Plant Archives Vol. 25, Supplement 1, 2025 pp. 3091-3098 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.422

OPTIMIZING *IN VITRO* PROPAGATION PROTOCOL AND ENSURING GENETIC FIDELITY OF STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.) CULTIVAR 'CHANDLER' THROUGH ISSR MARKER ANALYSIS

Vibhu Pandey[1*], Arvind Kumar[1], Khushboo Sharma[2], Deepesh Keshari[3], Amit Kumar[1] and Anuj Pal[1]
[1]College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, India, 250110
[2]Faculty of Horticulture, Sher-e-Kasmir University of Agriculture Sciences & Technology, Srinagar, India, 190025
[3]College of Agriculture, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttrakhand, India, 263145
*Corresponding author Email: pandey.vibhu007@gmail.com
(Date of Receiving : 07-11-2024; Date of Acceptance : 10-01-2025)

ABSTRACT

The cultivated strawberry, an octaploid hybrid, holds significant commercial value globally owing to its aroma and color. The research aimed to develop an efficient *in vitro* protocol for propagation and assess the genetic integrity of tissue culture-derived plantlets using molecular markers. Results showed that treatment T_5 (3.0 mg/l 2,4-D + 2.0 mg/l BAP) was the most effective for callus induction, exhibiting the shortest duration and highest callus formation percentage. For shoot initiation and regeneration, treatment T_5 (SNP 1.0 mg/l + BAP 3.0 mg/l + NAA 0.5 mg/l) demonstrated superior performance, producing the highest shoot number, length and regeneration percentage. Root induction was most efficient with treatment T_4 (NAA 2.0 mg/l + BAP 0.2 mg/l), showing the shortest time for root initiation and highest root development percentage. Genetic fidelity was evaluated using ISSR markers, with 12 primers initially screened and 6 ultimately selected for analysis. Results revealed a high degree of similarity (91.66%) between *in vitro* raised plants and mother plants, indicating genetic uniformity. Somaclonal variation was minimal, with ISSR markers proving effective in detecting any discrepancies. The study underscores the significance of *in vitro* propagation for commercial cultivation of strawberries with implications for crop improvement programs and global competitiveness. This protocol, coupled with molecular marker analysis, offers a robust approach to ensure clonal fidelity and genetic stability in micro-propagated plants, supporting advancements in strawberry cultivation and germplasm preservation.

***Keyword*:** Strawberry**,** *In vitro,* Clonal fidelity, Germplasm preservation, ISSR primers

Introduction

The cultivated strawberry (*Fragaria x ananassa* Duch.) a hybrid between the 'scarlet' or 'virginia' strawberry (*Fragaria virginiana* Duch.) and grown in most agricultural regions of the world, the pistillate South American strawberry (*Fragaria chiloensis* L. Duch.) is a perennial low growing herb that is dicotyledonous (Debnath, 2013). It belongs to subfamily Rosoideae and family Rosaceae. It holds a special place among the fruits that are cultivated. The fruit is highly valued for its distinct aroma and bright red colour (Lal and Sharma, 2003). There are at least 15 species in this genus with two wild species, *F. vesca* and *F. moschata*, being commercially farmed on a small basis. Despite its recent origins and limited geographic distribution of progenitors, breeders have evolved new cultivars that can withstand a wide variety of climatic conditions (Hancock, 1991). This esteemed fruit is primarily consumed fresh and is cherished by millions across various climates, in temperate, Mediterranean, sub-tropical and taiga regions. In India, the cultivation of strawberries spans across regions such as Maharashtra, Himachal Pradesh, Jammu and Kashmir, Haryana, Rajasthan, Delhi, West Bengal, Punjab, and the Nilgiri hills, with Maharashtra's Panchgani-Mahabaleshwar belt leading the production

(NHB, 2021-22). The micro-propagation technology was standardised for the first fruit crop, possibly the strawberry (Sharma and Singh, 1999).

Growth regulators, including auxins and cytokinins, are used in the *in vitro* production of plants. Cytokinins are recognised to be important for the process of organogenesis and shoot multiplication. According to Haddadi *et al.* (2010), the cytokinin that is mostly used for strawberry shoot growth is N6-benzylamino-purine (BAP). The type of plant growth regulator used determines the success of callus culture. In tissue culture, it is known that auxins and cytokinins stimulate the production of callus. According to Biswas *et al.* (2007), auxin has a number of effects on plant development and morphogenesis. The most effective combination for inducing and multiplying shoots was discovered to be kinetin, BA, and NAA (Karim *et al.,* 2015). Compared to plants grown by the conventional approach, *in vitro* plants are more uniform, generate more runners, have greater field survival, and have a 24% increase in fruit output (Kikas *et al.,* 2006). Using many molecular markers, including RAPD and ISSR markers, the current study will determine the genetic integrity and homogeneity of the tissue culture-derived plantlets in strawberries. In the proposed study, an efficient *in vitro* protocol for callusing and regeneration/micro-propagation and the genetic fidelity analysis will be performed in micro-propagated plantlets, if any in, strawberry using ISSR markers.

