MICROPROPAGATION OF STRAWBERRY

(*Fragaria×ananassa* Duch.)

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CERTIFICATE

This is to certify that the thesis entitled ***MICROPROPAGATION OF STRAWBERRY Fragaria×ananassa Duch.*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE IN BIOTECHNOLOGY,*** *embodies the result of a piece of bonafide research work carried out by* ***MD. MONIRUL ISLAM, Registration No. 17-08257,*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

I further certify that any help or sources of information as has been availed of during the course of this work has been duly acknowledged & style of the thesis have been approved and recommended for submission.

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MICROPROPAGATION OF STRAWBERRY (*Fragaria×ananassa* Duch.)

ABSTRACT

The present research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of May 2018 to November 2018 for *in vitro* renegaration of strawberry. The healthy, disease free shoot tips of 0.50-1.0 cm length were used as explants, It was sterilized by 0.1% $HgCl_2$ mixing with few drops Tween-20. The explant was inoculated in MS media supplemented with different combination of the BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and IBA (0.50, 1.00, 1.50, 2.00 and 2.50 mg/l) either alone or in combination. The maximum days for shoot induction were recorded in control treatment. The highest number of shoots (3.60) in BA 2.0 mg/l and leaves (9.60) per explant observed in BA 3.0mg/l. In contrast combine treatment of 2.0 mg/l BA+1.00 mg/l IBA performed best in case of percentage shoot induction (80%) and number of shoots (3.60).The highest length of shoots (3.66 cm) in BA 2.0 mg/l+ IBA 2.0mg/l . It was noticed that the minimum days to shoot induction, percent shoot initiation and no. of shoot were recorded in 2.0 mg/l BA+1.0 mg/l IBA. The highest number of leaves per explant was recorded in 2.0 mg/l BA+0.5 mg/l IBA. The highest number of root was observed in 2.0 mg/l BA+ 1.5 mg/l IBA and the highest percent root initiation in 3.0 mg/l BA+1.5 mg/l IBA Overall, micropropagation was found to be very effective and promising method in the proliferation of strawberry and this experiment can be a useful tool for breeding program of strawberry. Finally, the survival rate of micropropagated plantlets were observed in open atmosphere 75% and 80% in controlled condition.

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LIST OF ACRONYMS

AEZ	=	**Agro-Ecological Zone**
BARI	=	**Bangladesh Agricultural Research Institute**
BBS	=	**Bangladesh Bureau of Statistics**
LAI	=	**Leaf area index**
ppm	=	**Parts per million**
et al.	=	**And others**
N	=	**Nitrogen**
TSP	=	**Triple Super Phosphate**
MP	=	**Muriate of Potash**
RCBD	=	**Randomized complete block design**
DAS	=	**Days after sowing**
ha^{-1}	=	**Per hectare**
G	=	**gram (s)**
Kg	=	**Kilogram**
µg	=	**Micro gram**
SAU	=	**Sher-e-Bangla Agricultural University**
SRDI	=	**Soil Resources and Development Institute**
HI	=	**Harvest Index**
No.	=	**Number**
Wt.	=	**Weight**
LSD	=	**Least Significant Difference**
^0C	=	**Degree Celsius**
mm	=	**millimeter**
Max	=	**Maximum**
Min	=	**Minimum**
%	=	**Percent**
cv.	=	**Cultivar**
NPK	=	**Nitrogen, Phosphorus and Potassium**
CV%	=	**Percentage of coefficient of variance**
Hr	=	**Hour**
T	=	**Ton**
viz.	=	**Videlicet (namely)**

CHAPTER I

INTRODUCTION

Strawberry *(Fragaria×ananassa* Duch.) is one of the most popular and a valuable fruit of the world belongs to the family Rosaceae, subfamily Rosoideae. It is a perennial, stoloniferous herb growing in the Northern hemisphere in temperate and sub temperate environment (*Biswas et.al*., 2008). The cultivated strawberry (*Fragaria×ananassa*) is a hybrid between the Scarlet or Virginia strawberry (*F. virginiana*) and the pistillate South American *F. chiloensis* Duch. There are about 20 recognized species of strawberries in five chromosome groups (x = 7): ten diploids, four tetraploids, one pentaploid, one hexaploid and four octoploids (Staudt 1999; Jiajun *et al*. 2005). The cultivated strawberry is an octoploid (2n = 8x = 56).

The *F.*x *ananassa* is a perennial which arises from a crown of meristematic tissue or compressed stem tissue. Leaves, stems, runners, axillary crowns, inflorescences, and roots all arise from the crown. The plant has trifoliate leaves which spiral around the crown, with buds in the leaf axils giving rise to the runners. Runners have two nodes with a plant produced at the distal node (Darnell, 2003). Strawberry blossoms contain many pistils, each with its own style and stigma attached to the receptacle. When fertilization occurs the receptacle develops into a fleshy fruit. The fruit is called an achene which contains the seeds. The edible part is an accessory type fruit. The seeds are arranged on the outside of the receptacle tissue. The growth of the receptacle is dependent on successful fertilization of the ovules with its size and shape dependent on the number of achiness formed. Strawberry plants are day length dependent with cultivars being long day, short day or day neutral (Palei *et. al.,* 2015).

Strawberries had been a favorite among the fruits of the temperate world. They were valued for delicious flavor and fragrance, for health restoring qualities and as harbinger of spring (Wilhelm *et. al*., 1974).

Strawberry is one of the most consumed fruits owing to its fragrance, taste, antioxidant capacity resulting from high levels of anthocyanins and other nutritional properties (Giampieri *et. al.,* 2016). The berry is valued for its low-calorie carbohydrate and high fiber contents. The benefits of these high antioxidant activity fruit include reduction of carcinogens in humans, protection against tumor development and reversal of age related effects on memory (Bickford *et al.,* 2010). These qualities have ensured that the

economic importance of this crop has increased throughout the world and, now-a-days, it remains as a crop of primary interest for both research and fruit production (Mercado, et al., 2007).

