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INDUCTION OF ROOT THROUGH MEDIATION OF STRAIN OF *AGROBACTERIUM RHIZOGENES* IN CRYSANTHEMUM

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ABSTRACT
Rooting induction in chrysanthemum cultivar Arka Suvarna through the infection of *A.rhizogenes* strain RPB13 was demonstrated. Without the application of IAA and IBA *A.rhizogenes* strain RPB13 induced rooting with increasing survival percentage (100%)along with highest no. of roots (max. 15/explants). In contrast, hormonal treated showed survival percentage 88% and no. of roots was max. 9 per explants. Hence induction of rooting in chrysanthemum micropropogated cuttings was improved following inoculation with RPB13 suggesting that this rooting treatment has opened an avenue.

INTRODUCTION

Chrysanthemum is the world's second most economically important floricultural crops and has been cultivated for more than 200 years (Teixera da silva, 2003). It Occupies a prominent place in the national and international florist trade and mainly grown for cut flower and loose flower for making garlends, veni and for worshipping (Mamta B. and Ajit K., 2014). It is one of the most important global cut flower and pot plants. It is commonly known as autumn queen, belongs to the family Compositeae (Astaraceae), native to northern hemisphere, chiefly Europe and Asia (Anderson 1987, Kiran K. *et al*., 2013). Due to high popularity and demand for chrysanthemum, become one of the first commercial targets for micro propagation and thus tissue culture can be utilized for its large scale production. Micro propagation is one of the important contributions of plant tissue culture to commercial plant propagation and has vast significance.

Traditionally the crop is propagated by vegetative method. However, Nalini R., 2012, demonstrated that chrysanthemum plants can overcome the difficulties face by many farmers while raising chrysanthemum in nursery, in open field condition, due to climatic condition prevailing in that particular area, pest and disease incidence. Therefore, in vitro propagation of chrysanthemum appears to be a better option for rapid multiplication and supply of planting material to the farmers. In some cases, it was possible to improve in vitro rooting with hormone application, etiolation, or the use of polyamines (Damiano *et al*., 1991; Rugini *et al*., 1991; Damino and Monticell, 1998). In addition, rooting percentage of micropropagated explants is relatively low (Lloyd and Mc Cown, 1980, Anderson, 1984, Banko and Stefani, 1989, Isutsa *et al*., 1991, Mackay, 1996).

To overcome this problem recently many attempts have been carried out using *A. rhizogenes*. This bacterium induces adventitious root formation at the site of infection in a large no. of plants (Chilton *et al*., 1982). Microbes play an important role during the induction of rooting. To enhance adventitious root formation in almond (Bassil *et al*. 1991), pine and larch (McAfee *et al*., 1993) *A. rhizogenes* has been used. As there are no reports on induction of root and establishment of roots in chrysanthemum through *A.rhizogenes*. The experiment was conducted to study the induction of root in chrysanthemum by *A.rhizogenes*.

MATERIALS AND METHODS

Bacterial culture medium, growth and maintenance

Bacteria strain used for the rooting induction was isolated from nodules of pea collected from MPKV Rahuri. On the basis of morphological and biochemical characterization this organisms confirmed as *Agrobacterium rhizogenes* ((Anonymous (1957) and Bartholomew and Mittewar (1950), Cappuccino and Sherman (1987)). The strain was cultured on Yeast Mannitol broth, Luria Burnitti (LB), Nutrient broth (NB) and Yeast extract peptone broth.The bacterial strains showed excellent growth in Yeast Mannitol agar medium and was used in all further experiment.

Designation of *A.rhizogenes*

The *A.rhizogenes* strain was designated as *Agrobacterium rhizogenes* RPB13.

Plant material

Invitro micropropogated Chrysanthemum explants of variety Arka Suvarna was selected for induction of roots. The juvenile segments were surface sterilized with 0.1 % mercuric chloride cultured MS basal media (Murashige and Skoog medium 1962), supplemented with cytokinines in (Table1). Culture conditions were $21 \pm 2^{o}C$, photoperiod of 16 hrs. with a light intensity of 37 $\mu molm^{-2}$ s^{-1}.

Establishment of root culture

For treatment of micropropagated explants, 48 hrs old cultures of *A.rhizogenes* strain RPB13 were taken and suspended in required quantity of sterile water to obtain 0.1 OD (read at 620 nm).

Co-cultivation of explants

The micropropogated raised explants were punctured with hypodermic needles attached to a syringe containing overnight culture of *A.rhizogenes* strain RPB13. The explants were co-cultivated with overnight bacterial cultures and incubated at 24°C for two days in dark. Then, the explants were transferred to fresh MS medium without hormones. Successive transfer were made to make the incubating explants to free from *A.rhizogenes* and incubated under fluorescent light up to 25 days for root induction. The excess of bacterium present in the roots was eliminated by continuous sub culturing

RESULTS

Effect of A. rhizogenes for Rooting response, Survival of explants and number of roots per explants

The rooting response of micropropogated cutting treated with *A.rhizogenes* without hormones and MS medium with hormone was positive (Fig. 1). Whereas, the micropropogated explants without hormones or *A.rhizogenes* could not developed rooting. The survival percentage of chrysanthemum explants treated with *A.rhizogenes* was 100% with max no. of roots/explants (12-15 no. of roots /explants). The hormone treated showed 88% survival with 6-9 no. of roots/explants (Table 2). It was interesting to note that either hormones or *A.rhizogenes* required for rooting as it showed negative response with zero percentage of survival.

