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Effect of plant growth regulators on rooting behaviour of *in vitro* strawberry cv. Winter Star.

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Abstract
An experiment was carried out to examine the Effect of plant growth regulators on rooting behaviour of *in vitro* strawberry cv. Winter Star. It was observed that in direct regenerated microshoots with application of IBA (1.5 mg/ l) in 1/2 MS media takes minimum days (8.08) for root initiation followed by RM_2 (8.92) and RM_5 (9.74) with maximum rooting percentage (94.35 %) was observed in RM_3 (1/2 MS + 1.5 mg/ l IBA) followed by RM_2 (89.65 %) and RM_5,(84.50 %). Maximum number of roots/ explant (8.49) followed by RM_2 (7.83) and RM5 (6.75), and length of roots (5.37 cm) was also recorded in 1/2 MS + IBA (1.5 mg/l) followed by 1/2 MS + IBA (1 mg/l) media.

Keywords: Strawberry, *in vitro*, IBA and NAA

Introduction
Strawberry (*Fragaria* × *ananassa* Duch.), a member of the family Rosaceae, is a soft fruited, short-day herbaceous perennial plant that can successfully be grown at optimum day temperatures of 22°C to 25°C and night temperatures of 7°C to 13°C (De and Bhattacharjee, 2012) [1]. Strawberry is cultivated in Himachal Pradesh, Uttarakhand, Jammu and Kashmir, West Bengal (Darjeeling hills), Western Uttar Pradesh, and Haryana, with Maharashtra being the leading producer (Sharma and Budiyala, 1980) [3]. The fruit is widely appreciated mainly for its characteristic aroma and bright red colour (Lal and Sharma, 2003) [2]. It is valued for its low-calorie carbohydrate and high fiber contents. It is good source of natural antioxidants, including carotenoids, vitamins, phenols, flavonoids, dietary glutathionine metabolites and exhibits a high level of antioxidant capacity against free radicals, that are believed to reduce carcinogens in humans, protect against tumor development (Kersty *et al*., 2001) [4], and reverse age related effects on memory (Bickford *et al.*, 2000) [5]. Strawberry is a perennial herb that propagated by runners (Biswas *et al.*, 2008) [6]. Since runners retain all of the characteristics of the parent plant, they also result in the dissemination of over 30 viral and phytoplasmic diseases (Gautam *et al.*, 2001; Martin and Tzanetakis, 2006) [7, 8] and a yield capacity loss of up to 80%. (Thompson and Jelkman 2003) [10]. The conventional way of production is not adequate to meet the commercial demand. As a consequence, biotechnological activity is needed to sustain production capacity. Nurserymen use tissue culture plants for multiplication by runner production under safe cultivation, which farmers use for fruit production. Strawberry may be the first fruit crop to benefit from a standardised micro-propagation technique (Sharma and Singh, 1999) [9]. Explants such as meristem-tip, anthers, immature embryos, and first axillary buds of stolon have been used to develop millions of plants in a year (Boxus, 1989) [11].

Material and methods
The *in vitro* studies were carried out in the Plant Tissue Culture Laboratory of Department of Horticulture. The plants of strawberry cultivar 'Winter Star' grown under Hi-tech greenhouse, Department of Horticulture, CCS Haryana Agricultural University, Hisar was selected for the present study. 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 and also 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. The working table of the laminar airflow chamber was first surface sterilized with absolute alcohol and then by switching on the UV light for 20-30 minutes before work started. The petridishes as well as instruments used for inoculation were earlier steam sterilized in an autoclave at 15 psi pressure and 121°C for 20 minutes and then flame sterilized before each inoculation. Hands were also swabbed in 70% alcohol before inoculation. 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 IBA and NAA (shown in Table-1) for root induction. After rooting, hardening of the plantlets was carried out in a media consisting of a mixture of Cocopeat, Perlite, Vermicompost, Vermiculite and FYM 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.

Table 1: Combination and concentration of plant growth regulators for root induction in strawberry

Sr. No.	Medium code	Basal Medium	Growth regulators (mg/l)	
			IBA	NAA
1	RM_1	1/2 MS	0.5	-
2	RM_2	1/2 MS	1.0	-
3	RM_3	1/2 MS	1.5	-
4	RM_4	1/2 MS	-	0.5
5	RM_5	1/2 MS	-	1.0
6	RM_6	1/2 MS	-	1.5
7	RM_7	1/2 MS	-	-

Results

The induction of rooting in strawberry also significantly influenced and improved with the use of various hormonal concentrations and combinations in 1/2 MS media (Figure 1 - 4). According to the data in Figure- 1, the period required for the initiation of rooting in the strawberry cultivar Winter Star varied between 8.08 and 15.17 days in different treatments. The minimum number of days (8.08) required for root initiation was recorded with RM_3 (1/2 MS +1.5 mg/l IBA) treatment followed by RM_2 (8.92) and RM_5 (9.74) while, maximum number of days taken for root initiation (15.17) was recorded with RM7 (1/2 MS basal) followed by RM_4 (13.57).