Strawberry is also a good choice for production of pharmaceutical and recombinant proteins as oral vaccine. Strawberry is rich in vitamins C, A, anthocyanin and useful amino acids which makes it a medicinal and anticancer compound. Their use in processed forms such as cooked and sweetened preserves, jams or jellies and frozen whole berries or sweetened juice extracts or flavorings, and their use in making a variety of other processed products made them one of the most popular berry crops, more widely distributed than any other fruit (Samir *et al*., 2006).

Cultivation of the strawberry has greatly increased and this fruit has become one of the most cultivated fruit crops worldwide. Strawberry is cultivated in 73 countries worldwide on 3,95,844 hectors and produced 9,223,815 metric ton strawberry (FAO, 2017). It has been commercially cultivated in Canada, USA, Japan, Spain, Germany, Korea, Italy, Poland, Thailand and so many countries in the world (Biswas *et. al*., 2007). In Bangladesh, strawberry cultivation is very new and a significant number of farm households are now cultivating strawberry successfully, and so it is assumed that it would be profitable for growers in near future.

First variety of strawberry was brought to Bangladesh in 1996 and it took 12 years to develop the varieties suitable for cultivation in Bangladesh. Bangladesh Agriculture Research Institute (BARI) has invented a high yielding variety called 'BARI Strawberry-1. Besides Rajshahi University developed some strawberry varieties—RU straberry-1, 2, and 3 while modern horticulture center in Natore developed Modern Strawberry-1, 2, 3, 4 and 5 all suitable for cultivation in Bangladesh, according to DAE. According to Bangladesh Strawberry Association, around 6,500 bighas of land have been brought under strawberry cultivation throughout the country. Rajshahi district is ahead of other districts in farming the fruit (Anon., 2016)

Conventionally, strawberry is propagated by runners (Sakila *et al.,* 2007), which limits the number of propagules and the obtained propagules are susceptible to plant diseases. Strawberry mottle virus (SMoV), and Strawberry mild yellow edge virus (SMYEV), which gravely threaten strawberry production (Martin and Tzanetakis, 2013). Moreover, the conventional way of planting material production is not adequate to meet the commercial demand as because it does not produce viable seed. In contrast, mass

multiplication through tissue culture results higher yield and disease free plant material (Mohan *et al.*, 2005) and proved to be the best alternative approach to conventional propagation method (Mahajan *et al.*,2001).

Appropriate combination and optimum concentration of growth regulators are required in the culture media for callus initiation and plantlet regeneration from explants. Some workers reported high concentration of BAP is the best for strawberry micro propagation (Morozova, 2002) while other authors suggested that IAA + BAP + GA3 and BA + IBA (Bozena, 2001) is the best for strawberry micro propagation. A few researches on strawberry have been conducted at different institute in Bangladesh but which is not sufficient to find out suitable planting materials for agro-climatic condition of Bangladesh.

Based on the above mentioned context and prospects, the present investigation, therefore, has been carried out with the following objectives:

1. Assessment the combined effect of auxin and cytokinine for *in vitro* response of strawberry.
2. Identification of best hormonal treatment for *in vitro* culture of strawberry.
3. Establishment of *in vitro* regeneration protocol of strawberry.

CHAPTER II
REVIEW OF LITERATURE

Plant tissue culture forms the backbone of plant biotechnology, which is comprised of micropropagation, induction of somaclones, somatic hybridization, cryopreservation and regeneration of transgenic plants. Plant tissue culture is a technique through which any plant part is cultured on a sterile nutrient medium in controlled light and temperature with the purpose of obtaining growth. The totipotency which is exhibited by the apical meristem and the adjacent shoot tip region is the cornerstone for commercial micropropagation. Micropropagation of strawberry plants was introduced about thirty years ago (Boxus, 1974). Complete new plants can be derived from tissue either from pre-existing buds through shoot proliferation, following shoot morphogenesis through adventitious shoot regeneration or through the formation of somatic embryos. Micro propagation differs from all other conventional propagation methods in that aseptic conditions are essential to achieve success in the whole program. Now a day, it is very common practice all over the world to explore different aspects about strawberry using this technology. But unfortunately, it is very limited in Bangladesh. However, some. related works already performed by different institutes home and abroad have been reviewed and some of the most relevant literatures are cited below.

2.1 Explants

Shoot tip explants obtained from *Fragaria×ananassa* L were cultivated on solidified MS medium supplemented with 3% (w/v) sucrose and enriched with different doses of extracts obtained from seedlings of the same plant cultivar. Shoot tip were used as explants in this experiment. Large amount calli were formed after shoot tip cultured in basic MS medium with intermediate ratio of auxin to cytokinin, and the callus induction rate was up to 100%. Some differences were found between the treatments of lightness and darkness, the callus cultured in lightness represent more tight structure and regular shape with the color of Kelly, while that in darkness show itself less compact and irregular with the color of straw yellow. Subsequently, shoot regeneration was observed with the low ratio of auxin to cytokinin, which suggests that light wasn't the necessary element since shoot formed both in darkness and lightness and the No. of shoots had no significant difference. At the same time, slight

differences were found in surface color of callus and the state of shoots (Chao yanjie, 2011).

The aim of the strawberry regeneration study was to estimate the potential for the production of larger numbers of uniform, well-rooted strawberry plants by means of *in vitro* culturing. The study covered two cultivars – 'Carmen Rubin' and 'White Triumph'. The node explants were placed on two growth media containing the basic components of the MS medium as well as growth regulators. The first medium was supplemented with 1.0 mg dm-3 gibberellins and 0.1 mg dm-3 kinetin, while the second one – with 0.5 mg dm-3 IAA. The induction of organogenesis and regeneration of the plants took place on the same medium, with no passage. Within 9 weeks, 4 plants were produced from each primary explants in two multiplication cycles. The properties of the plantlets depended on the cultivar, weight of the explants and composition of the medium. The average weight of the 'Carmen Rubin' plants was higher than that of the 'White Triumph' ones. Moreover, the 'Carmen Rubin' plants produced longer shoots and more developed root systems. The sweet potato micro-plants displayed an ability to acclimatize quickly (Doliński and Olek, 2013).