Materials and Methods

Plant material

Selected disease and insect free branches that were 2-3 months old, healthy nodes were removed from them and these nodes were immediately immersed in a vitamin solution for 20 minutes to obtain the proper explant. For 15 minutes, explants were cleaned of field dust using running water. After a 10-minute soak in the 0.1% teepol solution, all of the explants were rinsed with tap water. Following a 20-minute Bavistin (0.1%) treatment, they were rinsed for 10 minutes under running water.

Culture conditions

All of the explants were collected and prepped as previously stated in a laminar air flow cabinet. For surface sterilization, the explants were then exposed to (Mercuric Chloride 0.1%) for 1 to 3 minutes. Explants were surface sterilized, then rinsed three times in distilled water before being inoculated. All the cultures were kept in the culture trollies of the culture room, which was kept at a temperature of 25±2°C with a daily light cycle of 16 hours of artificial white fluorescent light and 8 hours of darkness, with an intensity of 3000 lux at the plant level. The *in vitro* cultures were kept in a culture chamber throughout the duration of their establishment, multiplication and rooted.

Genomic DNA Isolation for molecular analysis

DNA was isolated using a modified version of Doyle and Doyle's (1990) CTAB (Cetyl-Trimethyl-Ammonium Bromide) technique. The specific chemicals used for PCR i.e., Taq. Polymerase, Taq Buffer, dNTPs and $MgCl_2$ were obtained from Bangalore Gei Nei. The primers used for molecular profiling were synthesized by Macflow Engineering Pvt. Ltd.

Statistical analysis

Analysis of variance (ANOVA), mean separation, and statistical analysis of the data were performed using Duncan's multiple range test. The significance threshold was determined to be ($p < 0.01$).

Results and Discussion

Optimization of *in vitro* protocol of Strawberry

The nodal segments of *Fragaria ananasa* cv. Chandler were subjected to various combinations of 2,4-D and BAP, resulting in differential effects on callus induction presented in table 1. Notably, treatment T_5 (3.0 mg/l 2,4-D + 2.0 mg/l BAP) exhibited the shortest duration for callus induction (16.77±0.56 days), followed by treatment T_4 (20.52±1.08 days). Conversely, the control treatment (T_1) showed the longest time for callus induction (41.98±1.24 days). In their study, Ara *et al.* (2012), different explant types and phytohormone combinations induced varying degrees of callus formation in strawberries. Interestingly, when grown on MS media supplemented with 1.0 mg/l of 2,4-D and 0.1 mg/l of BAP, the leaf disc explant showed an notably high frequency of callus induction. On the other hand, Howlader *et al.* (2016) found that the most efficient callus initiation occurred with a combination of 3.0 mg 2,4-D and 0.50 mg BAP/L, leading to callus formation in just 17.73 days. Callus formation percentage was highest in treatment T_5 (86.67±5.77%), followed by T_4 (66.67±5.77%), with minimal response in the control (20.00±10.00%). Ramdan *et al.,* (2013) observed high callus induction rates (100% and 83%) in citrus using combinations of 2,4-D at 1 mg/l + BAP at 0.5 mg/l and 2,4-D at 2 mg/l + BAP at 1 mg/l, respectively. Bakar *et al.* (2014) confirmed that the number of phytohormones utilized significantly influenced callus formation in *C. argentea*. Furthermore, callus weight and surface area were

significantly influenced by the PGR combinations showed in table 1. Treatment T_5 resulted in the highest callus weight (1570.73±73.11 mg) and surface area (1504.65±22.93 mm^2), followed by T_4, while the control showed minimal growth with the lowest values recorded. The optimization of appropriate doses and concentrations of growth hormones is vital for successful callus induction, indicating the necessity of both hormones in the explants. The levels of endogenous plant hormones in different plant parts may influence this process, as highlighted by Howlader *et al.* (2016), who achieved the most efficient callus weight of 1990.55 mg with 3.0 mg 2,4-D and 0.50 mg BAP/L and reported that the most efficient callus surface area (17.50 mm) occurred with a combination of 2.0 mg 2,4-D and 0.50 mg BAP/L

