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Effect of growth regulators on *in vitro* root formation in strawberry

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Abstract: An experiment was carried out to examine the effects of different combinations of plant growth regulators for *in vitro* root formation in strawberry shoots regenerated by direct and indirect via callus. It was observed that in direct regenerated microshoots, IBA 1 mg/l took minimum number of days (9.00) for root initiation with highest rooting percentage (95%) and significantly maximum root length (4.11cm.), while NAA 1 mg/l produced significantly maximum number of roots (7.00) followed by IBA (1 mg/l). whereas, microshoots regenerated from callus maximum rooting was observed on half strength MS within 8 days of inoculation. The highest number of roots were recorded on media supplemented with NAA at 1 mg/l, while highest length of roots was observed at 1 mg/l IBA.

Key words: Strawberry, *in vitro,* IBA, NAA,

Introduction

The cultivated strawberry (*Fragaria* x *ananassa* Duch.), a hybrid between the 'scarlet' or 'virginia' strawberry (*Fragaria virginiana* Duch.) and the pistillate South American strawberry (*Fragaria chiloensis* (L.) Duch.) is a dicotyledonous, perennial low growing herb grown in most arable regions of the world (Debnath, 2013). It belongs to subfamily Rosoideae and family Rosaceae. Strawberry is perhaps first fruit crop in which micropropagation technique has been standardized (Sharma and Singh, 1999). Tissue culture considered as an *in vitro* aseptic culture of cells, tissues, organs or whole plant below exact nutritional and ecological circumstances (Thorpe, 2007). that can be used for manufacturing clones of plants. The resulting clones are true-to kind of a selection of genotype. The organized circumstances often offer the culture of the environment helpful for their development and development. These circumstances have complete quantity of nutrients, pH medium, suitable temperature and complete gaseous and liquid condition. Plant tissue culture method can function for large amount of plant production. *In vitro* production of plants involves the application of growth regulators, such as auxins, cytokinin for process of organogenesis. Cytokinins are known to play a major role in shoot multiplication. N6-benzylamino-purine (BAP) is the cytokinin that is mostly used for *in vitro* shoot proliferation of strawberry (Haddadi *et al.,* 2010). A very highly competitive and encouraging result was observed regarding root initiation percentage as affected by different concentration of auxins such as IBA, NAA and IAA. Half strength MS media supplemented with the lower concentration (0. 1 and 0. 2 mg/l) of all the auxins responded well. Shoots obtained from nodal segments were rooted on half-strength agar-gelled medium with 1.0 µM NAA. Rooted shoots with fully expanded leaves acclimatized successfully and about 70% of plantlets survived *ex vitro* (Bhatt and Dhar, 2000). Low concentration of IBA was good for maximum number of roots and frequency of root formation (Biswas *et al.,* 2007).

After successful regeneration of microshoots of strawberry the next important step was to induce roots in the developed microshoots. Thus efforts were made to induce *in vitro* rooting on the regenerated shoots. Healthy shoots were transferred aseptically on Half MS medium fortified with different levels of IBA, IAA and NAA alone for rooting.

Materials and Method

The experiment was conducted in Centre of Plant Biotechnology, CCS Haryana Agricultural University, Hisar during the year 2014-15. Explants were collected from the healthy plants (in July) maintained at experimental polyhouse of the Department of Horticulture, CCS Haryana Agricultural University, Hisar. Some modifications was done in Bhanakher *et al.* (2008), Madhavrai *et. al.* (2014) disinfection procedure in which the young tender vegetative nodal segment of 5-10 cm length were excised. Explants were washed under running tap water and leaves were removed with the help of scalpel. The nodal segments of uniform size were prepared with the help of clean scalpel. The excised explants were washed with detergent (Teepol) followed by washing under running tap water. Explants were treated with 0.45% citric acid and 0.25% ascorbic acid for 10-12 minutes and were washed 4-5 times with distilled water. The washed explants were then treated with 0.40% bavistin and 0.40% streptocycline for 1.5-2.0 hour. The washed explants were surface sterilized in the laminar air flow with the help of sterilizing agent 0.1% mercuric chloride for 3 minutes. Further, the explants were given 5-6 washing with autoclaved sterilized water to remove the traces of sterilizing agent.

Nodal segments of 3-4 cm length were cultured on Murashiage and Skoog (MS) 1962 media supplemented with specific concentration of growth regulators *viz.* Indole Acetic Acid (IAA), Napthelene Acetic Acid (NAA), Benzylaminopurine (BAP) and Kinetin alone or in combinations, adding 30 g/L sugar and 8 g/L agar. The pH of medium was adjusted to 5.8 with 1N NaOH and 1N HCl using pH meter. The media were sterilized in autoclave at 121°C temperature and 15 psi pressure for 20 minutes and were stored at room temperature for further use. The bottles containing culture media were used within a week. The culture was incubated at temperature (25±1)^{0}C and light (4000 lux) for 16 hr photoperiod. Subculture was done at every 21 days interval. Nodal segments from proliferated shoots subcultured again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in Half MS medium containing different concentrations of IAA, IBA and NAA for root induction. After rooting, hardening of the plantlets was carried out in a media consisting of a mixture of Coco peat, vermicompost, vermiculite in different ration. Polythene bags with holes were filled with hardening media. Plantlets of two month old are ready to be transferred to the field.

