Original Research Article

Effect of different plant growth regulators for *in vitro* regeneration in strawberry

ABSTRACT:

The present study was carried out in College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya during 2017-18. The objective was to study the effect of growth regulators on *in vitro* regeneration in four varieties of strawberry *viz.,* Camarosa, Chandler, Festival and Winter Dawn. Best sterilization results were obtained for explants sterilized using Tween 20, 0.1% Bavistin, 0.1% Ascorbic acid + 0.1% Streptomycin and 0.1% Mercuric chloride for 30, 45, 40 and 8 minutes respectively. Sixteen different concentrations and combinations of growth regulators were used out of which regeneration from runners was successfully observed in six combinations of growth regulators. MS media supplemented with 5 mg/l BAP+ 1 mg/l IAA produced maximum percent survival of explants and maximum number of shoots while MS medium supplemented with 4 mg/l BAP + 1 mg/l IAA produced the highest shoot length. Rooting was observed in half strength MS media supplemented with 0.5 mg/l IBA. Rooted plantlets were transferred to soil where they showed 66.67% survival.

KEYWORDS: *Fragaria ananassa, morphological traits, in vitro regeneration,* growth regulators

ABBREVIATIONS: BAP- 6 Benzylaminopurine, NAA- Napthalene acetic acid, IAA-Indole-3-acetic Acid IBA-Indole-3-butyric acid mg/l- milligram per litre, MS media- Murashige and Skoog media

1. INTRODUCTION

Horticultural crops have contributed to agricultural development in the last few years due to high returns of income compared to annual cereal crops. The demand for horticultural crops is high in the domestic as well as global markets. Cultivation of fruits and vegetables is labour intensive, have low gestation period and provide greater returns [1]. In India, strawberry is cultivated in Maharashtra, Punjab, Himachal Pradesh, Uttarakhand, Uttar Pradesh, West Bengal, Nilgiri Hills, Delhi, Haryana and some parts of Rajasthan [2]. Cultivation of horticultural crops is essential in North Eastern Region in India as it provides abundant scope for diversification and acts as a source for entrepreneurial venture. Cultivation of strawberry also promotes eco-tourism resulting in generation of employment opportunities for the local youth [3].

Strawberry is a herbaceous member of genus *Fragaria* of Rosaceae family. Cultivated strawberry (*Fragaria x ananassa* Duch.) originated in Europe as a hybrid between the South American *Fragaria chiloensis* Duch and the North American *Fragaria virginiana* Duch [4]. There are about twenty recognized species with various ploidy levels. Cultivated strawberry is octoploid (2n=8x=56) [5]. Several varieties of strawberries are cultivated in India *viz.,* Camarosa, Festival, Winter Dawn, Sweet Charlie, Belrubi, Chandler etc [2]. Strawberry is propagated mainly by runners with the success of strawberry cultivation depending mainly on the availability of quality planting material [6]. Farmers use plants from meristem culture in order

to maintain the genetic constitution and desirable varietal characteristics and to obtain disease free planting material [7]. *In vitro* propagation of strawberry was first reported by Boxus in 1974 following which different types of medium, plant growth regulators, explants and genotypes has been used [8].

Therefore, given the importance of strawberry cultivation in Meghalaya in generating revenue for farmers especially in the Ri-Bhoi district of Meghalaya, the present study was proposed to assess the effect of different plant growth regulators for *in vitro* regeneration of strawberry. Standardization of *in vitro* propagation system provides an alternative to enhance the production of planting materials, including virus-free plants for large-scale planting. The establishment of an efficient *in vitro* regeneration system in strawberry could also make a significant contribution in improving the qualitative and quantitative characters of the plant.

2. MATERIALS AND METHODS

The study was conducted at College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University (Imphal), Umiam, Meghalaya in 2017-18 to study the effect of different plant growth regulators for *in vitro* regeneration in strawberry. Four varieties *viz.* Camarosa, Chandler, Festival and Winter Dawn were used. Runners were used as explants.