The use of MS media containing various combinations of SNP, BAP and NAA affected shoot initiation, shoot number, shoot length, number of leaves per explant and shoot regeneration percentage. Treatment T_5 (SNP 1.0 mg/l + BAP 3.0 mg/l + NAA 0.5 mg/l) showed the shortest duration for shoot initiation (19.57±1.25 days) and the highest values for shoot number, length, number of leaves per explant and shoot regeneration percentage. Conversely, the control treatment (T_1) exhibited minimal responses in all parameters. The observed minimum number of days required for shoot initiation with the combination of SNP, BAP and NAA underscores the importance of growth hormone optimization in tissue culture protocols. Similar findings have been reported in other plant species, such as Mexican lime (Jafari and Shahsavar, 2022), where SNP enhanced the positive effect of cytokinin on shoot initiation. The significant role of BAP in promoting shoot multiplication, especially when combined with SNP, is consistent with previous studies (Han *et al.,* 2009). The results align with research in other plant species like *Vanilla planifolia* (Tan *et al.,* 2013) and *Punica granatum* L. (Soni and Kanwar, 2016), highlighting the influence of growth hormone combinations on shoot length and multiplication. Furthermore, the observed increase in the number of leaves per explant with the supplementation of BAP and IBA corroborates findings in other plants like *Fragaria ananasa* Duch. (Daniel *et al.,* 2016). Conversely, the absence of growth hormones resulted in reduced shoot length, emphasizing the essential role of PGRs in regulating plant growth and development in *in vitro* cultures, as seen in studies on Stevia (Pradhan *et al.,* 2020) and cherry (Sarropoulou *et al.,* 2014). The high shoot regeneration percentage observed in present study is attributed to the action of SNP as a nitric oxide donor, enhancing micro-propagation, as reported in Blackberry (Lee *et al.,* 2018). The findings are consistent with previous studies in Strawberry (Sakila *et al.,* 2007) and Jamun (Naaz *et al.,* 2014), highlighting the importance of media composition, particularly MS medium with full salt strength, in promoting morphogenesis and shoot regeneration in *Fragaria ananasa* Duch.

Root induction on MS media containing various combinations of NAA and BAP resulted in table 4.3 showed differential effects on root initiation, root development percentage, number of roots per explant and root length. Treatment T_4 (NAA 2.0 mg/l + BAP 0.2 mg/l) exhibited the shortest time for root initiation (11.39±0.48 days) and the highest root development percentage (93.33±5.77%). Additionally, T_5 showed the highest number of roots per explant and the longest root length, while the control treatment (T_1) displayed minimal responses across all parameters. The observed shortest time for root induction with the combination of NAA and BAP aligns with the role of auxin (NAA) and BAP in meristematic tissue development and root elongation, respectively. These findings are supported by previous studies reporting similar observations in various plant species (Howlader *et al.,* 2016; Kurmi *et al.,* 2011). The highest percentage of root development observed in media optimized with specific combinations of PGRs highlights the importance of PGR concentration and composition in influencing root formation. Similar findings have been reported in pomegranates (Moradi *et al.,* 2011; Deepika and Kanwar, 2010), indicating a correlation between auxin levels and the position of the nodal segment on the shoot. Furthermore, the combination of NAA with BAP resulted in the highest number of roots per explant, consistent with previous studies in *Rhinacanthus nasutus* (L.) (Cheruvathur *et al.,* 2012). This suggests a synergistic effect of NAA and BAP in promoting root proliferation in *in vitro* cultures. The observation of maximum root length achieved with the combination of NAA and BAP is supported by studies in strawberries (Adak *et al.,* 2016) and pomegranates (Vala *et al.,* 2020). The optimization of the *in vitro* regeneration system in strawberries, as reported by Adak *et al.* (2016), further emphasizes the importance of PGR concentration and medium composition in achieving desirable root characteristics.

Genetic fidelity assessment of *in vitro* raised plants of strawberry (*Fragaria ananasa*) cv. Chandler by using ISSR markers

In this study, we used the ISSR primers (Inter Simple Sequence Repeats) assay to amplify the DNA of both mother and *in-vitro* raised strawberry (*Fragaria ananasa* Duch.) plants. Out of the 12 ISSR primers

screened, 6 primers (ISSR Primer-3, ISSR Primer-5, ISSR Primer-8, ISSR Primer-9, ISSR Primer-10, and ISSR Primer-12) successfully produced amplification. Data for each ISSR primer (Table 4) corresponding to the mother as well as *in vitro* plant were noteded as band present (scored as 1) and absent (scored as 0).