In regenerated shoots, the rooting percentage ranged from 61.35 % to 94.35 %. The data in Figure- 2 clearly demonstrate that the different hormone treatments improved the percentage of shoots with root formulation as compared to the control. The maximum rooting percentage (94.35 %) was observed in RM_3 (1/2 MS + 1.5 mg/ l IBA) followed by RM_2 (89.65 %) and RM_5,(84.50 %) whereas, minimum were recorded in control (61.35 %), followed by RM_7 (75.35 %).

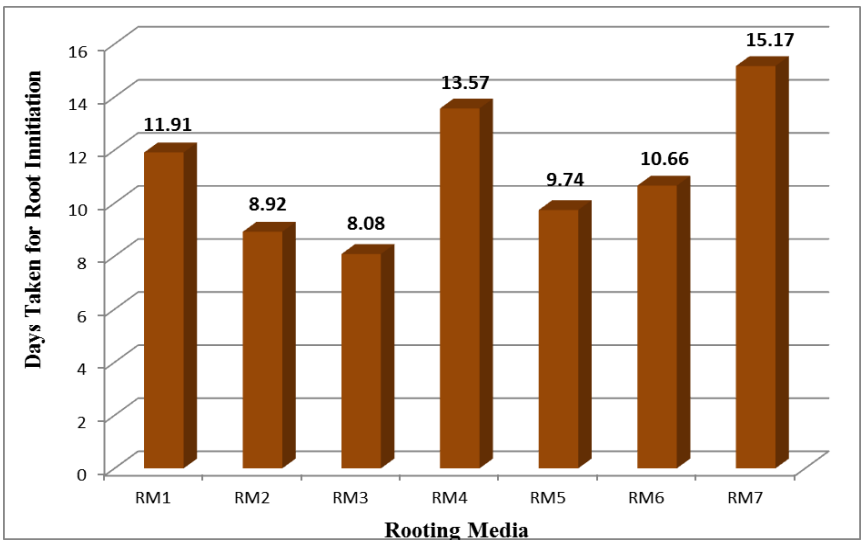

Fig 1: Effect of rooting hormones time taken for root initiation (days) in strawberry cv. 'Winter Star'

In regenerated shoots, the rooting percentage ranged from 61.35 % to 94.35 %. The data in Figure- 2 clearly demonstrate that the different hormone treatments improved the percentage of shoots with root formulation as compared to the control. The maximum rooting percentage (94.35 %) was observed in RM_3 (1/2 MS + 1.5 mg/ l IBA) followed by RM_2 (89.65 %) and RM_5, (84.50 %) whereas, minimum were recorded in control (61.35 %), followed by RM_7 (75.35 %).

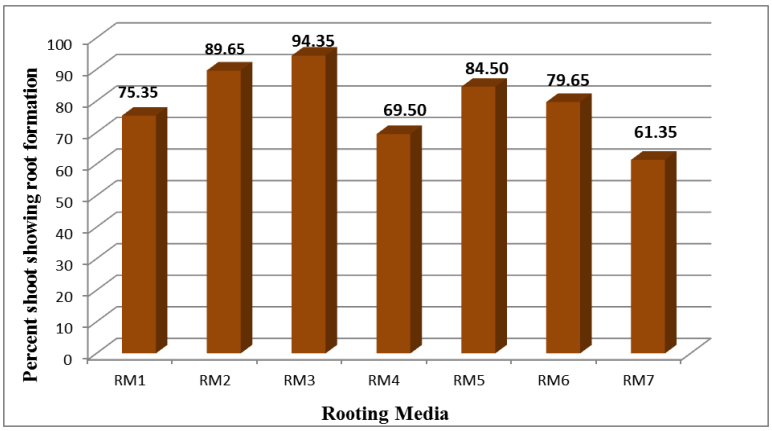

Fig 2: Effect of rooting hormones on number of shoot showing root formation (%) in strawberry cv. 'Winter Star'

Figure- 3 demonstrated that the increase of auxins to rooting media increased root initiation per shoot as compared to the control. The highest number of roots (8.49) were recorded with RM_3 (1/2 MS + 1.5 mg/l IBA) followed by RM_2 (7.83) and RM5 (6.75), while minimum number of roots (3.41) were observed with 1/2 MS basal followed by RM_4 (4.33) treatment. 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.