2.1. Explant preparation and sterilization

Different combinations of sterilants were used. The runners were first washed with running tap water. The explants were surface sterilized with different combinations of Tween 20, Bavistin, 0.1% Ascorbic acid + 0.1% Streptomycin and 0.1**%** mercuric chloride ($HgCl_2$) inside a laminar air flow chamber for different time periods. The explants were cut using new, sterile surgical blades to obtain clean excision with minimum injury to the explants.

2.2. Shoot induction

Sterilized explants were inoculated in bottles containing MS media supplemented with different concentrations of BAP (2–5 mg/l) alone and in combination with IAA (1 mg/l) and KIN alone (2-5mg/l) and in combination with IAA (1 mg/l) (Table 1). MS medium was used which was adjusted to pH 5.8 with 1N Sodium Hydroxide (NaOH) before autoclaving. Temperature was maintained at 28□C and subculturing was done after 45 days. Best concentration of growth regulators for shoot growth was found out by recording the rate of shoot multiplication, length of shoots and number of shoots per explant after 5 weeks. Serial sub-culturing in fresh medium was performed regularly after 5 weeks.

	Plant growth regulators (mg/l)		
Media	BAP	KIN	IAA
MS 1	2	-	-
MS 2	3	-	-
MS 3	4	-	-
MS 4	5	-	-
MS 5		2	-

MS 6		3	-
MS 7		4	-
MS 8		5	-
MS 9	2	-	1
MS 10	3	-	1
MS 11	4	-	1
MS 12	5	-	1
MS 13		2	1
MS 14		3	1
MS 15		4	1
MS 16		5	1
MS 17	Control		

Table 1. Different concentrations and combinations of growth regulators for direct regeneration

2.3. Preparation of hormones/ plant growth regulators

6-Benzylaminopurine (BAP), Kinetin, Napthalene acetic acid (NAA), Indole-3-acetic Acid (IAA), Indole-3-butyric acid (IBA) was prepared by dissolving 0.01g of BAP, Kinetin, NAA, IAA and IBA respectively in 5 ml of Sodium hydroxide and the volume was made to 100ml by autoclaved distilled water.

2.4. Rooting of *in vitro* regenerated shoots

Regenerated shoots were subcultured for rooting on basal half strength MS media alone and MS media supplemented with 0.5mg/l IBA. Observations on percent explant response, number of shoots, shoot length, number of roots and root length were taken.

2.5. Hardening of rooted plantlets

Sterilized soil and artificial soil mixture were used for hardening of *in vitro* regenerated plants. Perlite, vermiculite and peat were mixed at 1:1:1 ratio which was further mixed with sterilized soil at 1:1 ratio. Fully developed elongated shoots with roots were taken from the bottles and the base portion was washed thoroughly with running water to remove the medium attached to it. Regenerated seedlings were transplanted on Protrays containing soil mixture for acclimatization.

2.6. Statistical analyses

The experiments were conducted in a completely randomized design. The data recorded pertaining to different parameters was subjected to analysis of variance (ANOVA) using CRD. The statistical analyses were carried out using MS-Excel.

3. RESULTS AND DISCUSSION

3.1 *In vitro* regeneration

An economic study of costs and returns of strawberry cultivation on commercial scale in Meghalaya indicated that although strawberry fruit is highly profitable for the farmers, a major constraint in adaptation of

strawberry cultivation was the unavailability of runners [9]. Strawberry is commercially propagated using runners to obtain true-to-type plants. The success of strawberry planting depends on the availability of large numbers of runners and good planting stock. *In vitro* propagation system provides an alternative of enhancing the production of planting materials, including virus-free plants for large-scale planting. The establishment of an efficient *in vitro* regeneration system in strawberry could also make a significant contribution in improving the qualitative and quantitative characters of the plant [8].