For the majority of the molecular primers examined, the banding pattern of the PCR amplified product from micro-propagated plantlets was found to be monomorphic. No polymorphism was found in the DNA profile of tissue-cultured plants, Even though their frequency was quite low and they could not be replicated, several weak bands were missing from some of the regenerates. The mother plant and 10 *in vitro* raised plantlets both had the same ISSR banding pattern. The majority of primers had DNA profiles that were identical to those of the mother plant. We found no somaclonal variation. With an average of 2 bands per primer, the 6 ISSR primers yielded 12 unique and scorable bands. Every primer produced a distinct set of amplification products with sizes ranging from 100 to 600 base pair. Different genetic and epigenetic pathways cause variation, which is likely reflected in the banding pattern created by using various marker systems. However, the reliability and efficiency of molecular markers in detecting large scale genome arrangements have been frequently questioned. ISSR markers are thought to be appropriate for detecting variation among tissue-cultured generated plants because they target the rapidly changing and highly variable DNA sequence. Remarkably, there was no variation seen in the tissue culture plantlets in this investigation according to the ISSR test. Bands with the same mobility were considered identical, regardless of their band intensity. This absence of diversity suggests that the plants grown *in vitro* and the mother plant shared the same genetic makeup. The plants grown *in vitro* did not differ morphologically from one another either. Rai *et al.* (2012) emphasised that genetic integrity is an essential need for plant regeneration. Molecular genetic approaches were used, as recommended by Tyagi *et al.* (2010), Wang *et al.* (2012) and Singh *et al.* (2013), to insure the genetic integrity of plants regenerated *in vitro*.

Using molecular analysis, determine the genetic homogeneity of the Fig micro-propagated plants. 109 resolvable, reproducible, and scorable bands with sizes ranging from 250 to 1550 bp were obtained using a combination of six RAPD primers and five inter-simple sequence repeat (ISSR) primers. Of these bands, six (5.5%) were polymorphic while the remaining 103 (94.5%) were monomorphic. Mother plants and micro-propagated plants exhibit a high genetic similarity, suggesting that the in-vitro propagation strategy for fig trees is reliable, and the low polymorphism ratio between them suggests that somaclonal variations have minimal influence. Dessoky *et al.* (2016) and Kaleybar *et al.* (2018) used the ISSR molecular marker to assess the genetic diversity in micro-propagated plants from various tissues (after 8 subcultures). Of the 18 pre-selected primers, 10 showed distinct, repeatable, and illuminating bands. In the molecular profile of several explants, 88 unique bands with a polymorphism rate of 46% were formed.

A similarity index of 0.738 for stipule explants and 0.645 for petiole explants indicated which explants had the highest and lowest resemblance values to the mother plant. It's crucial to remember that, according to other researchers (Chowdari *et al.,* 1998; Leroy *et al.,* 2001; Rahman and Rajora, 2001), ISSR markers are still thought to be appropriate for identifying differences across tissue culture plants.

The degree of genomic (DNA) content in the mother plant and the in-vitro-raised plantlets determined the clustering pattern that developed. Comparing the current study's similarity coefficient value to previous research on strawberries and bananas, which ranged from 0.6250 to 1.000, revealed that it was comparatively higher (Rout *et al.,* 2009; Gantait *et al.,* 2010). Shenoy and Vasil (1992) have noted that the variety in similarity and dissimilarity patterns might be impacted by the particular plant parts and genotypes chosen. According to earlier research on bananas, genetic and epigenetic pathways can cause changes that are reflected in the banding patterns created using various marker systems (Rout *et al.,* 2009).

The strong genetic resemblance between all in-vitro grown and mother plants is further supported by the PIC (Polymorphism Information Content) values, which range from 0.083 to 0.793. Because ISSR markers target rapidly developing and highly variable DNA sequences, they are thought to be appropriate for identifying variation across plants grown in tissue cultures (Negi *et al.,* 2010; Joshi and Dhawan, 2007). Long-term in-vitro clonal uniformity preservation is highly significant from a commercial standpoint, particularly when fruits are difficult to start in-vitro cultures (Sharo *et al.,* 2019).

A disadvantage of in-vitro cloning and germplasm preservation techniques may be somaclonal variation. Thus, it is crucial for ensuring the genetic homogeneity of plants grown *in vitro* from an early age. Finally, the current ISSR-based genetic study showed that the

strawberry plants grown *in vitro* shared the same genetic makeup as the parent plant. It was found that the ISSR markers worked well for both clonal fidelity and identifying differences between plants grown in tissue cultures. The preservation of germplasm and commercial uses are significantly impacted by this.

Conclusion

In vitro propagation of strawberry provides significant opportunities for commercial cultivation. For callus induction, treatment T_5 (3.0 mg/l 2,4-D + 2.0 mg/l BAP) emerged as the most effective, exhibiting the shortest duration for callus induction and the highest callus formation percentage. This treatment also resulted in the highest callus weight and surface area, indicating robust growth. Similarly, for shoot initiation and regeneration, treatment T_5 (SNP 1.0 mg/l + BAP 3.0 mg/l + NAA 0.5 mg/l) demonstrated superior performance, producing the highest shoot number, length, number of leaves per explant, and shoot regeneration percentage. Root induction was most efficient with treatment T_4 (NAA 2.0 mg/l + BAP 0.2 mg/l), which showed the shortest time for root initiation and the highest root development percentage. This treatment also yielded the highest number of roots per explant and the longest root length. Genetic fidelity was evaluated using ISSR markers, with 12 primers initially screened, and 6 ultimately selected for analysis. Results revealed a high degree of similarity (91.66%) between *in vitro* raised plants and mother plants, indicating genetic uniformity. Somaclonal variation was minimal, with ISSR markers proving effective in detecting any discrepancies. The study underscores the significance of *in vitro* propagation for commercial cultivation of strawberries with implications for crop improvement programs and global competitiveness. This protocol, coupled with molecular marker analysis, offers a robust approach to ensure clonal fidelity and genetic stability in micro-propagated plants, supporting advancements in strawberry cultivation and germplasm preservation.

