The Pharma Innovation Journal 2023; 12(5): 3469-3473

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 3469-3473

www.thepharmajournal.com
Received: 22-03-2023
Accepted: 26-04-2023

Preeti Toppo
Department of Horticulture (Fruit Science), College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

GL Sharma
Department of Horticulture (Fruit Science), College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

Prabhakar Singh
Department of Horticulture (Fruit Science), College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

Hement Panigrahi
Department of fruit Science, College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

LS Verma
Department of floriculture and Landscape architecture, College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

Corresponding Author:
Preeti Toppo
Department of Horticulture (Fruit Science), College of Agriculture, I.G.K.V., Raipur, Chhattisgarh, India

Rapid *in-vitro* propagation of strawberry cv. Nabila

Preeti Toppo, GL Sharma, Prabhakar Singh, Hement Panigrahi and LS Verma

Abstract
The present investigation entitled "Rapid *in-vitro* propagation of strawberry cv. Nabila" which was carried out during the year 2018-19 and 2019-20 at Commercial Tissue Culture Laboratory Department of PMBB, College of Agriculture, IGKV, Raipur (C.G.) G.) Hence, a study was conducted to standardize stage wise *in-vitro* media for *in-vitro* rapid multiplication of strawberry cv. Nabila and to find out best combination of plant growth regulators for various stages of *in-vitro* multiplication experiments with 3 replications and 24 treatments combinations. Experiment is laid out in Completely Randomized Design. The present study demonstrates the combined effects various growth regulators viz., BAP, IBA, NAA, Kinetin and GA_3 at varied concentration with MS medium on various shoot prolifaction, multiplication and rooting media, . In shoot prolifaction media, MS medium supplemented with BAP (0.1 mg/l) and NAA (0.1 mg/l) resulted minimum number of days required for culture establishment and MS + Kinetin (1.0 mg /1) + NAA (0.5 mg/1) recorded maximum number of shoots/ clumps per explant. In shoot multiplication media, MS + Kinetin (1.0 mg /1) + NAA (0.5 mg/1) recorded maximum number of shoots/ clumps per explant. The maximum length of shoot of strawberry explant was observed with (MS + Kinetin (1.0 mg /1) + NAA (0.1 mg/1) and MS + BAP (0.1 mg /1) + NAA (0.1 mg/1) recorded minimum days to shoot. In shoot elongation and rooting media, minimum days required to root induction on strawberry explant was observed with MS+ NAA (0.50 mg/l) + Activated Charcoal (200 mg/l) and MS medium supplemented with NAA (1.00 mg/l) + Activated Charcoal (200 mg/l) was found to be the most effective and resulted higher roots and when coming to rooting percentage MS medium with NAA (1.00 mg/l) emerged as best during both the years of study (2018-19 and 2019-20).

Keywords: *Fragria* × *ananassa*, BAP, IBA, NAA, kinetin, GA_3, activated charcoal

Introduction
Strawberry (*Fragria* × *ananassa* Duch.), a fruit that emits a fragrantly sweet flavor, is the most widely adorned berry fruits through out the world. It belongs to the family Rosaceae and subfamily Rosoideae. It is native of temperate regions, but varieties are available which can be cultivated in sub-tropical climate conditions also. The name "strawberry" derived from the practice of using straw mulch for cultivation for avoiding direct touch of fruits to soil during old times. Alternatively, the name supposed to be derive from the Anglo-Saxon word "strew" meaning to spread, as strawberry plants spread by runners. The cultivated strawberry (*Fragaria*× *ananassa* Duch.) is a hybrid between (*Fragaria virginiana*× *Fragaria chiloensis*), obtained more than 300 years ago in Europe. Strawberry plants are perennial, stoloniferous herb, meaning that they spread through botanical structures known as stolons or "runners". The leaves are trifoliate and arise from the "crown" which is a reduced stem in the center of the plant. Leaflets are ovate or broadly oval, obtuse, dentate or coarsely serrate. The runners produce "daughter" plants at every other node. Inflorescence is borne terminally on crowns. Pollination occurs through insects or wind. The strawberry is an accessory fruit, since the edible portion is non-ovarian in origin. The fruit is an enlarge receptacles bearing numerous achenes, the so-called seeds.