3.2. Standardization of surface sterilization of strawberry explants

It was observed that Tween 20, 0.1% Bavistin, 0.1% Ascorbic acid+ 0.1% Streptomycin and 0.1% Mercuric chloride when used for 30, 45, 40 and 8 minutes respectively produced maximum response (83.33%) while lowest response (0%) was produced when sterilization was done using Tween 20, 0.1% Bavistin, 0.1% Ascorbic acid+ 0.1% Streptomycin and 0.1% Mercuric chloride for 5, 20, 15, 6 minutes;10, 25, 20, 7 minutes; 5, 20, 20, 6 minutes and 10, 25, 25, 7 minutes (Table 2).

Sl.No.	Exposure time (minutes)				Number of explants treated	Percent response to culture establishment (%)
	Tween 20	0.1% Bavistin	0.1% Ascorbic acid + 0.1% Streptomycin	0.1% Mercuric chloride		
1.	5	20	15	6	6	0
2.	10	25	20	7	6	0
3.	15	30	25	8	6	16.67
4.	20	35	30	6	6	33.33
5.	25	40	35	7	6	66.67
6.	30	45	40	8	6	83.33
7.	5	20	20	6	6	0
8.	10	25	25	7	6	0
9.	15	30	30	8	6	33.33
10.	20	35	35	6	6	50
11.	25	40	40	7	6	66.67
12.	30	45	45	8	6	66.67

Table 2. Effect of different sterilization treatments on strawberry explants

3.3. Effect of Media

Out of the sixteen concentrations and combinations of growth regulators used along with one control medium, shoot induction was observed in six combinations of the growth regulators i.e., 4 mg/l BAP alone and in combination with 1mg/l IAA; 5 mg/l BAP alone and in combination with 1mg/l IAA and 5 mg/l Kinetin alone and in combination with 1 mg/l IAA (Figure 1).