Table 1: Effect of different concentrations and combinations of 2,4-D and BAP on Callusing of Strawberry (*Fragaria ananasa*) cv. Chandler

Treatment	Medium Composition MS (Basal medium) + Growth regulator		Day taken for callus induction	Callus development percentage	Weight of Callus (mg)	Surface area of callus (sqm)
	2,4-D mg/l	BAP mg/l				
T_1 (Control)	0	0	41.98±1.24e	20.00±10.00a	367.66±25.08a	76.86±3.57a
T_2	0.5	2	38.44±3.02de	33.33±5.77ab	474.11±13.09abc	134.96±9.79b
T_3	1	2	26.92±1.04c	43.33±5.77bcd	842.42±58.23e	667.03±24.91e
T_4	2	2	20.52±1.08ab	66.67±5.77ef	1200.52±30.96f	1075.59±31.4f
T_5	3	2	16.77±0.56a	86.67±5.77f	1570.73±73.11g	1504.65±22.93g

Table 2: Effect of different concentrations and combinations of SNP, BAP and NAA on Shooting of Strawberry (*Fragaria ananasa*) cv. Chandler

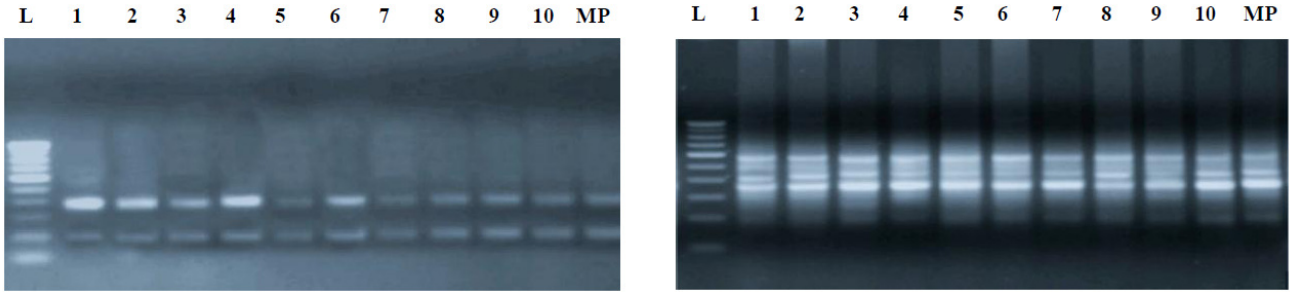
Treatment	Medium Composition MS (Basal medium) + Growth regulator			Day taken for shoot initiation	Number of shoots per explant	Length of shoot (cm)	Number of leaves per explant	Shoot regeneration percentage
	SNP mg/l	BAP mg/l	NAA mg/l					
T_1 (Control)	0	0	0	33.83±2.21e	0.30±0.04a	1.03±0.11a	0.19±0.02a	23.33±5.77a
T_2	1	0.5	0.5	29.73±1.86de	3.05±0.10b	3.54±0.19g	3.79±0.20b	30.00±10.00ab
T_3	1	1	0.5	25.33±1.76cd	5.26±0.16c	2.80±0.19ef	6.07±0.41cd	46.67±5.77bc
T_4	1	2	0.5	24.20±1.66bc	7.51±0.51e	2.02±0.27bcd	8.77±1.16ef	66.67±5.77de
T_5	1	3	0.5	19.57±1.25ab	7.84±0.41e	1.65±0.19b	9.20±1.04ef	70.00±10.00de

Table 3: Effect of different concentrations and combinations of NAA and BAP on Rooting of Strawberry (*Fragaria ananasa*) cv. Chandler

