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INFLUENCE OF PLANT GROWTH PROMOTING SUBSTANCES IN MICROPROPAGATION OF STRAWBERRY CV. FESTIVAL

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ABSTRACT
Strawberry cv. Festival nodal segments were excised and cultured for 21 days on the initiation medium supplemented with various levels of BAP and TDZ. Subculture was performed and finally rooting was instigated on the medium supplemented with IBA for 4 weeks. The upshots revealed that, shoot proliferation percentage was maximum in MS medium supplemented with 0.5 mg/L TDZ at 100%. The maximum number of shoots per explant and shoot length was obtained in MS medium supplemented with 1mg/L TDZ at 6.40±0.37 and 3.50±0.07 cm respectively, whereas, minimum number of days to shoot initiation was obtained in MS medium supplemented with TDZ 0.5 and BAP 1.5 mg/L at 8.40±0.40 and 8.40±0.31 days respectively. MS medium supplemented with 0.5 mg/L IBA unveiled highest percentage of rooting at 100%, however, highest number of roots per microcutting at 5.00±0.21, maximum root length of microcuttings at 3.25±0.10 cm and minimum number of days to root initiation at 9.80±0.29 was obtained in medium supplemented with 1mg/L IBA.

INTRODUCTION

The cultivated strawberry (*Fragaria* × *ananassa* Duch.) is a perennial, dicotyledonous low-growing herb. Micropropagation of strawberry plants was introduced more than thirty years ago (Boxus, 1974). Growth regulators added to the basal medium for culture plays an important role in determining the nature of growth and development of plantlets *in vitro* as proposed by Skoog and Miller (1957). Morozova (2002) reported that high concentration of BA (benzyl adenine) is the best for strawberry micropropagation, while Boxus (1999) suggested lower concentrations (0.5-1mg/l) of BA for strawberry micropropagation. The use of BAP for shoot induction and proliferation was also carried out in other crops, e.g., banana (Lalrinsanga *et al.,* 2013) and pomegranate (Singh and Patel, 2014). Thidiazuron (TDZ), a substituted phenylurea with cytokinin and auxin like effects, is considered as a highly efficacious bioregulator for morphogenesis in the tissue culture of many plant species (Murthy *et al.*, 1998). Assessment of the efficacy of both the growth regulators on various shooting responses would be interesting as it would ease the choice for a desired growth regulator for micropropagation of strawberry. IBA also influences the rooting of micropropagated plants (Sakila *et al.*, 2007; Mante *et al.*, 1989). Nevertheless, the standardization of IBA concentration needs to be done for effectual rooting of the microcuttings. Therefore, in the present study efficacy among the various concentrations of TDZ and BAP were evaluated for shoot proliferation of explants and the influence of IBA on rooting responses of the microcuttings will also be assessed.

MATERIALS AND METHODS

Explant preparation

The work was carried out at Indian Institute of Horticultural Research, Hessarghatta, Bangalore, India. *In vitro* cultures were initiated from field grown plants. A disinfection protocol was developed in which nodal segments (1-2 cm) obtained were pretreated with a mixture of 1% Bavistin, 0.1% antibiotic formulation (streptomycin + tetracycline) and 0.5% CTAB (Cetyl Trimethyl Ammonium Bromide) for 90 minutes. Washing of the explants for 3 times in sterile distilled water was done, followed by disinfection with 75% ethanol containing 2 drops of tween-20 and subsequently washed with distilled water. The pre-treated explants were treated with mercuric chloride (0.1%) for 3 minutes followed by washings in sterile distilled water for six times. After washing, the leaf sheaths were removed and the exposed ends were trimmed off, and the excised nodal explants (1-1.5 cm) were conveyed to a bottle containing sterile water. Additionally, the excised explants were further retreated with Mercuric chloride (0.1%) for 10 seconds, and subsequently washed 5 times with sterile distilled water. The explants were then inoculated in culture tubes containing 10mL of the prepared culture medium.

Shoot and root proliferation

Surface sterilized nodal segments obtained were cultured on MS (Murashige and Skoog, 1962) medium containing 0.5, 1.0, 1.5 and 2.0 mg/L of either TDZ or BAP (6-

Table 1: Effect of plant growth regulators on the shoot proliferation responses of strawberry cv. Festival

Plant growth regulators (mg/L)	Days to shoot initiation	Number of shoots per explant	Shoot length (cm)
Control	11.00±0.33	2.10±0.23	0.91±0.11
BAP 0.5	9.50±0.43	3.20±0.25	1.24±0.10
BAP1	9.20±0.55	4.50± 0.22	2.21±0.14
BAP 1.5	8.40±0.31	5.90±0.18	3.11±0.10
BAP 2	9.00±0.56	4.60±0.22	2.21±0.09
TDZ 0.5	8.40±0.40	5.60±0.31	3.08±0.09
TDZ 1	7.40±0.45	6.40±0.37	3.50±0.07
TDZ 1.5	7.60±0.37	5.60±0.27	3.06±0.09
TDZ 2	8.30±0.37	4.70±0.21	2.20±0.15
$CD_{p=0.05}$	1.20	0.72	0.30