Micropropagation of strawberry plants were introduced in 1974. Immediately, the most of important European nurseries started producing several million plants peryear, through this technique. The two basic reasons that gave the importance to this technique were: it gave definitive answer to the problems of soil fungi, causing a lot of damage to the strawberry fields and by another way, tissue culture plants seemed to produce more runners per mother plant in a short span of time. To obtain basic material for large scale, the micro-propagation is one of the effective means to achieve this goal. Tissue cultured strawberry has been introduced to reduced soil and plant transmissible diseases (Sharma and Sharma, 2004) [9]. *In-vitro* techniques are important tools for modern plant improvement programs to introduce new traits

into selected plants, to multiply elite selection and to develop suitable cultivars in the short time. Used in conjunction with classical breeding methods, an efficient *in-vitro* shoot proliferation and regeneration system could accelerate cultivar development programmes. The ability of regenerate plants is crucial to the successful application of *in-vitro* methods.

Material and Methods

The present investigation was conducted during the year 2018-19 and 2019-20 at Commercial Tissue Culture Laboratory Department of PMBB, College of Agriculture, IGKV, Raipur (C.G.), Experiment-1 is laid out in experiments with 3 replications and 24 treatments combinations Completely Randomized Design. Shoot prolification media- T_1 $_m$S+BAP (0.1 mg /1)+ NAA(0.1 mg/1), T_2 MS+BAP (0.1 mg /1)+ NAA(0.5 mg/)1, T_3 MS+BAP (0.5 mg /1)+ NAA(0.5 mg/1), T_4 MS+BAP (0.5 mg /1)+ NAA (0.1 mg/1), T_5 MS+BAP (1.0 mg /1)+ NAA (0.1 mg/1), T_6 M S + BAP(1.0 mg /1)+ NAA (0.5 mg/1), T_7 MS+Kinetin (1.0 mg /1)+ NAA (0.5 mg/1), T_8 MS+Kinetin (1.0 mg /1)+ NAA(0.1 mg/1). Shoot multiplication media- T_1 MS+BAP (1.0 mg/1), T_2 MS+BAP (1.50 mg/1), T_3 MS+BAP (2.00 mg/1), T_4 MS+BAP (1.00 mg /1) + GA3 (2.00 mg/1), T_5 MS+BAP (1.50 mg /1)+ +GA3(2.00 mg/1), T_6 M S + BAP (2.00 mg /1) + GA3 (2.00 mg/1), T_7 MS+BAP (1.00 mg/1)+ Kinetin (0.05 mg/1), T_8 MS+ BAP (1.00 mg/1) + Kinetin (0.50 mg/1) + GA3(2.00 mg), Shoot elongation and rooting media- T_1 MS+IBA (0.50 mg/1), T_2 MS +IBA (1.00 mg/1), T_3 MS+NAA(0.50 mg/1), T_4 MS+NAA (1.00 mg/1), T_5 MS+IBA(0.50 mg/1) +Activated Charcol (200 mg /1), T_6 M S + IBA(1.00 mg/1) +Activated Charcol (200 mg /1), T_7 MS+ NAA(0.50 mg/1) + Activated Charcol (200 mg/1), T_8 MS+ NAA(1.00 mg/1) +Activated Charcol (200 mg/1).

Mother plants

The plants of strawberry cultivar "Nabila" grown under and Centre of Excellence on Protected Cultivation and Precision farming under poly tunnel, IGKV, Raipur (C.G.) were used as source of explant in the present study. Runner tips of 1-2cm long were taken from mother plants for sterilization.

Media used for shoot proliferation

For shoot proliferation, sterilized explants were cultured on solidified MS basal media supplemented along with BAP, NAA, Kinetin, as per treatment combination.

Media used for Shoot induction

For rooting, regenerated shoots were sub-cultured in solidified MS medium supplemented along with concentration of BAP, GA_3 and Kinetin their combinations in accordance to treatment combination.

Media used for root induction

For rooting, regenerated shoots were sub-cultured in solidified MS medium supplemented along with concentration of IBA, NAA and Activated Charcol their combinations in accordance to treatment combination.

Culture room

The inoculated cultures were incubated at 25±2°C in an air-conditioned culture room with a light intensity of 2000-3000 lux by cool white fluorescent tubes. The light/dark cycles of photoperiod were maintained as 16/8 hours daily.