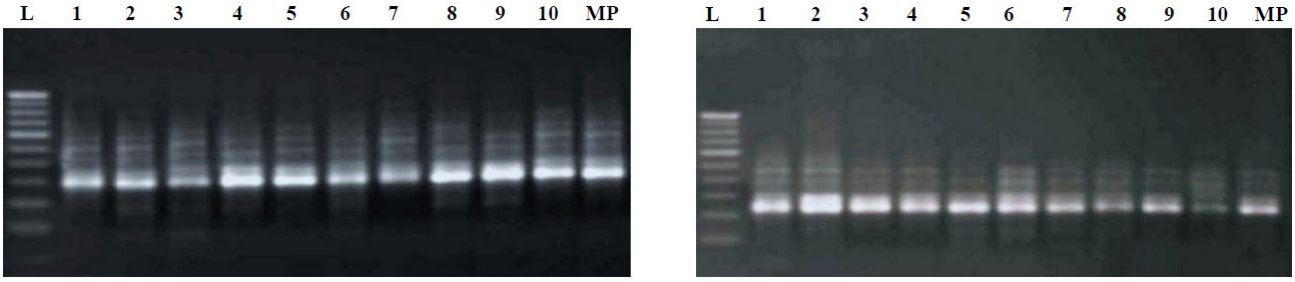
Treatment	Medium Composition MS (Basal medium) + Growth regulator		Day taken on in-vitro root development	Root development percentage	Number of root	Length of root (cm)
	NAA mg/l	BAP mg/l				
T_1 (Control)	0	0	23.24±2.43e	13.33±5.77a	1.97±0.24a	1.60±0.17a
T_2	0.5	0.2	18.28±0.72cd	36.67±5.77bc	6.21±0.28e	2.16±0.08ab
T_3	1	0.2	15.76±0.79bcd	63.33±5.77d	7.60±0.30f	1.76±0.09a
T_4	2	0.2	11.39±0.48a	93.33±5.77e	4.39±0.15c	3.29±0.14de
T_5	3	0.2	14.89±1.81abc	50.00±10.00bcd	3.51±0.23b	3.75±0.46e

Table 4: Details of amplification given by ISSR primer in *in-vitro* raised plant material of strawberry

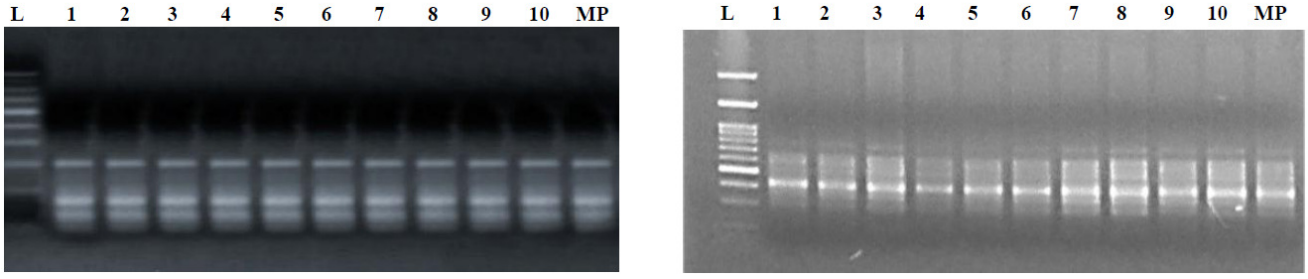
Sr. No.	Primer code	Nucleotide Sequence (5'-3')	Total number of amplified bands	Polymorphic bands	Monomorphic bands	Band Range
1.	**ISSR-3**	CACACACACACACACAT	2	0	2	200-400
3.	**ISSR-5**	GAGAGAGAGAGAGAGAT	3	0	3	200-400
4.	**ISSR-8**	AGAGTTGGTAGCTCTTGATC	1	0	1	320
5.	**ISSR-9**	CTCTCTCTCTCTCTCTAC	1	0	1	230
6.	**ISSR-10**	CTCTCTCTCTCTCTCTTTG	3	0	3	100-300
8.	**ISSR-12**	CACACACACACAGG	2	1	1	300-600
	Total		12	1	11	
	Average		2	8.34%	91.66%	



Gel image 1: ISSR pattern of mother plant (MP) and in vitro raised plants of Strawberry generated by Primer-3, Primer-5



Gel image 2: ISSR pattern of mother plant (MP) and in vitro raised plants of Strawberry generated by Primer-8, Primer-9



Gel image 3: ISSR pattern of mother plant (MP) and in vitro raised plants of Strawberry generated by Primer-10, Primer-12

Acknowledgement: We thank Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (UP, India) for providing laboratory, research field facilities and encouragement

References

Adak, N., Kaynak, L., & Adak, M. (2016). Optimization of *In Vitro* Regeneration System of Newly Developed Strawberry Hybrids. *International Journal of Agriculture Innovations and Research*, ***5***(3).

Ara, T., Karim, R., Karim, M. R., Islam, R., & Hossain, M. (2012). Callus induction and shoot regeneration in strawberry (*Fragaria x ananassa* Duch.). *International Journal of Biosciences*, ***2***(10), 93-100.

Bakar, D. A., Ahmed, B. A., & Taha, R. M. (2014). *In vitro* callus induction and plant regeneration of *Celosia argentea*-an important medicinal plant. *Brazilian Archives of Biology and Technology*, ***57***, 860-866.

Biswas, M. K; Hossain, M. and Islam, R. (2007). Virus free plantlets production of strawberry through meristem culture. *World J. Agric. Sci.* **3**(6):757-763.