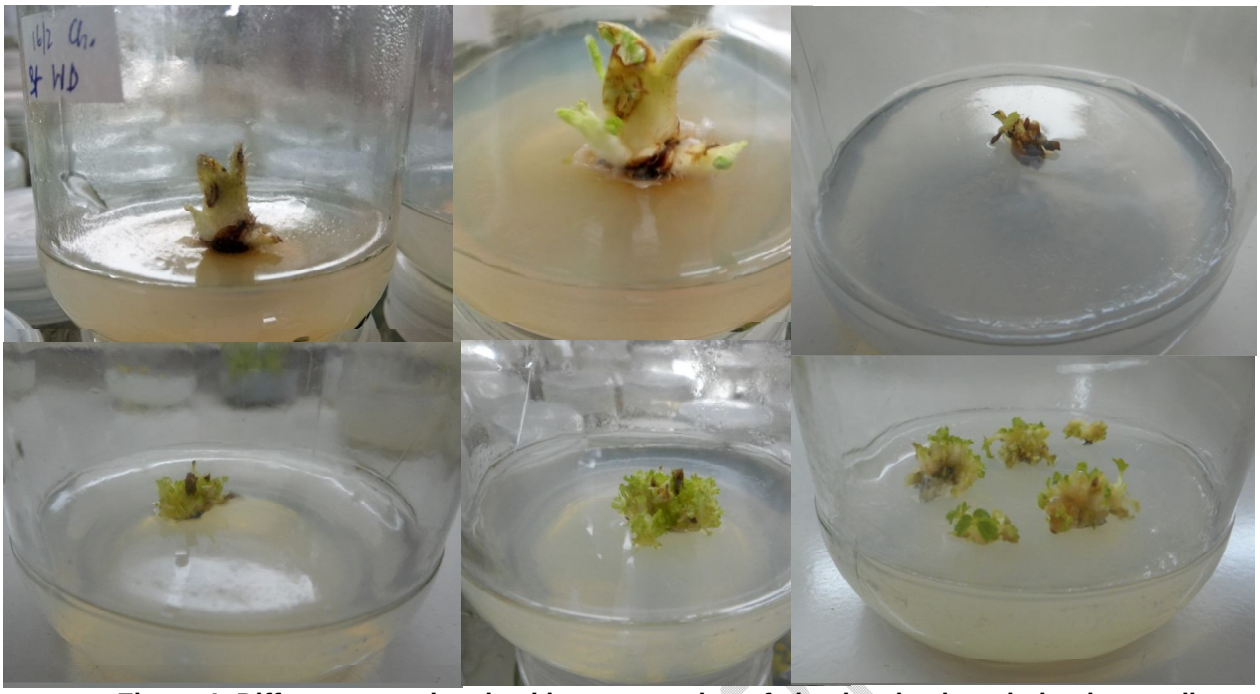

Figure 1. Different steps involved in regeneration of plantlets in shoot induction media

MS medium supplemented with 5 mg/l BAP + 1 mg/l IAA produced the maximum percent survival of explants (100%) in all the varieties studied. Lowest explant survival (57.14%) was observed in media supplemented with Kinetin 5 mg/l followed by media supplemented with Kinetin 5 mg/l + 1 mg/l IAA (60.71 ± 3.57). Media supplemented with 4 mg/l BAP + 1mg/l IAA produced 100 % survival in Winter Dawn and Festival followed by 85.71% in Camarosa and Chandler. Maximum survival (80.95 ± 7.97) of explants was observed in variety Winter Dawn followed by Camarosa and Chandler (78.57 ± 7.14). MS medium supplemented with kinetin were inferior in performance for shoot development to MS medium supplemented with various concentrations of BAP (Table 3).

Sl. No.	Media	Winter Dawn	Camarosa	Chandler	Festival	Mean ± SE	Variance
1.	MS 3	85.71	85.71	85.71	71.43	82.14 ± 3.57	51.02
2.	MS 4	85.71	85.71	71.43	71.43	78.57 ± 4.12	68.03
3.	MS 8	57.14	57.14	57.14	57.14	57.14	0
4.	MS 11	100	85.71	85.71	100	92.86 ± 4.12	68.03
5.	MS 12	100	100	100	100	100	0
6.	MS 16	57.14	57.14	71.43	57.14	60.71 ± 3.57	51.02
	Mean ± SE	80.95 ± 7.97	78.57 ± 7.14	78.57 ± 6.12	76.19 ± 7.97		
	Variance	380.95	306.12	224.49	380.95		

Table 3. Percent response of explants for the different combinations and concentrations of growth regulators for the varieties studied

It was found that MS Medium supplemented with 5mg/l BAP + 1mg/l IAA produced the best results both in terms of maximum percentage survival of explants and number of shoots in all the varieties studied. This concentration was much higher than the concentration of 1 mg/l BAP +1 mg/l IAA in half strength MS medium which produced highest number of shoots [10]. MS medium supplemented with 4mg/l BAP + 1mg/l IAA produced the longest shoot length (1.62± 0.03) followed by media supplemented with 5 mg/l BAP + 1mg/l IAA (1.48 ± 0.05). Minimum shoot length (0.76 ± 0.16) was observed in media supplemented with Kinetin 5mg/l followed by media supplemented with 5 mg/l Kinetin + 1 mg/l IAA (0.84 ± 0.05). Maximum shoot length was observed in variety Winter Dawn (1.29 ± 0.12) followed by Festival (1.27 ± 0.18) while minimum shoot length was observed in variety Camarosa (1.16 ± 0.15) (Table 4).