Stem cuttings of strawberry from 3 month old plants, young buds, rhizome cuttings with shoot bud primordial and juvenile shoots were cultured on ½ MS medium supplemented with different combinations of growth hormones. On the other hand, callus could not be induced on the stem explants, callus was induced on the juvenile shoots which on sub culturing to medium with varying concentrations of 2,4-D and BAP developed bud primordia. These buds on further sub culturing grew into small plantlets. Young shoot buds along with a portion of rhizome when inoculated on MS medium containing 2,4-D and BAP each @ 0.5 mg/L ruptured and some callus was produced. The callus on further sub culturing on media containing 0.1mg/L 2,4-D and 0.5 mg/L BAP and 0.1mg/l both of 2,4-D and BAP produced lateral buds and multiple shoots. Excised single shoot when transferred to a medium supplemented with 0.1 mg/l each of 2,4-D and BAP started rooting within 2-3 days. Young shoots inoculated on MS medium supplemented with 0.5 mg/l both of 2,4-D and BAP developed greenish white hard callus within 4 weeks. These calli when subculture on a media containing 0.1 mg/l of 2,4-D and 0.5 mg/l BAP produced good callus growth within a week. Shoot buds were observed after 4 weeks which ultimately grew into small plantlets (10-15 plantlets in each culture). Good callus growth was observed within 4 weeks when 2

weeks old shoot buds were inoculated on a medium supplemented with BAP and 2,4-D each @ 0.5mg/l. Callus growth stopped during sub culturing to plain basal medium and roots initiated from it, whereas the callus sub cultured on a medium containing BAP and 2,4-D @ 0.5 mg/L developed into greenish white compact callus masses (Ilahi and Jabeen, 1987).

2.2 Sterilization

For obtaining contamination free cultures the most important step is sterilization of explants. In the present study the sterilization procedure was standardize for strawberry cultivar Kufri Himalini. Comparison was done between two important sterilant sodium hypochlorite and mercuric chloride with three-time duration 2, 5 and 8 minutes. After sprouting the sprouts of 0.5 to 1 cm. were taken for the study and treated by chemicals of surface sterilization with three selected timings i.e. 2, 5 and 8 minutes. Sterilized explants were inoculated on without hormones MS medium to evaluate the response of different chemicals. The observations were recorded regularly till to 30 days for the non-growing cultures, infected cultures and healthy cultures. Result showed that amongst the two sterilants i.e. NaOCl and HgCl2, NaOCl was found better for controlling the infection and it had not any adverse effect on explants even in long duration. Sodium hypochlorite (NaOCl) for 8 minute (T3) was selected for suitable sterilization chemical after 5 minute of savlon wash, 30-second dip in ethanol and at last washed with double distilled water (Badoni and Chauhan, 2010).

2.3 Media:

Cultures can be initiated and maintained on medium containing Knop's (Knop, 1965) macronutrients and Murashige and Skoog (1962, MS) micronutrients and organic components, or MS medium supplemented with 2.2-4.4 μM 6-benzyladenine (BA), 0.5-2.5 μM indole- 3-butyric acid (IBA) and 0.3 μM gibberellic acid (GA_3) at 23-25°C during the light peri- od, and 17°C in the dark; the quantum irradiance is 46 μmol $m^{-2}s^{-1}$ for a 16 h photoperiod (Sowik *et al*., 2001). Runners can be initiated from *in vitro* culture on media containing $AgNO_3$ (10-20 mg/l). GA_3 (28.9-57.7 μM) increased the efficiency of $AgNO_3$ significantly. Borkowska (2001) initiated strawberry cultures according to Boxus (1974, 1992), but modified medium by lowering the concentration

of cytokinin (BA, 2.2 μM) and auxin (IBA, 0.5 μM). Although agar (0.6-0.8%, w/v) is the most commonly used gelling agent for *in vitro* strawberry culture on semi-solid medium. The agar/galactomannan mixture in the proportion of 0.3/0.3 (w/v) in MS medium showed better performance and enhanced shoot proliferation compared to medium containing agar (0.6%, w/v) only. Cultures were maintained at 23°C under a photosynthetic photon flux density (PPFD) of 30 μmol $m^{-2}s^{-1}$ from'warm-white' fluorescent lamps and 16 h photoperiod. The use of light-emitting diodes, or LEDs, in particular 70% red and 30% blue at 60 μmol $m^{-2}s^{-1}$ re- sulted in greatest shoot proliferation, plantlet and total fresh weight when three-leaved explants of cv.

2.4 Effect of Growth Regulators

Manipulation of the composition and ratio of plant growth regulators (PGRs) is often the primary approach used for optimization of *in vitro* micropropagation methods (Shukla *et al*., 2012).

Frequency of shoot proliferation was maximum at 2.5 mg/l BAP and 0.5mg/l Kn and the number of shoot was 22-25 per explant. It took 26 days for shoot induction and 30 days for root induction. Multiplication rate in the treatment with BAP 0.5 mg/l which showed 2 plantlets were lowest. Among the BAP-2,4 D formulations, maximum multiplication was observed at BAP 1.0 and 0.5 mg/l 2,4 D where the number of shoots was 15. Numerous adventitious shoot primordia were observed near the basal portion of the shoot cluster. Augmentation of MS-medium with 4.5 mg/l BAP recorded the highest number of shoots and leaves (8.0 and 15.50 respectively). Shoot lets were highly rooted on half strength of B5 medium supplemented with 1.0 mg/l NAA. The maximum percentage of acclimatization, hardening and rhizomes production of *in vitro* derived plants in greenhouse was 80–100% (Mohammed *et al*., 2011).

