J Krishi Vigyan 2020, 9 (1) : 193-201 DOI : 10.5958/2349-4433.2020.00158.0

In vitro Protocol Standardization for Growth and Rooting in Strawberry

Harpreet Kaur[1]*, Jaismeen Kaur[2] and Bhagwant Singh Chahil[3]

Department of Horticulture, College of Agriculture
Guru Kashi University, Talwandi Sabo, Bathinda – 151302 (Punjab)

ABSTRACT

Strawberry is commercially propagated via runner multiplication but this method being laborious and hectic could not meet the demand of local fresh market, processing and export. Also, the health and quality of plant is degenerated and not up to mark by this method. Hence, the present study was conducted at Guru Kashi University, Talwandi Sabo, Bathinda during 2017-2018 with an aim to study *in vitro* explant response and regeneration capacity of strawberry cultivar sweet Charlie using different media composition through micro propagation. For explant, meristem gave superior and significantly better results than nodals. Also, best effect came due to the treatment DEM_1 with respect to regeneration percentage, shoot length and leaves. The interaction between the two factors concluded that it was better to use meristem as explants and DEM_1 as media composition for better regeneration potential and vegetative growth. For rooting, REM_1 media composition showed best rooting response with minimum days taken (6) and highest rooting percentage (80%).

Key Words- *Fragaria***,** Micro propagation, Meristem, Nodes, Runners,Strawberry.

INTRODUCTION

Strawberry (*Fragaria X ananasa*) is a major berry fruit crop widely consumed throughout the world. It is a natural hybrid of *Fragaria chilonensis* LP Mill. and *Fragaria virginiana* Duch. India produced 5,000Mt strawberry during the year 2017-2018 and was cultivated on 1000 ha area (Anon, 2018) commercially in Maharashtra, Karnataka and Madhya Pradesh. Recently, strawberry cultivation in Northern India especially in Punjab, Haryana, Himachal Pradesh and parts of Uttar Pradesh is picking up fast due to availability of market in Delhi and other cities.

Strawberry is commercially propagated via runner multiplication but this method being laborious and hectic could not meet the demand of local fresh market, processing and export. Also the health and quality of plant is degenerated and not up to mark by this method. So micro propagation is the best tool to overcome all these barriers in strawberry growing to meet the demand of plant material in shortest possible time, supply of disease free healthy plant stock and best way to overcome the loss by soil causing fungi and viruses. Also, *in vitro* raised plants produce more number of runners per plant as compared to commercial method. A lot of research work has been done in the crop with variation in growth regulators, explant sources. In the present study, an attempt was made to develop and standardize a protocol for strawberry cultivar with an aim to identify the most suitable explant and best basal media composition for successful *in vitro* propagation

MATERIALS AND METHODS

The present investigation was conducted during 2017-2018 at Biotechnology laboratory, Department of Agriculture, Talwandi Sabo, Bathinda, Punjab, India. The Strawberry cultivar Sweet Charlie, obtained from the horticulture experimental field of University campus, was used as experimental material. Fresh Nodes and meristem tips were

Corresponding Author's Email: reet1221.me@gmail.com

used as explant. The roots and leaf sheaths of the meristem were removed with the help of a sharp knife. The meristem-tip explants were prepared by removing extraneous corm tissue from crown (Manchanda *et al*, 2012). The experiment was performed by following the completely randomized design (CRD) with 3 replications.

Preparation of explant

Young tender vegetative nodes of 4-6 cm and meristem tips (Fig. 1) were excised from the mother plant and immediately putted in the jar filled with distilled water so as to ensure the least contamination by direct contact of environment and sap of excised plant. These explants were first washed under running tap water for 20 min and then treated with labolene for 4-5 min. followed by repeated rinsing with sterile distilled water to remove the detergent. After washing with double distilled water properly the explants were subjected to chemical sterilization under the laminar air flow and were surface sterilized for 30min.with 0.1% Bavistin, followed by 20 min dip in 30% sodium hypochlorite solution. After this they were again sterilized by 0.1% mercuric chloride for 5-10 sec. After complete washing with sterile water, explants were trimmed to final size of 0.1-0.5 cm.