Collection of explants

The explants of strawberry used for present micro-propagation studies were nodal segment. These explants were excised from the runner. Explants were collected in clean polythene bags and brought to the laboratory. The nodal segment measuring about 1-1.5 cm long were cut from healthy runners and used as explants.

Surface sterilization of explants

Explants were rinsed under running tap water for 20-25 minutes. Followed by sterilized distilled water. Then the explants were subjected to incision into appropriate sizes (Around 1-1.5 cm long) and transferred to the laminar hood where the processed explants were sterilized in 0.1 percent Hg Cl_2 with 2 drops of twenty for five minutes followed by three rinsing with sterile distilled water for 4 minutes each. After sterilization, explants were resized to remove the surface of explants and meristems of 3-5 mm long were isolated as final explant with the help of scalpels and forceps. Trace of water remaining on the surface of the explants was soaked with sterilized filter paper. The sterilized material of explant was inoculated on appropriate nutrient medium.

Inoculation of explants into culture medium

The cut ends of the explants were trimmed off and surface sterilized and aseptically excised explants were finally placed on media by working in a laminar air flow cabinet. The bottles containing medium prepared as per different treatments, were unplugged by holding them over spirit lamp and inoculations were performed by placing explants on the surface of the medium with the help of flame sterilized long forceps and again plugged with screw cap of the bottles. During inoculation, the explants were properly positioned on the media and were gently pressed with forceps to secure their firm contact with the media.

Incubation of culture

The culture bottles after inoculation were kept in culture room at 25±2 °C temperature for germination. The explants incubated for shoot induction/ proliferation were maintained at 25±2 °C temperature and photoperiod (2000-3000 lux) of 16 hours light and 8 hours dark in culture room.

Results and Discussion

Days required for Culture Establishment

The data reveals that number of days required for culture establishment of strawberry explant under *in-vitro* condition had significant effect of various treatments. Further, the number of days for culture establishment of explant had significantly reduced with the application of BAP, NAA and Kinetin as compared to control.

However, the minimum number of days for required for culture establishment of strawberry explant was recorded at MS + BAP (0.1 mg /1) + NAA (0.1 mg/1) (T_1) treatment (9.2 days in 2018-19 and 8.83 days in 2019-20), which was significantly less than all other treatments. However, the maximum days required for culture establishment of explant (11.87 days in 2018-19 and 11.49 days in 2019-20) was recorded with MS +BAP (0.5 mg /1) + NAA (0.5 mg/1) (T_3) treatment. The pooled mean data for number of days required for culture establishment of strawberry explant under *in-vitro* condition also had significant effect by various treatments and were found maximum (11.68 days) in T_3 (MS + BAP (0.5 mg

/1) + NAA (0.5 mg/1)), whereas, the minimum number of days required for culture establishment (9.02 days) were recorded in T_1 (MS + BAP (0.1 mg/1) + NAA (0.1 mg/1)).
Similarly, results were obtained by Jones *et al.* (1988) [5], who reported that the days taken for culture establishment will be less if we use BAP and NAA along MS medium in low concentrations.

Shoot Multiplication

Days to Shoot induction

The data reveals that number of days required for shoot induction of strawberry explant under *in-vitro* condition had significant effect of various treatments. Further, the number of days for sprouting of explant had significantly reduced with the application of BAP, Kinetin and GA_3 as compared to control. However, the minimum number of days required for shoot induction of strawberry explant was recorded at MS + BAP (1.00 mg /l) + Kinetin (0.05 mg/l) (T_7) treatment (10 days in 2018-19 and 9.58 in 2019-20), which was significantly less than all other treatments . However, the maximum days required for shoot induction of explant (13.33 days in 2018-19 and 12.69 in 2019-20) was recorded with MS+BAP (1.00 mg/l)+GA_3 (2.00 mg/l) (T_4). The pooled data for number of days required for shoot induction in strawberry explant under *in-vitro* condition as influence by various samples of the MS medium supplemented with different levels of auxins and cytokinins also has significant effect and were found maximum (13.01 days) in treatment T_4 (MS + BAP (1.00 mg /l) + GA_3 (2.00 mg/l)),whereas, the minimum number of days required for shoot induction (9.79 days) was recorded in treatment T_7 (MS+ BAP (1.00 mg /l) +Kinetin (0.05 mg/l)).
In the present investigation, number of shoots per plant seems to be higher when compared to an earlier study of Zebrowska and Hortynski (2002) [13].