The plant tissue culture techniques are an applicable method for multiplication of this valuable medicinal plants. Thus, the aim of this study is to understand the effect of different auxin/cytokinin ratios on indirect shoot organogenesis of this plant. According to our results, the maximum callus induction frequency (100%) was obtained on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) plus 0.05 mg/l 6-benzylaminopurine (BAP) from

petiole segments. For shoot induction purpose, the yellow-brownish, friable, organogenic calli were inoculated on shoot induction medium. On MS medium supplemented with 1.5 mg/l BAP and 0.15 mg/l Indole-3-butyric acid (IBA), 96.66% of the petiole-derived calli responded with an average number of 3.56 shoots per culture. The highest root formation frequency (96.66%), root number (5.5), and root length (4.83 cm) were achieved on MS medium containing 2.0 mg/l IBA plus 0.1 mg/l Naphthaleneacetic acid (NAA). The rooted shoots were successfully transferred to field condition and the substrate with the mixture of cocopeat and perlite (1:1) had the highest survival rate (96.66%). This is the first report of an effective *in vitro* organogenesis protocol for *F. religiosa* by indirect shoot organogenesis through axenic seedling derived petiole explants, which can be efficiently employed for conservation of this important medicinal plant species as well as the utilization of active biomolecules (Hesami *et al*., 2018).

Strawberry *in vitro* propagation study investigated by Ling *et al* (2013) the effects of different plant growth regulators (PGRs) on *in vitro* stem and leaf explants of *L. pumila*. Methods. The capabilities of callus, shoot, and root formation were evaluated by culturing both explants on Murashige and Skoog (MS) medium supplemented with various PGRs at the concentrations of 0, 1, 3, 5, and 7 mg/L. Results: Medium supplemented with 3 mg/L indole-3-butyric acid (IBA) showed the optimal callogenesis from both leaf and stem explants with (72.34±19.55)% and (70.40±14.14)% efficacy, respectively. IBA was also found to be the most efficient PGR for root induction. A total of (50.00±7.07)% and (77.78±16.47)% of root formation were obtained from the in vitro stem and leaf explants after being cultured for (26.5±5.0) and (30.0±8.5) d in the medium supplemented with 1 and 3 mg/L of IBA, respectively. Shoot formation was only observed in stem explant, with the maximum percentage of formation ((100.00±0.00)%) that was obtained in 1 mg/L zeatin after (11.0±2.8) d of culture. Conclusions: Callus, roots, and shoots can be induced from in vitro leaf and stem explants of *L. pumila* through the manipulation of types and concentrations of PGRs.

Plant Micropropagation has also been used for the propagation of genetically manipulated superior clones. the attempt was made to develop micropropagation as suitable condition for cloning of *Catharanthus roseus*. After optimizing the culture media of explant culture, the repeated subculturing was performed at regular intervals for 5 weeks. The roots were developed within 10 days. IBA concentration and the

period of pulse treatment had significant effects on the average number of roots produced per shoot. Rooted plants were successfully acclimatized at room temperature in soil contained in pots. All plants flowered and set seeds in the greenhouse after 3 months. It is an attempt to highlight some of the important landmarks of tissue culture of medicinal plants and it is also as important recent development in vitro technology (Rajora, *et al*., 2013).

The medium was supplemented with different concentrations of NAA and BA. The highest frequency of callus induction was obtained on 2.7 μM NAA and 4.4 μM BA from 3-day-old explants of Yazd genotype. In addition, the effect of explant age for callus induction was a genotype-dependent characteristic. The highest number of direct shoot regenerations was obtained at low BA concentrations (<1 μM). Pearson's correlation coefficient identified that callus induction correlated significantly and positively (0.408) with direct shoot regeneration. It was also shown that the region near the petiole of cotyledon was the best for direct shoot regeneration from cotyledon explants of lettuce. Direct shoot regeneration from the region near to petiole of cotyledons may aid the use of genetic engineering to improve important characteristics of lettuce (Mohebodini *et al*., 2011)

Plant growth regulator is indispensable material in culture media, and is important to the plant tissue induction, organ differentiation and growth. Therefore, the effects of seven plant growth regulators such as BA, TDZ, KT, NAA, IBA, IAA and GA_3 with different concentrations on the leaf regeneration, subculture of shoots and rooting were studied in *Ziziphus jujuba* cv. 'Dongzao'. The results indicated that the efficiency of TDZ was significantly higher than that of BA in the induction of adventitious bud from leaf. Leaves should be first induced on MS medium supplemented with TDZ (1.0 mg/L) and IBA (0.1 mg/L) for 28 days, and then transferred to medium MS+IBA 0.1 mg/L+GA_3 0.05 mg/L. In this way, the regeneration rate reached 92.45%. MS+BA 1.0 mg/L+KT 0.5 mg/L+IBA 0.1 mg/L was suitable for subculture of shoots, with the multiplication coefficient of 3.64. Supplements of GA_3 at 0.5 mg/L in the medium could significantly increase the elongation of shoots. The efficiency of IAA was best among that of IAA, IBA and NAA in the induction of rooting. The regenerated plantlets rooted well in 1/2 MS medium plus IAA (1.5 mg/L), with rooting percentage of 95.3% (Zhou and Liu, 2009).