The tests were conducted under laboratory conditions. Observations was carried out using CRD with 10 explants were used per treatment and replicated three times.

Results and Discussion

The efforts were made to induce *in vitro* rooting on the regenerated shoots. The proliferated micro shoots were transferred to half strength MS medium modified with different levels of IBA IAA and NAA to find out their most appropriate concentrations for the regeneration of roots. It was observed that (Table 1) IBA 1 mg/l took minimum number of days (9.00) for root initiation with highest rooting percentage (95%) and significantly maximum root length (4.11cm.), while NAA 1 mg/l produced significantly maximum number of roots (7.00) followed by IBA (1 mg/l). In (Table 2). It was observed that all treatments induced roots, but maximum rooting was observed on half strength MS within 8 days of inoculation. The highest number of roots were recorded on media supplemented with NAA at 1 mg/l, while highest length of roots was observed at 1 mg/l IBA. It was observed that IBA at 1mg/l roots were strong and stout and shoots with thick stem, more leaf area and darker in colour as compared to other auxins. In this study half strength MS medium was used which induces stress condition to the plants which initiate roots earlier as compared to MS medium.

IBA at 1 mg/l was recorded best for root development in strawberry cultivar Ofra. There was positive correlation between the rooting percentage and root length. Ali *et al.* (2009) reported that like other developmental processes, cell elongation involves sequential changes in levels or activity of enzymes. Superior effects of IBA on root elongation as compared to NAA might be due to the several factors, such as preferential uptake, transport, metabolization and subsequent gene action. It was observed that type and concentration of auxin strongly influenced the quality of the shoot and the root system at the end of the rooting period. It was observed that the effect of auxin on rooting is promotory at optimum concentration and inhibitory at supra-optimal concentrations. The present findings are in agreement with the work of number of scientists viz Sakila *et al*. (2007); Haddadi *et al*. (2010); Diengngan *et al*. (2014), Madhavrai *et al.* (2014) and Haragude *et al*. (2014) on other cultivars of strawberry in which they found that the use of IBA at 1 mg/l gave best result for *in vitro* rooting of micropropagated shoots. Signifying this concentration is optimum for effective rooting of tissue culture derived shoots of strawberry.

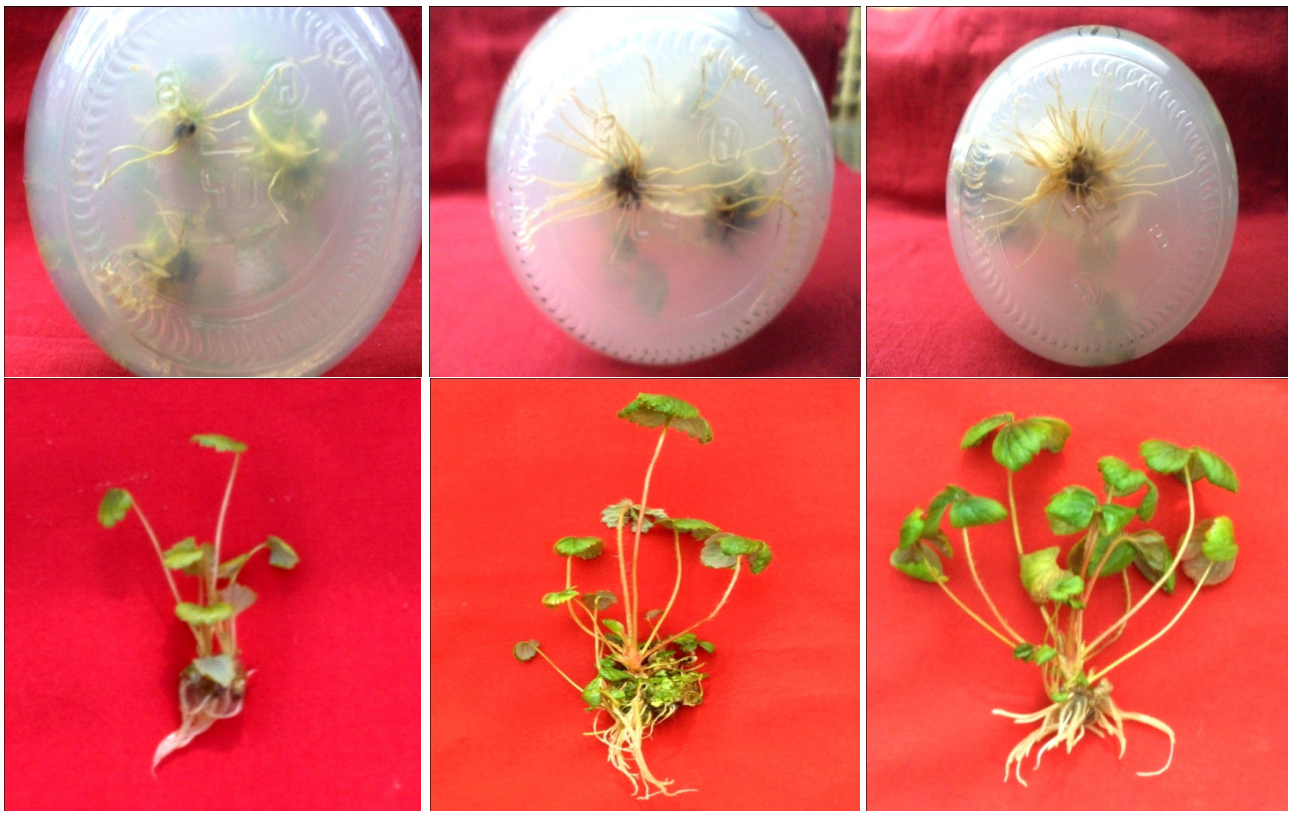
However, many scientists (Moradi *et al*., 2011; Haragude *et al*., 2014; Madhavrai *et al.,* 2014) reported that IBA at 1 mg/l was best for rooting. Addition of auxins in rooting media accentuates rooting but many a times microshoots develop callus at their base that hampers their proper root development. Similar results were