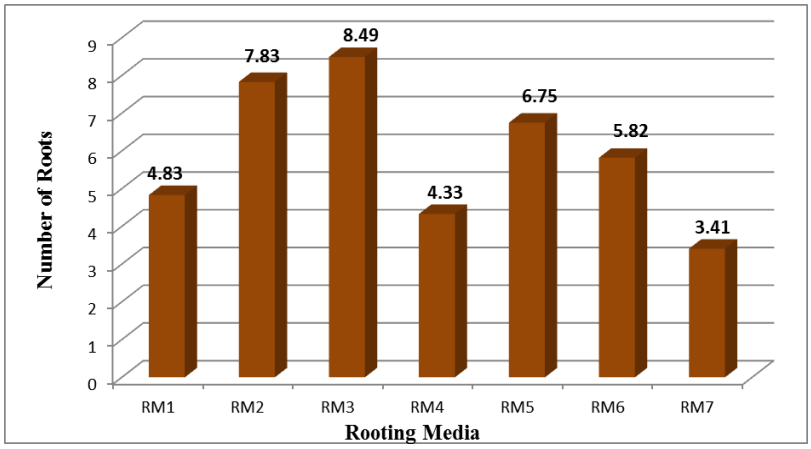

Fig 3: Effect of rooting hormones on number of roots per plant in strawberry cv. 'Winter Star'

The data in Figure- 4 clearly exhibited an increase in length of roots in all MS media treatments supplemented with auxins over control. Maximum root length (5.37 cm) was observed with (1/2 MS + 1.5 mg/l IBA) treatment, which was followed by 1/2 MS + 1 mg/l IBA (5.08 cm), whereas, smallest roots (1.96 cm) were recorded with (1/2 MS basal alone) followed by(1/2 MS + 0.5 mg/l NAA (2.44 cm) treatment.

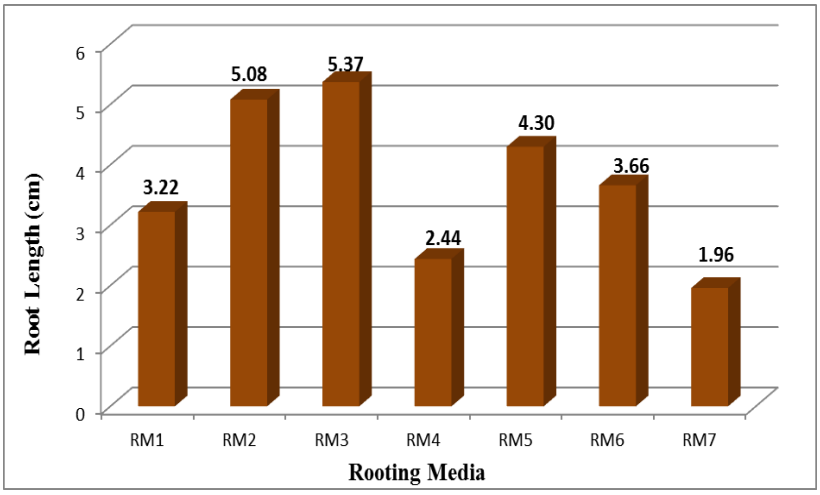

Fig 4: Effect of rooting hormones on root length (cm) in strawberry cv. Winter Star

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) [14]; Haddadi *et al.* (2010) [13]; Diengngan *et al.* (2014) [12], Madhavrai *et al.* (2014) [16] and Haragude *et al.* (2014) [15] on other cultivars of strawberry in which they found that the use of IBA at 1 -1.5 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. Sarwar and Flegmann (1989) [17] 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. 1/2 MS + 1.5 mg/l IBA proved to be better rooting hormone for strawberry cultivar 'Winter star' in terms of time taken to root initiation, number of shoot showing root formation, root length and number of roots as compared to NAA. NAA proves to be less effective for rooting due to more stable in nature as compared to IBA (as shown in Figure- 5)

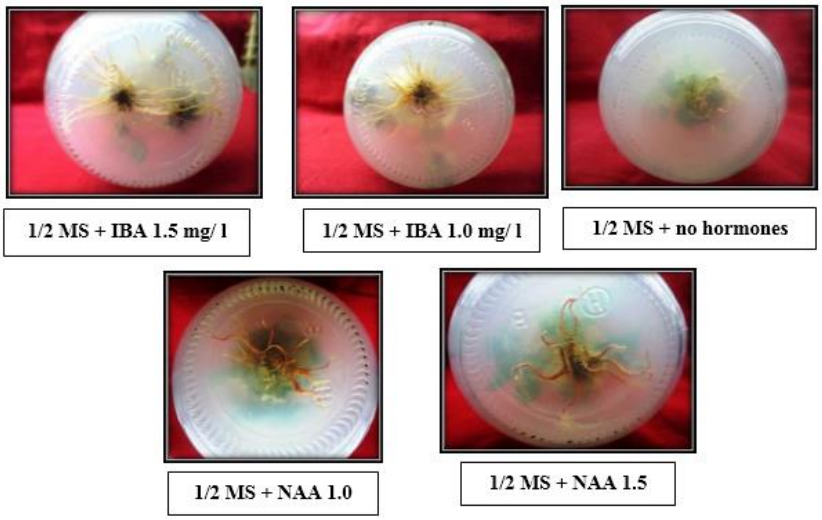

Fig 5: *In vitro* rooting on MS media with different hormones in strawberry

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