Table 2: Influence of various concentrations of IBA on the rooting responses of strawberry cv. Festival

IBA concentrations (mg/L)	Days to root formation	Number of roots per microcutting	Root length (cm)
IBA 0	16.10±0.53	1.50±0.17	0.92±0.11
IBA 0.5	12.70±0.45	3.00±0.26	2.00±0.09
IBA 1	9.80±0.29	5.00±0.21	3.25±0.10
IBA 1.5	9.90±0.31	4.00±0.21	2.62±0.07
IBA 2	11.00±0.45	3.00±0.26	2.00±0.09
$CD_{p=0.05}$	1.19	0.64	0.26

Benzylaminopurine). The cultures were examined daily and the data for shoot initiation and proliferation was recorded after 21 days of culture. The microcuttings obtained were subcultured on fresh medium for further shoot proliferation and root initiation. Growth characteristics such as percentage of explants showing shoot proliferation, days to shoot initiation, number of shoots per explant and average length of shoot were chronicled scrupulously and the average of all the observations recorded was worked out. The microcuttings produced from the shoot induction treatments were transferred to MS media containing 0.5, 1.0, 1.5 and 2.0 mg/L of IBA. The percentage of rooting, number of days to root initiation, number of roots per microcutting and average root length were recorded in each treatment after 4 weeks of subculture.

Hardening

After rooting, hardening of the plantlets was carried out in a media consisting of a mixture of sterilized sand, soil and cocopeat (1:1:2). The plastic cups punched with holes were filled with hardening media. The plantlets were transplanted in to plastic cups containing media and finally the cups were covered with an inverted punched holes plastic cup and placed under cool fluorescent light in the culture room. After 1 week, the inverted plastic cups covers were removed to permit further hardening, and one month after, hardened plantlets were relocated to glasshouse conditions. Plantlets of two months old are ready to be transferred to the field.

Statistical analysis

The tests were conducted under laboratory conditions. Analysis of variance for various observations was carried out using CRD with 10 replications using SPSS statistics 17.0. Test for significance ($p \leq 0.05$) was conducted among the various treatments of plant growth regulators.

RESULTS AND DISCUSSION

The shooting responses of the various cultivars of strawberry to in vitro culture using nodal explants were evaluated and TDZ (0.5 mg/L) when supplied to the medium resulted in the highest shoot proliferation percentage (Fig. 1). Maximum number of shoots per explant and shoot length was obtained in MS medium supplemented with 1mg/L TDZ, whereas, the minimum number of days to shoot initiation was obtained in 0.5mg/L TDZ which was *at par* with BAP 1.5 mg/L (Table 1). Thus, in the present investigation, TDZ was found to be significantly better than BAP in performance for various shooting responses. This is because TDZ devises a dual role, being a substituted phenylurea with cytokinin and auxin-like effects (Murthy *et al.*, 1998). Several other researchers (Thomas, 2003; Thomas and Puthur, 2004) also suggested that TDZ induces shoot regeneration better than other cytokinins. Debnath (2005) also inveterated that TDZ alone was more effective for plant regeneration from leaves of strawberry. Researchers applied TDZ between 0.5 (Debnath, 2005) and 18 μM (Husaini and Abdin, 2007) in regenerating explants and the effects of TDZ depends on the concentration and genotype. Husaini and Abdin (2007) found that 9 μM TDZ stimulated direct regeneration via *de novo* shoot bud formation in leaf disk and 18 μM induces direct embryogenesis. In the present investigation, 1mg/L TDZ was found to be effective in escalating the number of shoots per explant, shoot length and minimum number of days to shoot initiation.

Growth regulator IBA also had significant effects on the rooting responses of strawberry microcuttings. MS medium supplemented with 0.5 mg/L and 1mg/L IBA unveiled highest percentage of rooting of microcuttings (Fig. 2), whereas, highest number of roots per microcutting, maximum root length of microcuttings and minimum number of days to root initiation was obtained in medium supplemented with 1mg/L IBA (Table 2). The present findings are in agreement with Hemant *et al.* (2001) and Ritu *et al.* (2001) on other cultivars of strawberry in which they found that the use of IBA at 1mg/L gave the best result for *in vitro* rooting of micropropagated shoot. Thus, this concentration is optimum for effective rooting of microcuttings