Sl. No.	Media	Winter dawn	Camarosa	Chandler	Festival	Mean ± SE	Variance
1.	MS 3	1.35	1.225	1.47	1.4	1.36 ± 0.05	0.01
2.	MS 4	1.4	1.13	1.13	1.55	1.30 ± 0.10	0.04
3.	MS 8	1.23	0.57	0.6	0.65	0.76 ± 0.16	0.1
4.	MS 11	1.67	1.6	1.56	1.65	1.62± 0.03	0
5.	MS 12	1.36	1.47	1.54	1.57	1.48 ± 0.05	0.01
6.	MS 16	0.75	0.97	0.85	0.8	0.84 ± 0.05	0.01
	Mean ± SE	1.29± 0.12	1.16 ± 0.15	1.19 ± 0.16	1.27 ± 0.18		
	Variance	0.09	0.14	0.16	0.19		

Table 4: Shoot length for the different combinations and concentrations of growth regulators for the varieties studied

Maximum number of shoots (6.75 ± 0.25) were observed in media supplemented with 5 mg/l BAP+ 1 mg/l IAA followed by media supplemented with 4 mg/l BAP+ 1 mg/l IAA (6.5 ± 0.29) while minimum number of shoots (2.5 ± 0.29) was observed in media supplemented with 5 mg/l Kinetin. Maximum number of shoots (4.5 ± 0.85) was observed in variety Winter Dawn while minimum number of shoots (3.33 ± 0.84) was observed in variety Festival (Table 5).

Sl. No.	Media	Winter dawn	Camarosa	Chandler	Festival	Mean ± SE	Variance
1.	MS 3	4	4	3	2	3.25 ± 0.48	0.92
2.	MS 4	4	3	3	2	3 ± 0.41	0.67
3.	MS 8	3	3	2	2	2.5 ± 0.29	0.33
4.	MS 11	7	6	7	6	6.5 ± 0.29	0.33
5.	MS 12	7	7	7	6	6.75 ± 0.25	0.25
6.	MS 16	2	3	2	2	2.25 ± 0.25	0.25
	Mean ± SE	4.5 ± 0.85	4.33 ± 0.71	4 ± 0.97	3.33 ± 0.84		
	Variance	4.3	3.07	5.6	4.27		

Table 5: Shoot number for the different combinations and concentrations of growth regulators for the varieties studied after 45 days of inoculation

ANOVA for shoot length for the different varieties studied revealed that MS media supplemented with 5 mg/l Kinetin was significantly different from the other concentrations and combinations of growth regulators used (Tables 6 and7)(Figure 2.).

Sl. No.	Media	Media composition	Mean values
1.	MS 3	MS 11- 4 mg/l **BAP** + 1mg/l IAA	1.62
2.	MS 4	MS 12- 5 mg/l **BAP** + 1mg/l IAA	1.48
3.	MS 8	MS 3 - 4 mg/l **BAP**	1.37
4.	MS 11	MS 4 - 5 mg/l **BAP**	1.24
5.	MS 12	MS 16 - 5 mg/l **Kinetin** + 1mg/l IAA	0.86
6.	MS 16	MS 8 – 5mg/l **Kinetin**	0.79

Table 6: Mean values for shoot length for the different varieties studied

Source of Variation	SS	df	MS	F calculated	P-value	F table value (0.05%)
Between Groups	0.85	3	0.28	9.54	0.01	4.76
Within Groups	0.18	6	0.03			
Total	1.03	9				