Inoculation

Small nodes and excised meristem tissues were cultured on MS media supplemented with various concentration of different growth regulators (IAA, IBA, Kinetin, BA, NAA, 2,4-D, GA_3) in combination (Table 1). Ensure the pH of the media 5.7 before autoclaving and if not, adjust it by using 1 N NaOH or HCl accordingly. All the equipment to be used in inoculation and media were autoclaved at 121˚C and 15 psi for 30 min. The cultures were incubated under fluorescent white light and dark cycle of 16hr/8hr at 25±2°C. Data was recorded at every 7, 14 and 21 days intervals for the particular measures. For recording percent regeneration of explants and rooting percent the formula used are given below:-

$$\text{Percent regeneration} = \frac{\text{No. of explants regenerated}}{\text{Total no. of explants survived}} \times 100$$

$$\text{Percent rooting} = \frac{\text{No. of shoots rooted}}{\text{Total no. of shoots cultured on rooting media}} \times 100$$

Number of days taken for shoot initiation was calculated from the day of inoculation to the day explant showed shoot initiation at 7, 14 and 21d after inoculation. For calculating number of shoot and leaf, average number of shoots and leaves of all cultured explants were calculated at given days of interval. The regenerated explants were sub-cultured for 3-4 times on same media after interval of 20-25d for their multiplication. Number of days taken for root induction was calculated from inoculation of regenerated plant on rooting media up to appearance of first root (Table 2). The data was analyzed using the software OPSTAT developed by CCSHAU, Hissar and Critical difference (CD) values at 5% level of significance were used for checking the significance of effect of different factors on different parameters.

Factors	Stands for
A	Explants (nodal segnments and meristems)
B	Hormonal composition

RESULTS AND DISCUSSION

Percent regeneration

At 7th day, no significant difference was found between Meristem (10.83%) and Nodal segment (10.51%). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (30.46%) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest regeneration was obtained by the treatment DEM_1 from nodal segment (31.21%) which was at par with DEM_1 from meristem (29.72%) but significantly higher than other treatment combinations (Table 3).

At 14 days, there was no significant difference between percent regeneration from Meristem (28.53%) and Nodal segment (26.59%). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (54.17%) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest regeneration was obtained by the treatment DEM_1 from meristem (56.15%). However, there was no significant difference between all the treatment combinations (Table 4).

At 21 days, the per cent regeneration from meristem (55.05%) was found to be significantly higher than nodal segment (50.16%). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (93.30%) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest regeneration was obtained by the treatment DEM_1 from meristem (94.28%).However, there was no significant difference between all the treatment combinations (Table 5).

It was observed that the response in terms of regeneration has significantly varied in both explants as well as in different concentrations. On the each day of recorded observation meristem as explant and DEM_1 as basal composition showed superior results as compared to nodals and other basal compositions. Overall it was observed that meristems performed well on DEM_1. All the basal media supplemented with BA performed superior as compared to the media supplemented with KIN. Similar results have also been reported in *Fragaria indica* (Bhatt and Dhar, 2000), Papaya (Cononer and Litz, 1978). Khan and Spoor (2004) reported that standardized the protocol with MS Media supplemented with 2.5gm/L BA. However, these results contradicted those of Anuradha (2013) who recorded highest percent regeneration on 21st day on basal media supplemented with 2.5 mg KIN in strawberry cv. Ofra.

Shoot length

At 7 days, the average shoot length from Meristem (0.66 cm) was found to be significantly higher than from nodal segment (0.61 cm). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (1.45 cm) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest average shoot length was obtained by the treatment DEM_1 from meristem (1.54 cm). However, there was no significant difference between all the treatment combinations (Table 6).