The effects of auxins and cytokinin on callus formation, growth and regeneration of Gracilaria tenuistipitata Chang et Xia and G. perplexa Byrne et Zuccarello (Gracilariales, Rhodophyta) are reported. Plant growth regulators (PGR) in concentrations ranging from 0.1 to 100.0 μmol of indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and kinetin (K) were added to the ASP 12-NTA solid medium (0.7% agar), and apical and intercalary segments (5 mm long) were inoculated as initial explants. K stimulated growth rates of intercalary segments of *G. tenuistipitata* in a linear relation, and 2,4-D (1.0 μmol) and K (10.0 μmol) stimulated growth rates of apical and intercalary segments of *G. perplexa*, respectively. The simultaneous formation of apical, basal, and intermediate calluses is reported for the first time in axenic tissue cultures of red algae. With intercalary segments of *G. tenuistipitata*, basal callus induction rates were higher than those of apical and intermediate calluses in the majority of treatments, and auxins had stimulatory effects on the formation of all callus types. In apical segments of *G. perplexa*, intermediate callus formation was stimulated only by treatment with 1.0 μmol of K, while apical callus formation was stimulated by indole-3-acetic acid (1.0–10.0 μmol), 2,4-D (10.0–100.0 μmol), or K (0.1 μmol). Intercalary segments of *G. perplexa* developed only intermediate calluses, and the majority of treatments with PGR stimulated higher rates than those presented by apical segments. Potential for regeneration (development of adventitious plantlets originated from callus cells) was higher in apical calluses than in basal and intermediate calluses developed in intercalary segments of *G. tenuistipitata*. Moreover, auxins and cytokinin were essential to the induction of regeneration in intermediate calluses, while specific concentrations stimulated regeneration from basal and apical calluses. Plant regeneration in *G. perplexa* was observed only after transferring calluses from solid to liquid medium, and the majority of treatments with PGR had stimulatory effects. Regenerating plants of *G. perplexa* developed tetrasporangia, and released tetraspores giving rise to adult gametophytes. Our results indicate that auxins and cytokinin have a regulatory role in the growth and morphogenesis in *G. tenuistipitata* and *G. perplexa*, and diversity of responses presented by both species is related to specific developmental systems (Yokoya *et. al.*, 2006).

2.5 *In vitro* Regeneration

Plant regeneration from calli is possible by *de novo* organogenesis or somatic embryogenesis. Callus cultures also facilitate the amplification of limiting plant material. plant regeneration from calli permits the isolation of rare somaclonal variants which result either from an existing genetic variability in somatic cells or from the induction of mutations, chromosome aberrations, and epigenetic changes by the *in vitro* applied environmental stimuli, including growth factors added to the cultured cells (Flick, *et al*., 1983)

Regeneration ability of five *Nicotiana* varieties viz., Virginia, Jati, Motihari, CC Bengal and Sumatra were investigated via callus induction using leaf discs. Explants were cultured on MS medium which was supplemented with different concentrations and combinations of plant growth regulators. Callus formation frequency was 67.20%. Among the varieties used, Motihari induced the highest percentage (97.50%) of callus followed by Jati (92.50%) in 2.0 rng/L Kinetin and 2.0 mg/L IAA. Shoots were induced from calli cultured on the same medium. Maximum shoot formation from leaf discs was 82.50% on medium supplemented with 2.0 mg/L Kinetin and 2.0 mg/L IAA. It was also revealed from this study that Motihari was the best variety for callus formation and subsequent plantlet regeneration which is a pre-requisite for vector mediated transformation for varietal improvement of *Nicotiana* species. The rooting response of regenerated shoots was observed by using $^1/_2$ MS medium with IBA (0.0, 0.5, and 1.0 mg/L). The highest root formation was found in Motihari (90%) with ½ MS medium supplemented with 0.5 mg/L IBA. After that regenerated plantlets with plenty of roots were transferred successfully to pots and subsequently to the field (Rahman *et al*., 2010)

2.6 Rooting and acclimatization

Both in vitro and ex vitro methods have successfully been used to root and acclimatize the micropropagated strawberry shoots. Proliferated shoots can be rooted in vitro on Boxus (Borkowska 2001; Sowik et al. 2001), or modified cranberry medium without growth regulators, or on half-strength MS with activated charcoal (0.6 g/l) and IAA (5.7 μM) (Moore et al. 1991). The in vitro-formed roots are thick, posses no hairy roots, grow horizontally, and are fragile and easily damaged. In vitro-

grown plantlets have low photosynthetic activity, poor water balance and their anatomy and morpho- logy are far from being optimal. Kozai (1991) and Hayashi et al. (1997) developed a photoautotrophic micropropagation system for plant multiplication by simultaneously increasing the CO_2 concentration and light quality. Plantlets that are rooted ex vitro have a larger root system and more runners than those formed by in vitro-rooted strawberry plants. Similar results were observed by Nhut et al. (2000, 2003, 2006) when using either CO_2-enrichment with or without light-emitting diodes, or even when a simple im- proved aeration system was implemented in mixotrophic culture. When moved from in vitro culture conditions, micro- cuttings must be acclimatized gradually to ambient conditions to avoid mortality that might otherwise occur under an abrupt change in relative humidity, temperature or irradiance. For *in vitro*-rooted plantlets, standard procedure is to wash the plantlets and transfer to pots containing ProMix BX or 1 peat : 1 vermicu- lite (Zhou *et al*. 2005), and maintained in a humidity chamber and acclimatized by gradually lowering the humidity over 2 weeks.

Rooting can be induced *ex vitro* with complete success by transferring microshoots directly in rockwool (Borkowska *et al*. 1999). After one month, the plantlets rooted *ex vitro* are planted in 1 peat : 1 rockwool (v/v) and grown in the greenhouse for acclimatization . *In vitro*-derived strawberry shoots were rooted *ex vitro* in granulated, water-repelling and water absorbing mineral wool (Grodan) mixed in a proportion of 1:1 (v/v) by Sowik *et al*. (2003).

Debnath (2006) developed a protocol that enables strawberry micropropation in one step, i.e. multiplying shoots and having them rooted in the same culture medium. The use of microcuttings, giving both root and shoot growth in a medium containing cytokinin, is emerging as a better choice for micropropagation of strawberries than multiple shoot proliferation, (using a cytokinin supplement- ted medium) with subsequent rooting of shootlets. *In vitro*- derived strawberry shoots can be proliferated, elongated and rooted on zeatin-containing medium. Zeatin alone at very low levels (1-2 µM) produced two to three shoots per explant, averaging 88% rooting incidence in a single me- dium in ‘Bounty’ strawberry.

CHAPTER III
MATERIALS AND METHODS

3.1 Time and location of the experiment:

The present research was carried out in Biotechnology Laboratory in the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of May 2018 to November 2018.

3.2 Experimental materials:

3.2.1 Source of material

The planting materials of strawberry (*Fragaria×ananossa* Duch.) were collected from Horticulture Farm of Sher-e-Bangla Agricultural University.