Cheruvathur, M. K., Sivu, A. R., Pradeep, N. S., & Thomas, T. D. (2012). Shoot organogenesis from leaf callus and ISSR assessment for their identification of clonal fidelity in *Rhinacanthus nasutus* (L.) Kurz., a potent anticancerous, ethnomedicinal plant. *Industrial Crops and Products*, ***40***, 122-128.

Chowdari, K. V., Ramakrishna, W., Tamhankar, S. A., Hendre, R. R., Gupta, V. S., Sahasrabudhe, N. A., & Ranjekar, P. K. (1998). Identification of minor DNA variations in rice somaclonal variants. *Plant cell reports*, ***18***, 55-58.

Danial, G. H., Ibrahim, D. A., & Omer, M. S. (2016). Response of running shoot tips of strawberry (*Fragaria x ananasa*) for *in vitro* propagation in Kurdistan Region of Iraq. *Int. J. Environ. Agric. Biotechnol*, ***1***(2), 164-169.

Debnath, S. C. (2013). Propagation strategies and genetic fidelity in strawberries. *International journal of fruit science*, *13*(1-2), 3-18.

Deepika, R., & Kanwar, K. (2010). In vitro regeneration of *Punica granatum* L. plants from different juvenile explants. *J Fruit Ornamental Plant Res*, *18*(1), 5-22.

Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*, 13-15.

ElKichaoui, A. Y. (2014). *In vitro* propagation of strawberry (*Fragaria× annanasa* Duch.) through organogenesis via runner tips. *Ann. Plant Sci*, *3*(3), 619-627.

Gantait, S., Mandal, N., & Das, P. K. (2010). Field performance and molecular evaluation of micro-propagated strawberry. *Recent Research in Science and Technology*, *2*(5).

Haddadi, F., Abd Aziz, M., Saleh, G., Abd Rashid, A., & Kamaladini, H. (2010). Micro-propagation of strawberry cv. Camarosa: Prolific shoot regeneration from *in vitro* shoot tips using thidiazuron with N6-benzylamino-purine. *HortScience*, ***45***(3), 453-456.

Han, X., Yang, H., Duan, K., Zhang, X., Zhao, H., You, S., & Jiang, Q. (2009). Sodium nitroprusside promotes multiplication and regeneration of *Malus hupehensis* in vitro plantlets. *Plant cell, tissue and organ culture*, ***96***, 29-34.

Hancock, J. F; Maas, J. L; Shanks, C. H; Breen, P. J. and Luby. J. J. (1991). Strawberries (*Fragaria ananasa*). *Acta Hort*. **290**: 491–548.

Howlader, P., Kumar, S., & Robbani, M. (2016). In vitro plantlets regeneration of Strawberry through runner tip culture. *Bangladesh Hort*, ***2***(2), 51-57.

Jafari, M., & Shahsavar, A. R. (2022). Sodium nitroprusside: its beneficial role in drought stress tolerance of "Mexican lime" (*Citrus aurantifolia* (Christ.) Swingle) under *in vitro* conditions. *In Vitro Cellular & Developmental Biology-Plant*, ***58***(1), 155-168.

Joshi, P., & Dhawan, V. (2007). Assessment of genetic fidelity of micro-propagated *Swertia chirayita* plantlets by ISSR marker assay. *Biologia plantarum*, ***51***, 22-26.

Kaleybar, B. S., Nematzadeh, G. A., Ghasemi, Y., Hamidreza, S., & Petroudi, H. (2018). Assessment of genetic diversity and fingerprinting of strawberry genotypes using inter simple sequence repeat marker. *Horticult. Int. J*, ***2***, 264-269.

Karim, R; Ahmed, F; Roy, U. K; Ara, T; Islam, R. and Hossain, M. (2015). Varietal improvement of strawberry (*Fragaria x ananassa* Duch) through somaclonal variation using *in vitro* techniques. *J. Agric. Scl. tech.* **17**: 1-10.

Kikas, A; Libek, A. and Vasar, V. (2006) Influence micro-propagation on the production of strawberry runner plants, yield and quality. *Acta Hort.* **708**: 241-244.

Kurmi, U. S; Sharma, D. K; Tripathi, M. K; Tiwari, R; Baghel, B. S. and Tiwari, S. (2011). Plant regeneration of *Vitis vinifera* (L.) via direct and indirect organogenesis from cultured nodal segments. *Journal of Agricultural Technology,* **7**(3), 721-737.

Lal, M., Sharma, S., & Hegde, M. V. (2003). Micro-propagation of strawberry (*Fragaria x ananassa* Duch.). *Indian Journal of Agricultural Research*, ***37***(3), 231-234.