At 14 days, the average shoot length from Meristem (1.91 cm) was found to be significantly higher than from nodal segment (1.63 cm). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (3.00 cm) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest average shoot length was obtained by the treatment DEM_1 from meristem (3.14 cm) which

Table 1. Direct establishment media (DEM) supplemented with various growth regulators for direct shoot regeneration.

Media	Hormonal composition (mg/l)
DEM_0	MS Media (control, no growth regulators)
DEM_1	MS Media + BA (2) + GA (0.1) + IBA (0.1) + Charcoal (0.5 gm/l)
DEM_2	MS Media + BA (1) + GA (0.1) + IBA (0.1) + Charcoal (0.5 gm/l)
DEM_3	MS Media + BA (1.5) + GA (0.1) + IBA (0.1) + Charcoal (0.5 gm/l)
DEM_4	MS Media + KIN (1) + GA (0.1) + IBA (0.1) + Charcoal (0.5 gm/l)
DEM_5	MS Media + KIN (2) + GA (0.1) + IBA (0.1) + Charcoal (0.5 gm/l)
DEM_6	MS Media + BA (1.5) + GA (0.1) + IBA (0.1)

Table 2. Rooting media (RM) and its concentration (mg/l) .

Media	Media concentration (mg/L)
RM_0	Half strength MS media, control
RM_1	Half strength MS media + IBA (1.0) + NAA (1.0)]
RM_2	Half strength MS media + IBA (1.5) + NAA (1.5)

was found to be at par with DEM_1 from nodal segment (2.86 cm) but significantly higher than other treatment combinations (Table 7).

At 21 days, the average shoot length from Meristem (2.60 cm) was found to be significantly higher than from nodal segment (2.18 cm). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (4.02 cm) which was significantly higher than other treatments. Amongst the combination of both the factors, the highest average shoot length was obtained by the treatment DEM_1 from meristem (4.43 cm) which was significantly higher than other treatment combinations (Table 8).

As evident, DEM_0 was not responsive and does not cause any regenerated affect or shoot multiplication induction in both the explants. However the meristems on DEM_1 gave significantly higher responses and proved to be a better combination for shoot multiplication. It might be due to suitable amount of cytokinin and auxin which played an important role for early shoot initiation. The present results were not in agreement with Anuradha (2013) and Khaldoun (2015) as they obtained better shoot growth and multiplication on media supplemented with KIN then BA in strawberry and cucumber respectively.

No. of leaves

At 7 days, the no. of leaves from Meristem (1.04) was found to be significantly higher than from Nodal segment (0.88). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (2.15) which were significantly higher than other treatments. Amongst the combination of both the factors, the highest no of leaves were obtained by the treatment DEM_1 from meristem (1.36) which was significantly higher than other treatment combinations (Table 9).

At 14 days, the no. of leaves from Meristem (2.16) was found to be significantly higher than from Nodal segment (1.61). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (3.02) which were significantly higher than other treatments. Amongst the

Table 3. Effect of different concentrations of growth regulators on percent regeneration from meristem and nodal segments of strawberry cultivar sweet Charlie at 7 days.

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	Mean Explants
Meristem	0	29.72	16.44	10.36	8.99	6.24	4.03	10.83
Nodal segment	0	31.21	15.19	12.57	6.99	4.57	3.02	10.51
Mean Hormonal composition	0	30.46	15.81	11.47	7.99	5.41	3.53	
C.D. (Factor A) = N.S.		C.D. (Factor B) =1.193			C.D. (Factor A x B) =1.687			

Table 4. Effect of different concentrations of growth regulators on percent regeneration from meristem and nodal segments of strawberry cultivar Sweet Charlie at 14 days

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	Mean Explants
Meristem	2.50	56.15	48.20	36.42	29.83	16.25	10.39	28.53
Nodal segment	0	52.20	45.64	38.02	26.30	16.74	7.20	26.59
Mean Hormonal composition	1.25	54.17	46.92	37.22	28.07	16.50	8.79	
C.D. (Factor A) =N.S		C.D. (Factor B) = 3.713			C.D. (Factor A x B) = N.S.			