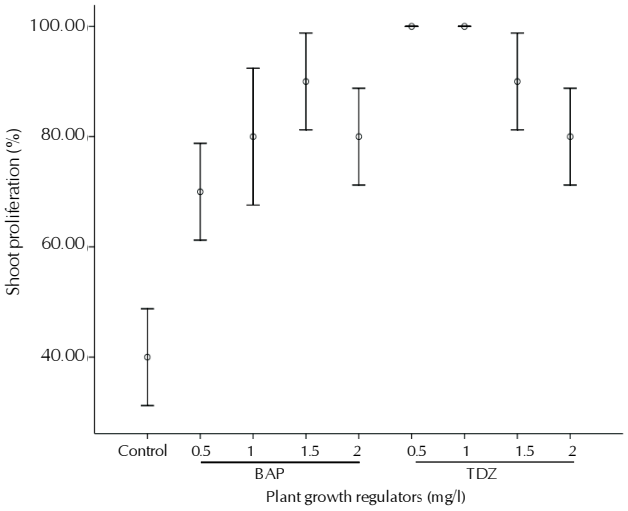

Figure 1: Shoot proliferation of strawberry cv. Festival

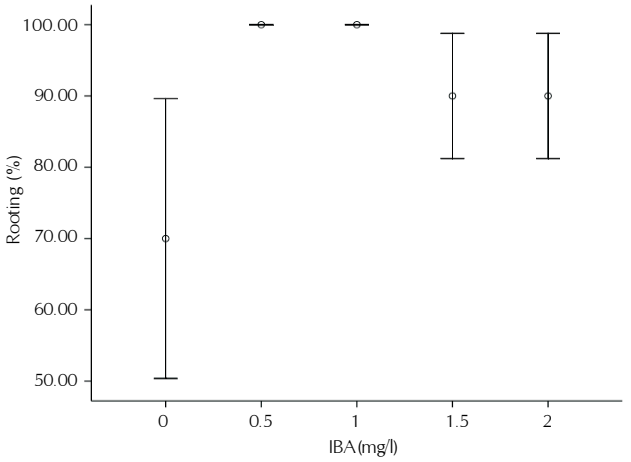

Figure 2: Rooting percentage of strawberry under the influence of IBA

of strawberry cv. Festival.

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REFERENCES

Boxus, P. 1974. The production of strawberry plants by *in vitro* micropropagation. *J. Hortic. Sci.* **49:** 209-210.

Boxus, P. 1999. Micropropagation of strawberry via axillaryshoot proliferation. In: *Plant Cell Culture Protocols*. Methods in Molecular Biology. Part III. Plant Propagation *in vitro*. *Hall R. D. (ed.) Humana Press Inc., Totowa NJ.* **111:** 103-14.

Debnath, S. C. 2005. Strawberry sepal: another explant for thidiazuron-induced adventitious shoot regeneration. *In Vitro Cell. Dev. Biol. Plant.* **41:** 671-676.

Hemant, G., Kaur, R., Sharma, D. R., Neetu, T., Gautam, H. and Thakur, J. N. 2001. A comparative study on *in vitro* and *ex vitro* rooting of micropropagated shoots of strawberry (*Fragaria* x *ananassa*). *Pl. Cell Biotech. Mol. Biol.* **2(3-4):** 149-152.

Husaini, A. M. and Abdin, M. Z. 2007. Interactive effect of light, temperature and TDZ on the regeneration potential of leaf discs of *Fragaria x ananassa* Duch. *In vitro Cell. Dev. Biol. Plant.* **43:** 576-584.

Lalrinsanga Robert, Vanlaldiki, H. and Meitei, W. I. 2013. *In vitro* shoot tip culture of banana cultivar 'Meitei Hei'. *The Bioscan.* **8(3):** 839-844.

Mante, S., Scorza R. and Cordts, J. M. 1989. Plant regeneration from cotyledons of *Prunus persieg, P. domestica* and *P. cerasus*. *Pl. Cell Tiss. Org. Cult.* **19:** 1-11.

Morozova, T. 2002. Genetic stability of Pure lines of *Fragaria vesca* L. in micropropagation and long term storage *in vitro*. *Acta Hort.* **567:** 85-87.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plantarum.* **15:** 473-497.

Murthy, B. N. S., Murch, S. J. and Saxena, P. K. 1998. Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. *In vitro Cell. Dev. Biol. Plant.* **34:** 267-275.

Ritu, M., Kaur, R., Anju, S., Sharma, D. R., Mahajan, R. and Sharma, A. 2001. Micropropagation of strawberry cultivar Chandler and Fern. *Crop Improvement.* **28(1):** 19-25.

Sakila, S., Ahmed, M. B., Roy, U. K., Biswas, M. K., Karim, R., Razvy, M. A., Hossain, M., Islam, R. and Hoque, A. 2007. Micropropagation of strawberry (*Fragaria ananassa* Duch) a newly introduced crop in Bangladesh. *Amer. Eu. J. Sc. Res.* **2(2):** 151-154.

Singh Pushpraj and Patel, R. M. 2014. Factors influencing *in vitro* growth and shoot multiplication of pomegranate. *The Bioscan.* **9(3):** 1031-1035.

Skoog, F. and Miller, C. D. 1957. Chemical regulation of growth and organ formation in plant tissue cultivated *in vitro*. *Symp. Soc. Expt. Boil.* **11:** 118-131.

Thomas, T. D. 2003. Thidiazuron induced multiple shoot induction and plant regeneration from cotyledonary explants of mulberry. *Biol. Plant.* **46:** 529-533.

Thomas, T. D. and Puthur, J. T. 2004. Thidiazuron induced high frequency shoot organogenesis in callus from *Kigelia pinnata* L. *Bot. Bull. Acad. Sin.* **45:** 307-313.