Table-1: Effect of different concentrations of auxins on root development from the direct regenerated shoots of strawberry cultivar Ofra

Media composition (mg/l)	Days taken for root initiation	Root length (cm)	Number of roots/shoot	Rooting percentage (%)
Half MS (no growth regulators)	15.33 ± 0.33	1.67 ± 0.19	2.78 ± 0.29	54.33 ± 0.66
Half MS+1.0 IBA	9.00 ± 0.58	4.11 ± 0.20	5.67 ± 0.69	94.00 ± 0.57
Half MS+2.0 IBA	11.00 ± 0.58	2.36 ± 0.12	4.11 ± 0.11	80.66 ± 0.88
Half MS+1.0 NAA	9.67 ± 0.33	3.03 ± 0.03	7.00 ± 0.58	73.00 ± 1.00
Half MS+2.0 NAA	10.67 ± 0.33	2.39 ± 0.06	5.44 ± 0.11	62.66 ± 1.14
Half MS+1.0 IAA	13.00 ± 0.58	2.49 ± 0.03	3.33 ± 0.11	70.33 ± 0.66
Half MS+ 2.0 IAA	13.00 ± 0.58	2.36 ± 0.03	5.00 ± 0.00	61.67 ± 0.66
CD (P=0.05)	1.49	0.36	1.13	2.80

Table-2: Effect of different concentration of auxins on root development from regenerated shoot derived from callus of strawberry cultivar Ofra

Hormonal composition of media (mg/l)	Number of days taken to root initiation	Root length (cm.)	Number of roots	Rooting percentage
Half MS (Control, no growth regulators)	8.00 ± 0.58	3.78 ± 0.22	3.86 ± 0.15	89.67 ± 0.33
Half MS + 1.0 IBA	10.67 ± 0.67	4.33 ± 0.00	6.60 ± 0.30	79.00 ± 0.57
Half MS + 2.0 IBA	14.67 ± 0.33	2.55 ± 0.40	2.85 ± 0.15	53.67 ± 0.88
Half MS + 1.0 NAA	14.33 ± 0.33	3.56 ± 0.29	7.50 ± 0.28	74.67 ± 0.33
Half MS + 2.0 NAA	15.00 ± 0.58	2.44 ± 0.29	4.77 ± 0.40	64.33 ± 0.66
CD (P=0.05)	1.64	0.88	0.88	1.90



(a) Half MS (Control) (b) Half MS + 1 mg/l NAA (c) Half MS + 1mg/l IBA

Fig. 1: Effect of rooting media on number and length of roots in directly regenerated shoots of strawberry

reported by Ara *et al.* (2012) and Karim *et al*. (2015). Sarwar and Flegmann (1989) also reported that inorganic salts in MS medium were enough to support the maximum root formation and use of IBA and other auxins was not necessary.

IBA proved to be better rooting hormone for strawberry cultivar Ofra in terms of root initiation , rooting percentage and root length as compared to NAA. NAA proves to be less effective for rooting due to more stable in nature as compared to IBA.

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