Table 5. Effect of different concentrations of growth regulators on percent regeneration from meristem and nodal segments of strawberry cultivar Sweet Charlie at 21 days.

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	Mean Explants
Meristem	9.12	94.28	85.27	76.89	62.86	35.67	21.26	55.05
Nodal segment	0	92.32	81.37	73.13	58.31	31.37	14.61	50.16
Mean Hormonal composition	4.56	93.30	83.32	75.01	60.59	33.52	17.94	
C.D. (Factor A) =3.484		C.D. (Factor B) = 6.518			C.D. (Factor A x B) = N.S.			

Table 6: Effect of different concentrations and combination of growth regulators on average shoots length (cm.) from nodal segments & meristem portion explants of strawberry at 7 days.

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	Mean Explants
Meristem	0	1.54	1.17	0.96	0.45	0.37	0.11	0.66
Nodal segment	0	1.36	1.20	0.98	0.39	0.27	0.05	0.61
Mean Hormonal composition	0	1.45	1.18	0.97	0.42	0.32	0.08	
C.D. (Factor A) = 0.037		C.D. (Factor B) = 0.069			C.D. (Factor A x B) =N.S.			

Table 7. Effect of different concentrations and combination of growth regulators on average shoots length (cm.) from nodal segments & meristem portion explants of strawberry at 14 days.

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	Mean Explants
Meristem	2.55	3.14	2.46	1.87	1.24	1.17	0.96	1.91
Nodal segment	0	2.86	2.59	1.98	1.51	1.48	0.96	1.63
Mean Hormonal composition	1.28	3.00	2.53	1.93	1.38	1.323	0.96	
C.D. (Factor A) = 0.125		C.D. (Factor B) = 0.234			C.D. (Factor A x B) = 0.331			

Table 8. Effect of different concentrations and combination of growth regulators on average shoots length (cm.) from nodal segments & meristem portion explants of strawberry at 21 days.

Explants\Hormonal composition	DEM_0	DEM_1	DEM_2	DEM_3	DEM_4	DEM_5	DEM_6	**Mean Explants**
Meristem	2.37	4.43	3.70	2.63	2.05	2.00	1.03	2.60
Nodal segment	0	3.61	3.42	2.49	2.64	2.07	1.00	2.18
Mean Hormonal composition	1.19	4.02	3.56	2.56	2.34	2.03	1.02	
C.D. (Factor A) = 0.138		C.D. (Factor B) = 0.258			C.D. (Factor A x B) = 0.365			

Table 9. Effect of different concentrations and combination of growth regulators on number of leaves from meristem portion and nodal segments of strawberry at 7days.

Explants\Hormonal composition	**DEM_0**	**DEM_1**	**DEM_2**	**DEM_3**	**DEM_4**	**DEM_5**	**DEM_6**	**Mean Explants**
Meristem	0	2.32	1.67	0.65	1.09	0.98	0.56	1.04
Nodal segment	0	1.98	1.94	0.76	0.95	0.34	0.17	0.88
Mean Hormonal composition	0	2.15	1.81	0.71	1.02	0.66	0.37	
C.D. (Factor A) = 0.083		C.D. (Factor B) = 0.155			C.D. (Factor A x B) = 0.22			

Table 10. Effect of different concentrations and combination of growth regulators on number of leaves from meristem portion and nodal segments of strawberry at 14 days.