Number of Shoot per clump

The data clearly indicate the supplementation of MS medium with different concentration of BAP, GA_3 and Kinetin had significantly increased the number of shoots/clumps per explant of strawberry under *in-vitro* condition. The maximum number of shoots/clumps (6/explant in 2018-19 and 5.71/explant in 2019-20) was recorded at MS+BAP (1.00 mg/l) + Kinetin (0.05 mg/l) (T_7) treatment which was higher than all other treatments. Whereas, the minimum number of roots (2/explant in 2018-19and 1.91/explant in 2019-20) was observed with MS + BAP (1.50 mg/l) (T_2) treatment. The pooled mean data for number of shoots per explant also had significant effect by various treatments and were found maximum (5.86) in treatment T_7 (MS+BAP (1.00 mg/l) + Kinetin (0.05 mg/l)), whereas, the minimum number of days required for culture establishment (1.96) were recorded in treatment 2(MS+ BAP (1.50 mg/l)).
In the present investigation, number of shoots per plant seems to be higher when compared to an earlier study of Zebrowska and Hortynski (2002) [13].

Shoot Length

It is evident from the data that shoot length of strawberry explant under *in-vitro* condition significantly increase with the application of BAP, GA_3 and Kinetin treatment at different concentration in MS medium as compared to control. The maximum length of shoot (3.27 cm in 2018-19 and 3.15 cm in 2019-20) was recorded with MS + BAP (1.00 mg/l) + GA_3 (2.00 mg/l) (T_4) treatment which was closely followed by MS+BAP (2.00 mg /l) + GA_3 (2.00 mg/l) (3.13 cm) (T_6) treatments. The T_4 and T_6 treatments were found significantly higher than all other treatments. The minimum length of shoot was recorded with MS+BAP (2.00 mg/l) (2.17 cm in 2018-19 and 2.06 cm in 2019-20) (T_3) treatment. The pooled mean data for shoot length also had significant effect by various treatments and were found maximum (3.21 cm) in treatment T_4 MS + BAP (1.00 mg /l) +GA_3 (2.00 mg/l)), whereas, the minimum shoot length (2.11 cm) was recorded intreatment T_3 (MS+ BAP (2.00 mg/l)).
The findings were in accordance with that reports of Karim *et al.* (2011) [6], Zebrowska and Hortysnki (2002) [13] but in contrast to the findings of Khan and Spoor (2004) who reported maximum shoot length was there when using BAP +NAA along with MS medium at very low concentration.

Root induction

The data clearly indicate the supplementation of MS medium with different concentration of IBA, NAA and Activated Charcoal had significantly decreased the number of days required for root induction per explant of strawberry under *in-vitro* condition. The maximum number of days required for root induction (13.33 in 2018-19 and 12.91 in 2019-20) was recorded with MS+NAA (1.00 mg/l) + Activated Charcoal (200 mg/l) (T_8) treatment) which was significantly higher than all other treatments. Whereas, the minimum number of days required for root induction (9 in 2018-19 and 8.57 in 2019-20) was observed at (T_7) sample MS + NAA (0.50 mg/l) + Activated Charcoal (200 mg/l). The pooled mean data for root induction per explant of strawberry also has significant effect and were found maximum (13.12 days) in treatment T_8 (MS + NAA (1.00 mg/l) + Activated Charcoal (200 mg/l)), whereas, the minimum number of days taken for root induction per explant of strawberry (8.78 days) was recorded in treatment T_7 (MS+NAA (0.50 mg/l) +Activated Charcoal (200 mg/l)).
In the present investigation, the findings are in accordance with that of Lopez Aranda *et al.* (1994) [11], El-Kichaoui (2014) [2], Letouze (1987) [10] who reported highest root induction by addition of charcoal along with MS medium.