Lee, K.S., Kim, H.J., Park, D.H., Oh, S.C., Cho, H.J., & Kim, E.Y. (2018). Establishment of optimal conditions for micro-propagation by node culture and multiple shoots formation from sucker explants of thornless Blackberry (*Rubus fruticosus* L. cv. BB21). *Journal of Plant Biotechnology*, ***45***(2), 110-116.

Leroy, X. J., Leon, K., Hily, J. M., Chaumeil, P., & Branchard, M. (2001). Detection of *in vitro* culture-induced instability through inter-simple sequence repeat analysis. *Theoretical and Applied Genetics*, ***102***, 885-891.

Moradi, K., Otroshy, M., & Azimi, M.R. (2011). Micro-propagation of strawberry by multiple shoots regeneration tissue cultures. *Journal of Agricultural Technology*, ***7***(6), 1755-1763.

Naaz, A., Shahzad, A., & Anis, M. (2014). Effect of adenine sulphate interaction on growth and development of shoot regeneration and inhibition of shoot tip necrosis under in vitro condition in adult *Syzygium cumini* L. a multipurpose tree. *Applied biochemistry and biotechnology*, ***173***, 90-102.

Negi, D., & Saxena, S. (2010). Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. using inter simple sequence repeat markers. *New Forests*, ***40***, 1-8.

NHB (2021-22). Annual reports of National Horticulture Board, Haryana.

Pradhan, N., Singh, P., Dwivedi, P., & Pandey, D.K. (2020). Evaluation of sodium nitroprusside and putrescine on polyethylene glycol induced drought stress in *Stevia rebaudiana* Bertoni under *in vitro* condition. *Industrial Crops and Products*, ***154***, 112754.

Rahman, M., & Rajora, O. (2001). Microsatellite DNA somaclonal variation in micro-propagated trembling aspen (*Populus tremuloides*). *Plant Cell Reports*, ***20***, 531-536.

Rai, M. K., Phulwaria, M., Harish, Gupta, A. K., Shekhawat, N. S., & Jaiswal, U. (2012). Genetic homogeneity of guava plants derived from somatic embryogenesis using SSR and ISSR markers. *Plant Cell, Tissue and Organ Culture (PCTOC)*, ***111***, 259-264.

Rout, G. R., Senapati, S. K., Aparajita, S., & Palai, S. K. (2009). Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers. *Plant Omics*, ***2***(6), 250-285.

Sarropoulou, V., Dimassi-Theriou, K., & Therios, I. (2014). *In vitro* plant regeneration from leaf explants of the cherry rootstocks CAB-6P, Gisela 6, and MxM 14 using sodium nitroprusside. *In Vitro Cellular & Developmental Biology-Plant*, ***50***, 226-234.

Sharma, R. R., & Singh, S. K. (1999). Strawberry cultivation: a highly remunerative farming enterprise. *Agro India*, ***3***(2), 29-31.

Sharo, A. A., & Al-Tawaha, M. S. (2019). Prediction of engineering properties of basaltic rocks in Jordan. *International Journal of Civil Engineering and Technology (IJCIET)*, ***10***(1), 1731-1739.

Shenoy, V.B., & Vasil, I.K. (1992). Biochemical and molecular analysis of plants derived from embryogenic tissue cultures of napier grass (*Pennisetum purpureum* K. Schum). *Theoretical and Applied Genetics*, ***83***, 947-955.

Singh, P; Patel, R.M. and Kadam, S. (2013). *In vitro* mass multiplication of pomegranate from cotyledonary nodal explants cv. Ganesh. *African J. Biotech,* **12**(20), 2863-2868.

Soni, M., & Kanwar, K. (2016). Rejuvenation influences indirect organogenesis from leaf explants of Pomegranate (*Punica granatum* L.) 'Kandhari Kabuli'. *The Journal of Horticultural Science and Biotechnology*, ***91***(1), 93-99.

Tan, B. C., Chin, C. F., & Alderson, P. (2013). Effects of sodium nitroprusside on shoot multiplication and regeneration of *Vanilla planifolia* Andrews. *In Vitro Cellular & Developmental Biology-Plant*, ***49***, 626-630.

Tyagi, P., Khanduja, S., & Kothari, S. L. (2010). In vitro culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. *Biologia plantarum*, ***54***, 126-130.

Vala, A. G., Tejas, B., Kalathiya, I. U., Gajera, S. B., Parakhia, M. V., Rathod, P. J., & Golakiya, B. A. (2020). Optimization of regeneration using different plant growth regulator in Pomegranate (*Punica granatum* L.) cv.'Bhagwa'cultivar. *IJCS*, ***8***(5), 1654-1662.

Wang, Q. M; Goa, F. Z; Goa, X; Youzou, F; Siu, X; Wang, M; Junhui,Y. and Wang, L; (2012). Regeneration of *Clivia miniata* and assessment of clonal fidelity of plantlets. *Plant Cell, Tissue and Organ Culture (PCTOC)*, ***109***, 191-200.