Table 7: ANOVA for shoot length for the different varieties studied

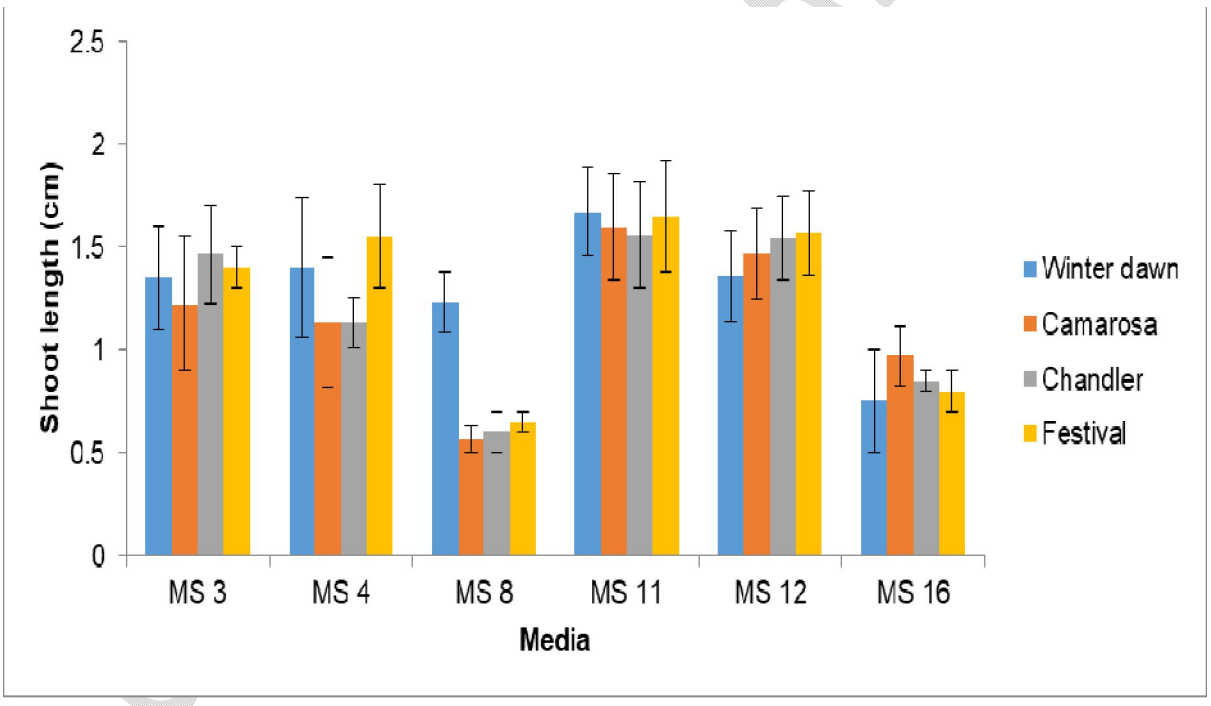

Figure 2: Mean values for shoot length for the different varieties studied. Error bars= ±S.E.

ANOVA for shoot length for the various concentrations and combinations of media studied showed that media supplemented with 4 mg/l BAP alone and in combination with 1mg/l IAA; 5 mg/l BAP alone and in combination with 1mg/l IAA were statistically at par with each other but not with media supplemented with 5 mg/l Kinetin alone and in combination with 1 mg/l IAA (Table 8).

Source of Variation	SS	df	MS	F calculated	P-value	F table value (0.05%)
Between Groups	7.73	5	1.55	6.15	5.94E-05	2.31
Within Groups	22.87	91	0.25			
Total	30.60	96				

Table 8: ANOVA for shoot length for the various concentrations and combinations of media studied

3.4. Root induction and hardening

Rooting was observed in half strength MS media supplemented with 0.5mg/l IBA while rooting was absent in half strength MS media without any growth regulators. Maximum root length (0.68 ± 0.19) was observed in variety Winter Dawn while minimum root length (0.36 ± 0.03) was observed in variety Camarosa. Maximum number of roots (1.13) was observed in variety Winter Dawn while minimum number of roots (1.0) was observed in variety Camarosa, Chandler and Festival (Table 9). ANOVA for root length showed no significant difference for the different varieties studied (Table10).