Number of roots per plantlets

The data clearly indicate the supplementation of MS medium with different concentration of IBA, NAA and Activated Charcoal had significantly increased the number of roots per explant of strawberry under *in-vitro* condition. The maximum number of roots/ plantlets (4.33/explant in 2018-19 and 4.12/explant in 2019-20) were recorded with MS + NAA (1.00 mg/l) + Activated Charcoal (200 mg/l) (T_8) treatment, which was significantly higher than all other treatments. Whereas, the minimum number of roots (2.31/explant in 2018-19 and 2.22/explant in 2019-20) was observed with (T_3) treatment (MS + NAA (0.50 mg/l)). Further, the statistical difference between these two treatments (T_8 and T_3) was found to be significant indicating that the response of both treatment with respect to number of roots/explants is at par. The pooled mean data for number of roots/plantlets per explant of strawberry also has significant effect and were found maximum (4.23/explant) in treatment T_8 (MS + NAA (1.00 mg/l) + Activated Charcoal (200 mg/l)), whereas, the minimum number of roots/ plantlets per explant of strawberry (2.28/explant)was recorded in treatmentT_3(MS +NAA (0.50

mg/l)).

Such beneficial effects of application of various growth regulators along with MS medium in *in-vitro* conditions have been established in the findings of Isac *et al.* (1994) [4] and Kaushal *et al.* (2004) [9] but in contrast with the findings of Diengngan *et al.* (2014) [1] who reported less root induction on usage of IBA with MS medium.

Rooting Percentage

It is evident from the data that percentage of explant rooted had significantly influenced by various treatments. The supplementation of MS medium with IBA, NAA and Activated Charcoal at different concentration resulted higher percentage of explant sprouted over control (MS medium). The maximum rooted explant of 92 percent in 2018-19and88.50 percent in 2019-20 was recorded at treatment T_2 (MS+IBA (1.00 mg/l)) which was significantly higher than rest of the treatment attempted in the present investigation. Whereas the minimum 71.33 percent in 2018-19 and 68.50 percent in 2019-20 explant rooted at sample (T_1) (MS + IBA (0.50 mg/l)). The pooled mean data for percentage of explant rooted also has significant effect and were found maximum (90.25 %) in treatment T_2 (MS + IBA (1.00 mg/l)), whereas, the minimum number of roots/ plantlets per explant of strawberry (69.69 %) was recorded in treatment T_1 (MS +IBA (0.50 mg/l)).

The findings were in contrast with that reports of Haddadi *et al.* (2010), Murti *et al.* (2013), Karim *et al.* (2015) [3, 7, 8] who reported that highest rooting percentage is obtained with sole usage of MS medium with out any growth regulators.

Table 1: Effect of various treatments on Shoot Prolifaction media for *in-vitro* establishment parameter of strawberry cv. Nabila

S.No.	Treatment	Treatment Details	Days Required for Culture Establishment		
			2018-19	2019-20	Pooled
1	T_1	MS + BAP (0.1 mg /1) + NAA (0.1 mg/1)	9.2^{e}	8.83^{d}	9.02^{e}
2	T_2	MS + BAP 0.1 mg /1) + NAA (0.5 mg/1)	10.2^{bc}	9.73^{bc}	9.97^{bc}
3	T_3	MS + BAP (0.5 mg /1) + NAA (0.5 mg/1)	11.87^{a}	11.49^{a}	11.68^{a}
4	T_4	MS + BAP 0.5 mg /1) + NAA (0.1 mg/1)	9.73^{cde}	9.44^{bcd}	9.59^{cd}
5	T_5	MS + BAP 1.0 mg /1) + NAA (0.1 mg/1)	9.47^{de}	9.14^{cd}	9.31^{de}
6	T_6	MS + BAP (1.0 mg /1) + NAA (0.5 mg/1)	10bcd	9.5^{bc}	9.75^{c}
7	T_7	MS + Kinetin 1.0 mg /1) + NAA (0.5 mg/1)	11.47^{a}	11.12^{a}	11.29^{a}
8	T_8	MS + Kinetin (1.0 mg /1) + NAA (0.1 mg/1)	10.53^{b}	10.05^{b}	10.29^{b}
		S.E.M.	0.19	0.21	0.14
		C.D. 5%	0.58	0.65	0.41

Table 2: Effect of various treatments on Shoot Multiplication Media parameters of strawberry cv. Nabila

