For pathological screening another medium used was Casamino acid casein hydrolysate medium [9]. This medium was prepared by using 1g/l Casamino acid casein hydrolysate, 10g/l peptone, 5g/l glucose and solidified with 17g/l agar. After autoclaving it was cooled in to 55^{0}c and added 1% stock solution of Triphenyl tetrazolium chloride (TTC/TZC). To each litre of basal medium, added 5ml of TZC/TTC solution. The stock solution of TTC/TZC was poured in to light proof bottle, autoclaved for only 8 minutes and stored in refrigerator. During plating, about 20ml of media can be poured in to each petriplate.

RESUTS AND DISCUSSION

Microrhizome induction

During the *in vitro* incubation all the cultivars showed variation in their culture responses.

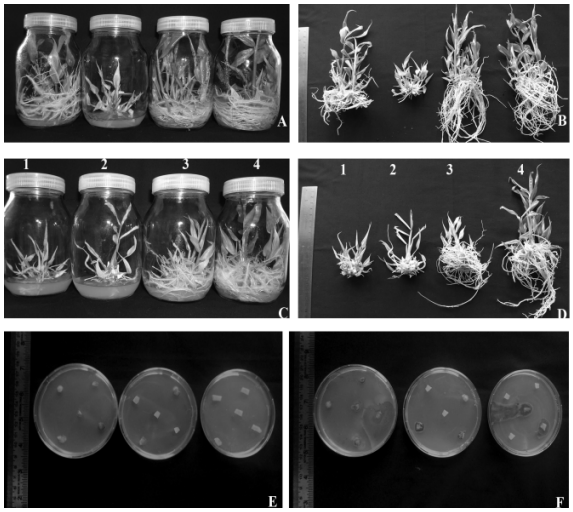
Table 1: Culture responses of three cultivars of *Zingiber officinale* after 3 months of *in vitro* growth

Combinations on MS(-NH_4NO_3)+9% sucrose+8gl^{-1} agar/ photoperiod	Number of shoots in three cultivars		
	Mahima	Rejatha	Varada
Control medium/ a	10.86±4.91	3.5±0.71	1.0±0.0
0.413gl^{-1} NH_4NO_3/ a	12.75±3.59	2.0±1.41	1.0±0.0
0.825 gl^{-1} NH_4NO_3/ a	7.67±3.51	3.0±1.0	3.33±2.31
1.650gl^{-1} NH_4NO_3/ a	14.0±0.01	3.0±0.0	1.67±0.58
3.300gl^{-1} NH_4NO_3/ a	7.0±2.83	8.8±0.28	2.0±0.0
Control medium/ b	6.33±2.25	1.33±0.58	3.67±1.53
0.413gl^{-1} NH_4NO_3/ b	7.0±3.0	1.67±1.15	1.67±0.58
0.825 gl^{-1} NH_4NO_3/ b	7.0±2.83	3.5±0.71	6.25±2.22
1.650gl^{-1} NH_4NO_3/ b	5.0±1.41	2.5±0.71	1.5±0.71
3.300gl^{-1} NH_4NO_3/ b	4.0±1.0	5.0±0.0	2.67±0.58

a- under light; b- under dark

During *in vitro* incubation, *Mahima* incubated at light showed maximum responses in the medium with 0.413mgl^{-1} of NH_4NO_3 during 1, 2 and 3 months of growth. Among the three cultivars, Mahima was the most responded one. In case of cv. *Varada* after three months of growth, more growth was observed in medium supplemented with 0.825mgl^{-1} NH_4NO_3, 9% sucrose and 8gl^{-1} agar; incubated at dark but more lengthy shoots were produced in control medium containing only 9% sucrose and 8gl^{-1} agar (7.20±1.18cm). More leaves were produced in the medium containing 0.413mgl^{-1} of NH_4NO_3 *i.e.*, 2.33±2.89. In case of cv. *Rejatha* more shoots and leaves were produced in cultures incubated at light, but more lengthy shoots were produced at dark (Table 1).

Behrend and Mateles [2] reported the culturing of tobacco cells on medium, in which ammonia was the sole source of nitrogen. As observed in ginger in the present study, in this case also, maximum growth is taking place in increased period of lighting or day length *e. g.*, in *Cucumis sativus* and *Ranunculus asiaticus*. But photoperiod of 8 hour is better than 18 hours for the micropropagation of *Malus* spp., [6]. Pelacho *et al*. [12] reported the positive effects of photoperiod (8-12hr) along with some organic acids like acetic acid, propionic acid, ascorbic acid and acetylsalicylic acid on *in vitro* tuber induction in potato. As observed in ginger, Sudhir and Mukundan [17] reported the influence dark incubation along with BA and increased sucrose to induce *in vitro* tuberization of *Armoracia rusticana*. There were so many reports on the positive effect of nitrogen or photoperiod on the micropropagation of plants [7,16]. Gorret *et al*. [7] reported the successful culture of oil palm suspension cells in a bioreactor and effects of different nitrogen sources and inoculum size and medium on biomass production in flask cultures. The optimum growth was attained at 1.45gl^{-1} NH_4NO_3 and 206mgl^{-1} glutamine. Dobranszki and Mandi [4] reported the effect of light on microtuber induction in potato. They concluded that tuberization can be induced by addition of 8% sucrose and the dark treatment (0h) after short days (8h) of incubation. Dobranszki in [4] reported the effect of different combinations of short days, dark treatment and different light intensities on *in vitro* tuberization of 3 potato cultivars. Tuberization in Desiree, Cleopatra and Gracia was induced by short day treatment after 4 weeks culture under long days. In the shorter photoperiod, high light intensity was necessary to induce tuber initiation.



A,B- Cultures grown under dark; C,D- cultures grown under dark (1-4- various NH_4NO_3 concentrations-given in materials and method); E- Pathological screening of conventional rhizome; F- Pathological screening of microrhizomes

Tsujita and Okubo [18] reported the effects of low nitrogen on *in vitro* shoot formation of *Cymbidium kanran* on MS medium supplemented with different combinations of 1mgl^{-1} BA, 0.1mgl^{-1} NAA, $AgNO_3$ with reduced NH_4NO_3 (25%) and KNO_3 (50%). Hussain *et al*. [8] reported the effect of sucrose, BAP and photoperiod on *in vitro* tuber induction of potato. Maximum tuberization was observed at zero photoperiod. Niedz and Evens [11] reported the effect of NN_4NO_3 and Fe on growth and

development of non-embryogenic callus of *Citrus sinensis*. Zakaria *et al* [19] reported the effect of nitrogen and potassium on *in vitro* tuberization of potato. Concentration of nitrogen at 60meq and potassium at 40meq in medium gave large microtubers. Malkawi *et al*. [10] reported the effect of photoperiod and temperature on tuber induction in *Solanum tuberosum* cv. Katahdin. 28^0C day and 13^0C night with an 18-hour photoperiod was used to induce tuber formation.

Pathological screening

In triphenyl tetrazolium chloride medium bacteria *Ralstonia solanacearum* was seen as brown coloured growth in the conventional rhizome discs. But it was absent in microrhizome induced rhizomes. In case of *Rejatha*, its conventional rhizomes showed the presence of 100% growth of *Ralstonia* sp. The conventional rhizomes of *Mahima* and *Varada* showed 40% pathogen growth.

CONCLUSION

In the present study pathogen free microrhizome induction protocol was developed for the large scale production of ginger using MS medium supplemented with externally added NH_4NO_3.

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