3.2.2 Explant Collection

The explant was collected from Horticulture field of Sher-e-Bangla Agricultural University. The healthy, disease free runner tips of 0.50 cm length were used as explants for the study of *in vitro* regeneration of strawberry.

Plate 1. Source of material (runner) in Petridish

3.2.3 Instruments

Metal instruments *viz*., forceps, scalpels, needless, spatulas and aluminum foils were sterilized in an autoclave at a temperature of 121^0C for 30 minutes at 1.06 kg/cm^2 (15 PSI) pressure.

3.2.4 Glass ware

The Borosil glassware was used for all the experiments. Oven dried (250^0C) Erlesn meyer flasks, culture bottles, flat bottom flasks, pipettes, petridishes, beaker and measuring cylinders (25 ml, 50 ml, 100 ml, 500 ml and 1000 ml) were used for media preparation**.** The glassware's were first rinsed with the liquid detergent (Trix) and washed thoroughly with tap water, until the detergent was removed completely. Finally they were rinsed with distilled water and sterilized in oven at 160-180^0C for 3-4 hours.

3.2.5 Culture medium

The degree of success in micropropagation is mainly related to the choice of nutritional components and growth regulators. Presence of plant growth regulators plays a significant role in a successful regeneration of any plant species. Media for tissue culture should contain all major and minor elements, vitamins as well as growth regulators which are essential for normal plant growth. Explants were inoculated onto media composed of basal MS (Murashige and Skoog, 1962) medium supplemented with the plant growth regulators. Composition of MS media have been shown in appendix I. Hormones were added separately to different media according to the requirements. To do so, stock solutions of hormones were prepared ahead of media preparation and stored at 4^0C temperature.

Treatments:

1. BA treatments (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) were used for shoot proliferation and plantlet regeneration.
2. BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) in combination with IBA (0.0, 0.50, 1.00, 1.50, 2.00 and 2.50 mg/L) were used for *in vitro* regeneration.
3. IBA treatments (0.50, 1.00, 1.50, 2.00 and 2.50 mg/l) were used for root formation.

3.3 The preparation of the stock solution of hormones:

To prepare these hormonal supplements, they were dissolved in proper solvent as shown against each of them below.

Hormones (Solute)	Solvents used
BA	1 N NaOH
IBA	70% ethyl alcohol

In present experiment, the stock solution of hormones was prepared by following procedure. 100 mg of powder hormone was placed in a small beaker and then dissolved in 10 ml of 70% ethyl alcohol or 1 (N) NaOH solvent. Finally, the volume was made upto 100 ml by the addition of sterile distilled water using a measuring cylinder. The prepared hormone solution was then labeled and stored at 4 ± 1^0C for use upto two month.

3.4 The preparation of culture media:

To prepare 1000 ml of culture media the following steps were followed:

Step-1. Seven hundred ml of sterile distilled water was poured into 1000 ml beaker.

Step-2. Five gm of MS media and 30 gm of sucrose was added and gently stirrered to dissolve these ingredients completely with the help of a Hot Plate Magnetic Stirrer.

Step-3. Different concentrations of hormonal supplements were added to the solution either in single or in combinations as required and mixed well.

Step-4. The volume was made up to 1000 ml with addition of sterile distilled water.

Step-5. The pH was adjusted at 5.8.

Step-6. Finally, 8 gm agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar.

Experiments:

3.5 Steam heat sterilization of media (Autoclaving)

For sterilization the culture medium was poured in 200 ml culture bottles and then autoclaving was done at a temperature of 121^0C for 15 minutes at 1.06 kg/cm^2 (15 PSI) pressure. After autoclaving the media were stored in at 25±2 ℃ for several hours to make it ready for inoculation with explants.

3.6 Preparation of explants

The trimmed strawberry shoot tips were washed thoroughly under running tap water and then with sterilized distilled water for several times. Subsequently the explants were transferred to laminar airflow cabinet and kept in a 250 ml sterilized beaker. The beaker with explants was constantly shaken during sterilization. They were treated with 70% ethanol for 1-2 minute and rinsed with sterilized distilled water for 3-4 times. Then the explants were immersed in 0.1% $HgCl_2$ within a beaker and added 3-4 drops of Tween-20 for about 4-5 minutes with constant shaking in clockwise and anticlockwise direction. Then the strawberry explants were washed 3-4 times with autoclaved distilled water to make the material free from chemical and ready for inoculation in culture media.

3.7 Inoculation of culture

The sterilized explants were inoculated carefully following proper sterilization process within laminar airflow cabinet. Prior to use, the surface of the laminar flow bench was swabbed down with 70 % ethyl alcohol and the interior sprayed with the same alcohol. All glassware, instruments and media were steam-sterilized in an autoclave. During the course of the work, instruments in use were placed in a beaker containing 70 % ethanol and were flamed repeatedly using a spirit burner. The worker's hands and forearms were washed thoroughly with soap and water and repeatedly sprayed with 70% alcohol during the period of work. The mouth of all culture vials were flamed before and after positioning of the explant on the medium.

For inoculation, explants were transferred to large sterile glass Petridis or glass plate with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer leaves were removed with sterile scalpel blade to make suitable size. After cutting explants into suitable size (0.25-0.50 cm), explants are transferred to culture bottles containing MS medium with plant growth regulator (Plate 2). After vertically inoculating the explants in culture bottle, the mouth of bottle is quickly flamed and capped tightly. After proper labeling, mentioning media code, date of inoculation etc. the bottles were transferred to growth room.