Effect of *A. rhizogenes* on period requirement for root induction and root density

A.rhizogenes treated explants required 14-15 days for root induction showed highest no. of roots after six weeks of infection (35 no. of roots). Whereas, in hormone treated required 22-25 days for root induction showed 24 no. of roots after six weeks of infection (Table 3). The percent increase in root numbers in micropropogated explants of chrysanthemum due to *A. rhizogenes* was 45-83% more over hormonal treatment. Thus it indicated that the *A.rhizogenes* was more effective for root induction in micropropogated cuttings and time required for root induction was less as compare to hormonal treatment.

DISCUSSION

The results showed that *A.rhizogenes* could significantly affect survival percentage and root number. For instance, the survival and rooting was poorest in *A.rhizogenes* treated explants. Interestingly, when these micro-cuttings were inoculated with *A. rhizogenes* without application of auxins cultures in ½ MS rooting medium, these significantly had higher number of roots as compared to hormonal treated. Thus exogenous auxin was not required for the *A. rhizogenes* strain to induce higher root number and root length. Bassil *et al.*, 1991 worked on Hazelnut soft wood cuttings and found an extensive root system with *Agrobacterium* inoculation in hazelnut cuttings. Hatta *et al.*, 1996 worked on jujube soft wood cuttings. They found that *A.rhizogenes* helps in induction of root in green house condition. It is possible that micro- cuttings inoculated with the bacterium produced auxin since *A. rhizogenes* is known to encode genes that increase auxin sensitivity to the plant tissue (McAfee *et al.*, 1993; Hatta *et al.*1996). In this study the

Table 1: Hormonal and vitamins used in MS media for micropropagation of Chrysanthemum

Cultivars	Basal composition Multi-plication	Rooting	Vitamins (mg/l)	Hormonal composition(mg/l) Multi-plication	Rooting
Chrysanthemum	Arka Suvarna	MS	MS 1/2	MS, Inositol	BAP-1.0 GA3- 0.2

BAP- Benzyl Amino Purine IAA- Indole Acetic Acid; GA_3- Gibberelic acid IBA- Indole Butyric Acid; KN-Kinetin

Table 2: In vitro performance of Agrobacterium rhizogenes on micro propagated cuttings of test plant for rooting

Explants	Treatment	No. of explants*	Rooting Response	Survival (%) in plant	No. of roots/ explants
Micro propagated cuttings	*A. rhizogenes*	9	+	100	12-15
	With Hormone	9	+	88.88	6-9
	Control(without Hormone)	9	-	0	-

(+) - Induction of roots * Infection was done in Petriplates; (-) - No root induction

Table 3: Performance of A. rhizogenes on induction of roots in micropropagated cuttings of chrysanthemum

Explants	Treatment	Days for induction of roots	No. of roots after six weeks of infection	% increase in root over hormoneTreatment
Micro propagated cuttings	*A. rhizogenes*	14-15	35	45.83
	With Hormone	22-25	24	-

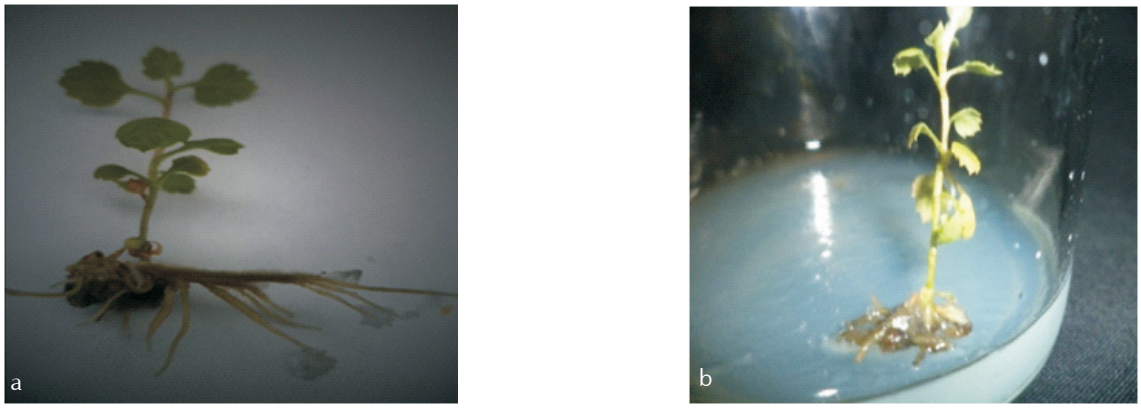

Figure 1: Rooting induction in micropropogated explants of chrysanthemum (a) treated with *A.rhizogenes* (b) treated with hormone

best rooting response was obtained when the micro- cuttings were inoculated with *A. rhizogenes* strain before they cultured in semi-solid ½ MS medium supplemented with 30 g/L sucrose without any hormone application. Monticelli *et al.*, 1997 also found that Almond and Ferragens with different rooting ability when infected *in vitro* with *Agrobacterium rhizogenes* 1855 induced root formation.

The conclusion of our study is that the use of *A.rhizogenes* can be a successful approach to improve rooting in Chrysanthemum. In Ferragnes, root induction was strongly increased (55.6%) with *A.rhizogenes* by infection without plant growth hormones in comparison to control (6.9 %) and the root induction was probably due to an improvement of rooting environment by the bacterium (McAfee *et al.*, 1993; Li and Leung, 2003). Giri and Narasu 2001, also stated that *A. rhizogenes* can be used for the efficient rooting of the cutting from recalcitrant woody species, as under highly defined experimental conditions, the process of root formation can be easily manipulated and the rooting response could be relatively high. Thus the application of *A.rhizogenes* appears to be very useful for micropropagation of chrysanthemum.

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