Variety/ Parameter	Root length	Root length	Number of roots	Number of roots
	Mean ± SE	Variance	Mean	Variance
Winter Dawn	0.68 ± 0.19	0.29	1.13	0.13
Camarosa	0.36 ± 0.03	0.01	1	0.00
Chandler	0.43 ± 0.04	0.01	1	0.00
Festival	0.44 ± 0.05	0.02	1	0.00

Table 9: Mean value root length and root number for the different varieties studied

Source of Variation	SS	df	MS	F calculated	P-value	F table value (0.05%)
Between Groups	0.44	3	0.15	1.66	0.20	2.98
Within Groups	2.31	26	0.09			
Total	2.75	29				

Table 10: ANOVA for root length for different varieties studied

The regenerated shoots were sub cultured in media containing half strength MS medium alone and in combination with 0.5mg/l IBA [Figure 3]. Highest percentage of rooting (100%) was observed in media supplemented with 0.5mg/l IBA while no rooting was observed in half strength MS basal medium. Similar results were reported also by [11]. Both 0.5 and 1.0 mg/l IBA concentration required the shortest time (8-10 days) for root induction and also produced the highest number of roots/culture and longest roots while no roots were produced in control treatment without IBA [12]. MS medium containing 0.5 mg/l IBA produced highest percentage of rooting (100%) while highest number of roots per microcutting, maximum root length of microcuttings and minimum number of days to root initiation was observed in medium containing 1mg/l IBA [13]. After successful rooting, the plantlets were transplanted into the potting mixture for hardening where the plantlets showed 66.67% survival.

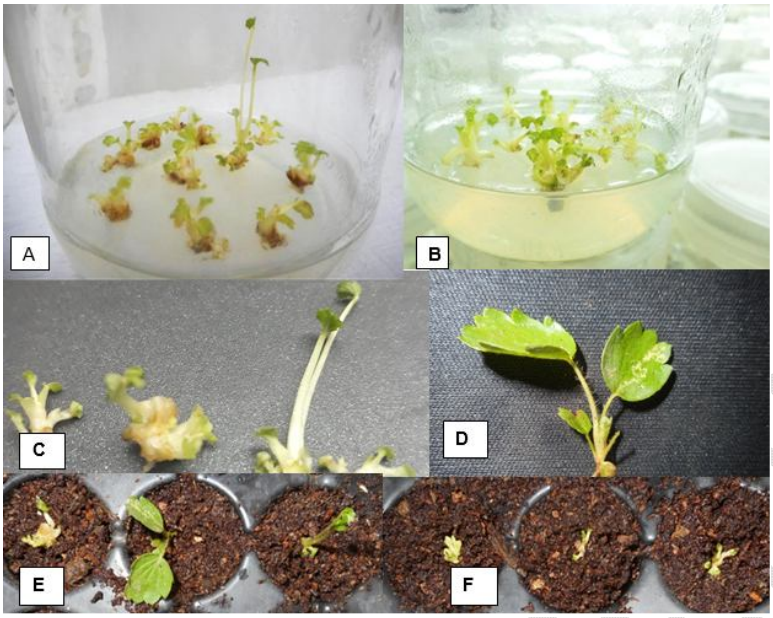

Figure 3: Rooting and hardening (A-B) Regenerated shoots in rooting media (C-D) Shoots showing rooting in *in vitro* regenerated seedlings (E-F) Hardening

4. CONCLUSION:

In vitro regeneration revealed that MS Medium supplemented with 5 mg/l BAP + 1 mg/l IAA produced the best results both in terms of maximum percentage survival of explants and number of shoots in all the varieties studied. The regenerated shoots sub cultured in media containing half strength MS medium supplemented with 0.5 mg/l IBA gave the highest percentage of rooting (100%) After successful rooting, the plantlets were transplanted into the potting mixture for hardening where the rooted plantlets showed 66.67% survival. Surface sterilization of explants with Tween 20, 0.1% Bavistin, 0.1% Ascorbic acid + 0.1% Streptomycin and 0.1% Mercuric chloride when used for 30, 45, 40 and 8 minutes respectively gave maximum response (83.33%). From *in vitro* studies, it was concluded that MS medium supplemented with Kinetin were inferior in performance for shoot development to MS medium supplemented with various concentrations of BAP when runners are used as explants for regeneration.

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