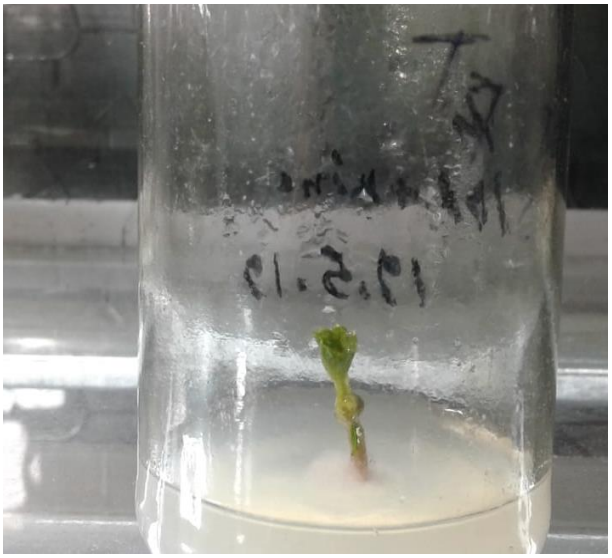

Plate 2. Inoculation of explants in the culture vial

3.8 Incubation

The bottles were kept to the culture racks and allowed to grow in controlled condition. The cultures were maintained at 21±1 °C with light intensity varied from 4000–5000 lux (23 W white bulbs). White fluorescent lamps were used for growth of the culture. The photoperiod was generally 14 hours' light and 10 hours dark having 70% relative humidity (RH).

3.9 Shoot proliferation

The explants were cultured on MS nutrient medium supplemented with different concentration of BA alone or in combination of IBA. Percentage of explants showing shoot proliferation, days for shoot induction, number shoots per explants, average length of shoots, and number of leaves per explants were considered as parameter for evaluating this experiment. After successful shoot proliferation, subculture was done with newly form shoots. Shoots ware excised in aseptic condition with help of sterile scalpel blade and sterile forceps and transferred to new MS media which was supplemented with same concentration of growth hormones in order to increase budding frequency. The observations on development pattern of shoots were made throughout the entire culture period. Data recording was started after 7, 14, 21, 28 days from inoculation.

3.9 Rooting of shoots

Newly formed shoots with adequate length were excised individually from the culture vial and transferred to rooting media. Different concentration of BA alone or in combination of IBA was used with MS media. The observations on development pattern of roots were made throughout the entire culture period. Data were recorded from 14, 21, 28 days of inoculation.

3.10 Acclimatization

Acclimatization, also known as "hardening is a process by which *in vitro* propagated plants are made to adapt to in natural environment.

Step-1: After 30 days of culture on rooting media, the plantlets were taken out from culture vial with forceps with utmost care in order to prevent any damage to newly formed roots and dipped in gentle warm water to remove any traces of solidified agar media for acclimatization. Plastic pots (6×6 cm) were kept ready filled with garden soil and compost in the proportion of 1:1 respectively. Immediately after removing solidified agar media from newly formed roots, the plantlets were then transplanted in to the pots with special care.

Step-2: After planting, the plantlets were thoroughly watered and were kept at 23±1 °C with light intensity varied from 3000–5000 lux. The photoperiod was generally 14 hours light and 10 hours dark and 70% RH for 7 days with consecutive irrigation.

Step-3: Then the plants were shifted to shade house with less humidity and indirect sunlight. The tops of the pots were covered with transparent plastic sheet and grew at room temperature and 70% RH for 14 days with periodic irrigation (2days intervals).

Step-4: After 3 weeks, the plants were transferred to the soil following depotting and poting into different pot having bigger pot size. The plants were watered periodically and upper layer of the soil mulched occasionally whenever necessary.

3.11 Data recording:

The observations on development pattern of shoots and roots were made throughout the entire culture period. Five replicates each of them containing 4 bottles were used per treatment. Data were recorded after 14, 21 and 28 days of culture, starting from day of inoculation on culture media in case of shoot proliferation. In event of root formation, it

was done every day starting from 7 days to 28 days of culture. The following observations were recorded in cases of shoot and root formation under *in vitro* condition.

1. Days for shoot induction
2. No. of shoots per explants
3. Length of shoot
4. No. of leaf per explants
5. Length of leaf (cm)
6. Days for root induction
7. No. of roots per explants
8. Length of root

3.12.1 Calculation of days to shoots and roots induction

Days to shoots and roots induction was calculated by counting the days from explants inoculation to the first induction of shoots/roots.

3.12.2 Calculation of number of shoots and roots per explant

Number of shoots and roots per explants was calculated by using the following formula,

$$\text{Number of shoots / roots per explants} = \frac{\text{Number of shoots / roots per explant}}{\text{Number of observation}}$$

3.12.3 Calculation of number of leaf

Numbers of leaves produced on the plantlet were counted. After that, the mean was calculated.

3.12.4 Calculation of shoots and root length

Shoot and root length were measured in centimeter (cm) from the base to the top of the explants by a measuring scale. Then the mean was then calculated.

3.12.5 Length of leaf

Length of leaf of each sample plant was recorded and sum total of them was divided by the total number of leaves of the sample plant.

3.13 Statistical analysis

The experiment was one factorial set up in a completely randomized design (CRD) with five replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level using MSTAT-C (1990) program.

CHAPTER IV
RESULTS AND DISCUSSION

Different investigations were made for regeneration of strawberry ability. The results obtained from the experiment were described and discussed here and analyses of variance (ANOVA) presented in Appendix I-X. Presentation of results has been made in two phases. The first phase involves shoot regeneration potentiality of explant and the second phase involves root initiation from regenerated shoots.

4.1 Sub-experiment 1. Effect of BA on shoot induction potentiality in Strawberry

The result of the effect of different concentrations of BA has been presented under following headings with Figure (1.1-1.2) and Table (1-3).

4.1.1 Days to shoot induction

The different concentrations of BA showed the significant variation on days to shoot induction. The maximum days (18.00) to shoot induction were recorded in control treatment followed by 5.0 mg/l (15.0 days) and 4.0 mg/l (13.20 days). On the other hand, minimum (7.60 days) was required in 2.0 mg/l BA and 3.0 mg/l (11.0 days) (Figure 1.1).