S.No.	Treatment	Treatment details	Days to Shoot induction			Shoot Length (cm)			No. of Shoot per clump		
			2018-19	2019-20	Pooled	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
1	T_1	MS + BAP (1.0 mg/l)	10.01^{d}	9.68^{e}	9.84^{e}	2.57^{de}	2.49^{de}	2.53^{e}	3.67^{e}	3.49^{e}	3.58^{e}
2	T_2	MS + BAP (1.50 mg/l)	11^{c}	10.45^{d}	10.73^{d}	2.53^{e}	2.41^{e}	2.47^{e}	2g	1.91^{g}	1.96^{g}
3	T_3	MS + BAP (2.00 mg/l)	11.33^{bc}	10.97^{cd}	11.15^{cd}	2.17^{f}	2.06^{f}	2.11^{f}	3.67^{e}	3.51^{e}	3.59^{e}
4	T_4	MS + BAP (1.00 mg /l) + GA3 (2.00 mg/l)	13.33^{a}	12.69^{a}	13.01^{a}	3.27^{a}	3.15^{a}	3.21^{a}	3.33^{f}	3.17^{f}	3.25^{f}
5	T5	MS + BAP (1.50 mg /l) + GA3 (2.00 mg/l)	11.67^{bc}	11.22^{bc}	11.44^{bc}	3^{bc}	2.87^{bc}	2.94^{c}	5^{b}	4.84^{b}	4.92^{b}
6	T_6	MS + BAP (2.00 mg /l) + GA3 (2.00 mg/l)	12^{b}	11.4^{bc}	11.7^{b}	3.13^{ab}	2.98^{b}	3.06^{b}	4.7^{c}	4.44^{c}	4.55^{c}
7	T_7	MS + BAP (1.00 mg /l) + Kinetin (0.05 mg/l)	10^{d}	9.58^{e}	9.79^{e}	2.93^{c}	2.85^{c}	2.89^{c}	6a	5.71^{a}	5.86^{a}
8	T_8	MS + BAP (1.00 mg/l) + Kinetin (0.50 mg/l) + GA3 (2.00 mg/l)	12^{b}	11.59^{b}	11.8^{b}	2.7^{d}	2.58^{d}	2.64^{d}	4.33^{d}	4.19^{d}	4.26^{d}
		S.E.M.	0.3	0.19	0.17	0.05	0.04	0.03	0.07	0.06	0.05
		C.D. 5%	0.91	0.57	0.5	0.16	0.13	0.1	0.2	0.19	0.14

Table 3: Effect of various treatments on Rooting Media parameters of strawberry cv. Nabila

S. No.	Treatment	Treatment details	Root induction			No of root per plantlets			Rooting percentage (%)		
			2018-19	2019-20	Pooled	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
1	T_1	MS + IBA (0.50 mg/l)	10^{d}	9.58^{d}	9.79^{d}	3b	2.89^{b}	2.95^{b}	71.33^{e}	68.05^{e}	69.69^{e}
2	T_2	MS + IBA (1.00 mg/l)	9.33^{e}	9.05^{e}	9.19^{e}	2.33^{d}	2.24^{d}	2.29^{d}	92^{a}	88.5^{a}	90.25^{a}
3	T_3	MS + NAA (0.50 mg/l)	12^{b}	11.54^{b}	11.77^{b}	2.31^{d}	2.22^{d}	2.28^{d}	75de	72.15^{d}	73.57^{d}
4	T_4	MS + NAA (1.00 mg/l)	11.67^{b}	11.18^{b}	11.42^{b}	2.67^{c}	2.55^{c}	2.61^{c}	$88.33a^{b}$	84.8^{a}	86.57^{b}
5	T_5	MS + IBA (0.5 0 mg/l) + Activated Charcoal (200 mg /l)	11^{c}	10.65^{c}	10.82^{c}	2.67^{c}	2.53^{c}	2.6^{c}	80.33^{cd}	76.96^{bc}	78.65^{c}
6	T_6	MS + IBA (1.00 mg/l) + Activated Charcoal (200 mg /l)	10.33^{d}	9.96^{d}	10.15^{d}	3^{b}	2.9^{b}	2.95^{b}	82.33^{c}	79.7^{b}	81.02^{c}
7	T_7	MS + NAA (0.50 mg/l) + Activated Charcoal (200 mg/l)	9^{e}	8.57^{e}	8.78^{f}	2.67^{c}	2.57^{c}	2.62^{c}	83.33^{bc}	79.83^{b}	81.58^{c}
8	T_8	MS + NAA (1.00 mg/l) + Activated Charcoal (200 mg/l)	13.33^{a}	12.91^{a}	13.12^{a}	4.33^{a}	4.12^{a}	4.23^{a}	76.33^{de}	73.28^{cd}	74.81^{d}
		S.E.M.	0.19	0.17	0.12	0.06	0.06	0.04	`1.93	1.33	1.18
		C.D. 5%	0.56	0.52	0.36	0.19	0.17	0.12	5.87	4.04	3.42