Explants\Hormonal composition	**DEM_0**	**DEM_1**	**DEM_2**	**DEM_3**	**DEM_4**	**DEM_5**	**DEM_6**	**Mean Explants**
Meristem	0.86	3.17	2.98	1.76	2.65	2.78	0.94	2.16
Nodal segment	0	2.87	2.67	1.65	1.87	1.34	0.87	1.61
Mean Hormonal composition	0.43	3.02	2.83	1.71	2.26	2.06	0.91	
C.D. (Factor A) = 0.096		C.D. (Factor B) = 0.18			C.D. (Factor A x B) = 0.254			

Table 11. Effect of different concentrations and combination of growth regulators on number of leaves from meristem portion and nodal segments of strawberry at 21 days.

Explants\Hormonal composition	**DEM_0**	**DEM_1**	**DEM_2**	**DEM_3**	**DEM_4**	**DEM_5**	**DEM_6**	**Mean Explants**
Meristem	1.23	5.52	3.94	2.58	3.89	2.96	1.17	3.04
Nodal segment	0	5.98	3.65	2.47	2.89	2.33	1.07	2.63
Mean Hormonal composition	0.62	5.75	3.79	2.52	3.39	2.65	1.12	
C.D. (Factor A) = 0.184		C.D. (Factor B) = 0.343			C.D. (Factor A x B) = 0.486			

Table 12. Effect of different concentrations of auxins on root development from the direct regenerated shoots of strawberry.

Media concentration (mg/L)	Days taken for root initiation	Root length (cm)	Number of roots	Rooting percentage (%)
RM_0 (Half strength MS media, control)	13	2.54	3.55	60
RM_1 [(Half strength MS media + IBA (1.0) + NAA (1.0)]	6	3.98	4.76	80
RM_2 [(Half strength MS media + IBA (1.5) + NAA (1.5)]	8	3.21	4.25	76
C.D. (@5%) 1.591		0.559 0.727 12.132		

combination of both the factors, the highest no. of leaves were obtained by the treatment DEM_1 from meristem (3.17) which was at par with DEM_2 from meristem(2.98) but significantly higher than other treatment combinations (Table 10).

At 21 days, the no. of leaves from Meristem (3.04) was found to be significantly higher than from Nodal segment (2.63). Amongst the hormonal composition, the highest value was obtained by the treatment DEM_1 (5.75) which were significantly higher than other treatments. Amongst the combination of both the factors, the highest number of leaves were obtained by the treatment DEM_1 from nodal segment (5.98) which was at par with DEM_1 from meristem(5.52) but significantly higher than other treatment combinations (Table 11).

Explants inoculated on BA based medium exhibited enhanced and earlier response as compared to KIN based medium. However encouraging results were obtained when meristem was used as an explant on basal medium DEM_1 for obtaining maximum number of leaves. The results were in accordance with Negi *et al* (2008) who observed that MS media supplemented with kinetin (0.5 mg/l) and BAP (0.5 mg/l) was best for survival and earliest days to shoot initiation in strawberry. It was found that BA along with GA provided synergistic effect.

Root development

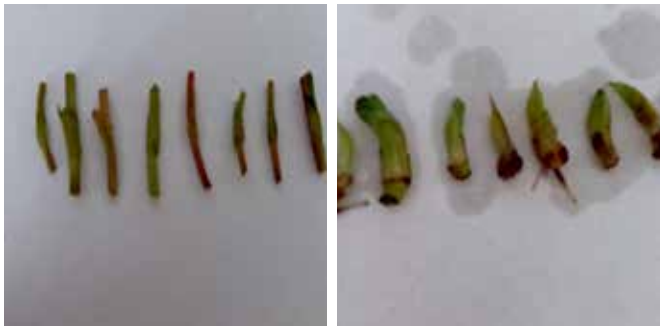
In cultivar Sweet Charlie, highest rooting percentage (80%) with highest root length (3.98 cm) and minimum days taken for root initiation i.e. 6, was found on RM_1 (Table 12) which was significantly better than the values obtained by the treatments RM_2 and RM_0. The No. of roots were also found to be highest in treatment RM_1 (4.76) which were at par with RM_2 but significantly higher than RM_0. All the four parameters followed the trend in values $RM_1>RM_2>RM_0$, in case of Rooting percentage, Number of roots and Root length (Table 12). It was observed that IBA responded much better as compared to any other auxin derivative used for root induction. Mereti *et al* (2002), Kaur *et al* (2005) and Sakila *et al* (2007) also succeeded in inducing roots in strawberry with IBA.