Conclusions

Based on the experiment result, it may conclude that the explants cultured on MS media supplemented with various growth regulators like BAP, NAA, GA3, IBA and Kinetin at desired concentration took less time for shoot prolification, days culture establishment, shoot multiplication, shoot induction, shoot length ,number of shoot, root induction, number of roots, rooting percentage.

References

1. Diengngan S, Murthy BNS. Influence of plant growth promoting substances in micro-propagation of strawberry cv. Festival. The Bioscan. 2014;9(4):1491-1493.
2. El-Kichaoui AY. *In-vitro*, propagation of strawberry (*Fragaria* × *annanasa* Duch.) through organogenesis via runner tips. Annals of Plant Sciences. 2014;3(03):619-627.
3. Haddadi F, Aziz MA, Saleh AA, Kamaladini H. Micropropagation of strawberry cv. Camarosa: Prolific regeneration from in vitro shoot tips using Thidiazuron with N6- Benzylamino-purine. Hort Science. 2010;45(3):453-456.
4. Isac V, Popescu AN, Coman M, Schmidt H. Studies on plant regeneration from tissue derived callus in *Fragaria X. ananassa* Duch. Progress in temperate fruit breeding. Proceeding of the Eucarpia fruit breeding section meeting, wodens will – Einsiedelna Switzerland, 30 August to, September, 395-398, developments in plant breeding. 1994. September; 1:395-398,
5. Jones OP, Waller BJ, Beech MG. The production of strawberry plants from callus cultures. Plant Cell. Tissue and Organ Culture, 1988;12(3):235-241.
6. Karim MR, Aziz MA, Roy UK, Hoque MA, Hossain MM. *In vitro* response of strawberry (*Fragaria x ananassa* Duch.) for callus induction and shoot regeneration. Int. J Agr. & Agri. R. 2011;1(1):29-36.
7. Karim R, Ahmed F, Roy UK, Ara T, Islam R, Hossain M. Varietal improvement of strawberry (*Fragaria* × *ananassa* Duch.) through somaclonal variation using in vitro techniques. J Agr. Sci. Tech. 2015;17:977-986.
8. Karim R, Ahmed F, Roy UK, Ara T, Islam R, Hossain M. Varietal improvement of strawberry (*Fragaria* × *ananassa* Duch.) through somaclonal variation using in vitro techniques. J Agr. Sci. Tech. 2015;17:977-986.
9. Kaushal K, Nath AK, Kaundal P, Sharma DR. Studies on somaclonal variation in strawberry (*Fragaria X ananassa* Duch.) Cultivars. Acta Hort. 2004;662:275.
10. Letouze C. *In vitro* regeneration of strawberry plants from petiole segments and flower buds. Biol. Plant. 1987;29(6):409-414.
11. Lopez Aranda JM, Pliego Alfaro F, Lopez Navidad I, Barcelo Munoz M. Micro-propagation of strawberry (*Fragaria* × *ananassa* Duch.) effect of mineral salts, benzyl adenine levels and number of subcultures on the in-vitro and field behavior of the obtained micro plants and the fruiting capacity of their progeny. Journal of Horticultural Science. 1994;69(4):625-637.
12. Murti RH, Yeoung YR. Effects of BA and IBA concentrations and subculture frequent on meristem culture of strawberry. APRN. J Agric. Bio. Sci. 2013;8(5):405-410.
13. Zebrowska JI, Hortynsiki J. Plant regeneration from leaf explants in strawberry (*Fragaria X ananassa* Duch.). Proc. 4th Int. strawberry symp. Acta Hort. 2002;567:313-315.