After successful *in vitro* rooting the established plantlets were carefully taken out from the culture tube and thoroughly washed with running tap water and dipped in Bavistin 0.1 % for 10 min (Fig. 3.a and 3.b). For hardening they were transferred to pot containing sterile media of coco-peat and sand in equal ratio (1:1) , a week after a new leaf in pots were noticed (Fig. 3c).

CONCLUSION

It can be concluded that for *in-vitro* regeneration of Strawberry cultivar Sweet Charlie gave best results

on treatment combination DEM_1 with meristem used as explant for percent Regeneration ,Shoot growth and Vegetative growth which is indicated by the values of Regeneration potential (%), Average shoot length (cm) and number of leaves. Regarding the effect of different concentrations of auxins on root development from the direct regenerated shoots, RM_1 gave the best results as evident from the values obtained for various parameters *viz.,* days taken for root initiation, rooting percentage, root length and number of roots. Nodals used as explant gave very constant and uniform growth and also the mortality rate of nodes were less as compared to the meristem. Despite having comparatively higher mortality rate, the meristem showed very speedy growth once they were established on the medium. Hence, this is a new finding of this investigation. Also unlike all other strawberry cultivars propagated under in vitro conditions cultivar Sweet Charlie showed different morphology with dull pink to red coloured stems obtained unlike green in all other cultivars.



(a) (b)

Fig.1 Morphology of explants used foe experiment (a) nodes (b) meristem

(a) (b) (c)

Fig.2 (a) meristem on DEM_1 on day 14th (b) meristem on $DEMI_1$ on day 21st day (c) nodals on DEM_1

Fig.3 (a and b) root length and number of roots on sweet Charlie cultivar of strawberry on RM_1 (c) hardening.

REFERENCES

Anonymous (2018). *Horticulture Statistics at a Glance*. Government of India, Ministry of Agriculture and Farmers welfare, Department of Agriculture, Cooperation and Farmer's welfare, Horticulture statistics division. Pp: 38-388.

Bhatt I D and Dhar U (2000). Micropropagation of Indian wild strawberry. *Plant Cell Tissue and Org Cult* **60**: 83-88.

Cononer R A and Litz R E (1978). In vitro propagation of papaya. *Hort Sci* **13** (3) : 241-242.

Hu C Y and Wang P J (1983). Meristem, shoot tip and bud culture. In: Handbook of Plant Tissue Culture. Vol. I. (D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada) pp.177-227, Macmillan, New York.

Kaur R, Gautam H and Sharma D R (2005). A low cost strategy for micro propagation of strawberry (*Fragaria × ananassa* Duch.) cultivar Chandler. *Acta Hortic* **696**: 129-133.

Khan S and Spoor W (2004). A study of an in vitro callus culture and regeneration system from leaf disc explants in strawberry (*Fragaria X ananassa*) cultivar Tango. *Int JBiol and Biotechnol***1** (3) : 423-428.

Manchanda P, Kaur A and Singh S G (2012). Micropropagation of banana (*Musa acuminata*) through proliferation of axillary shoots. *Indian J Agric Sci* **82**(5) : 451-454.

Mereti M, Grigoriadou K and Nanos G D (2002). Micropropagation of the strawberry tree (*Arbutus unedo* L.). *Sci hortic* **93** (2) : 143-148.

Rattanpal H S, Gill M I S and Sangwan A K (2011). Micropropagation of Strawberry through Meristem Culture. *Acta hortic* **890**: 149-153.

USDA (2003). Economics, Statistics and Marketing information.

Received on 01/07/2020 *Accepted on 05/11/2020*