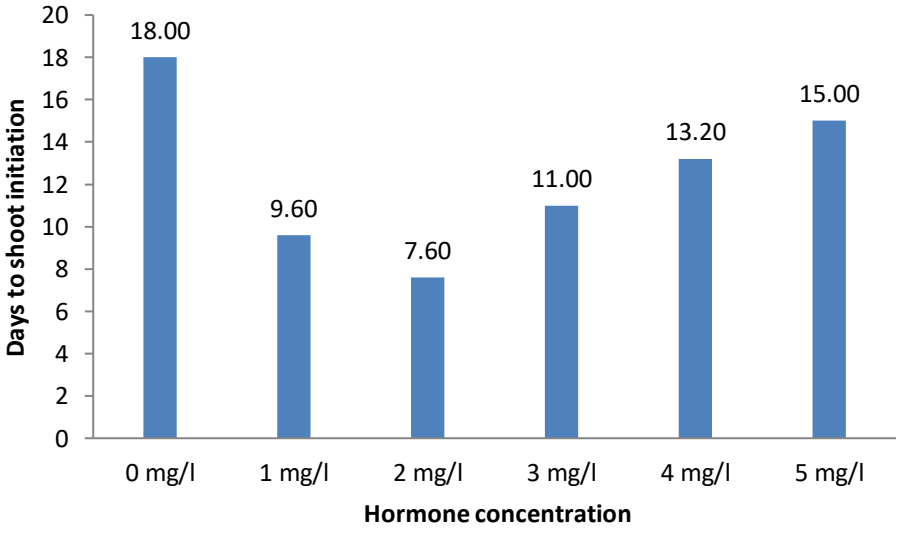

Figure 1.1 Effect of BA on days to shoot induction in Strawberry

4.1.2 Percentage of shoot initiation

Significant variations were observed among different concentrations of BA on percentage of shoot induction. The treatment BA 2.0 mg/l had produced the highest frequency of shoot (80.00%), while the lowest percentage (40.00%) of shoot was produced in control treatment (Figure 1.2). Waseem *et al.* (2009) carried out shoot multiplication of Strawberry from shoot tip explants in MS media supplemented with 1.0 mg/l BAP that produced maximum shoot initiation percentage (93.3%). This result partially supported present findings. These variation may be due to the age, nature, and the physiological state of the explants. (Bajaj**,** *et al.*, 1991).

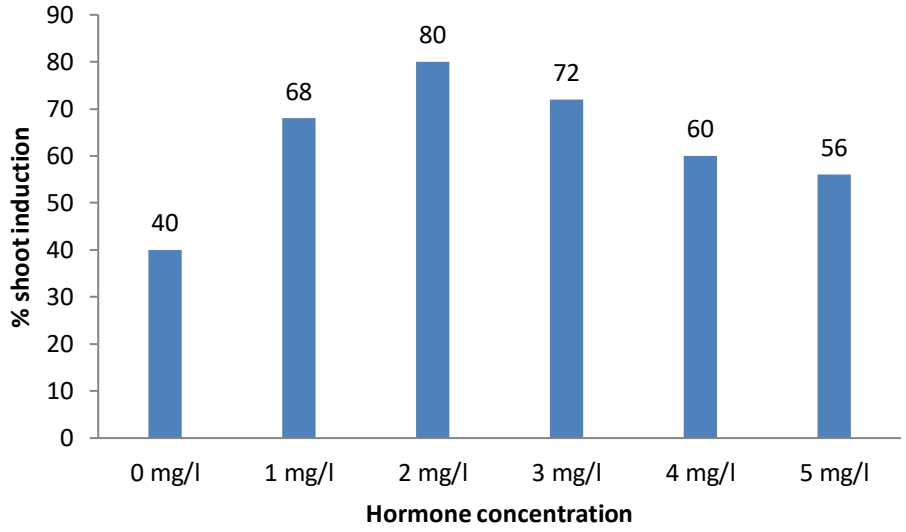

Figure 1.2. Effect of BA on percentage of shoot induction in Strawberry

4.1.3 Number of shoot per explant

There was significant influence of different concentrations of BA on the number of shoots per explant. The treatment BA 2.0 mg/l gave the highest number of shoot (2.40, 3.20 and 3.60) at 14, 21 and 28 DAI (Days after induction), respectively (Table 1 and Plate 3) and the control treatment showed the lowest number of shoot (1.20, 1.40 and 1.60) at 14, 21 and 28 DAI (Table 1). Himstedt *et al.* (2001) studied regeneration from leaf and stem explants of 19 Strawberry cultivars. Most of the cultivars regenerated shoots on MS medium supplemented with BAP 2.0 mg/1 with more than 10 regenerated shoots per explant in some of the cultivars. Waseem *et al.* (2009) carried out shoot multiplication of strawberry using MS media supplemented with 1.0 mg/l BAP which showed maximum number of shoot (4.1) per explants. The findings of their

study seems completely different from our result. This variation may be because of interaction between genotype, growth regulators and environmental factors. (Sen, *et al.*,2002).

Table 1. Effect of different concentration of BA on number of shoot at different DAI

BA (mg/l)	Number of shoot/explants		
	14 DAI	**21 DAI**	**28 DAI**
0.0	1.20c	1.40b	1.60b
1.0	1.60b	2.20b	2.60b
2.0	2.40a	3.20a	3.60a
3.0	1.60b	2.00b	2.20b
4.0	1.20bc	1.60c	1.80b
5.0	1.40c	2.00b	2.20b
CV (%)	32.96	34.21	37.14
$LSD_{(0.05)}$	0.06741	0.923	1.1796

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 3. Highest number of shoot induction at 28 DAI in 2.0 mg/l of BA treatment

4.1.4 Length of shoot

There was significant influence of different concentrations of BA at 5% level on the length of shoot. The highest length of shoot (1.42 cm, 2.44 cm and 3.12 cm) at 14, 21 and 28 DAI (Plate 5) was found in 3.0 mg/l BA and second highest length (1.14 cm, 2.18 cm and 2.90 cm) with 1.0 mg/l BA at 14, 21 and 28 DAI (Table 2) was observed. The control treatment gave the lowest length (2.04cm) of shoot which was statistically similar with 2.0 mg/l (2.14 cm) at 28 DAI (Table 2).Waseem *et al.* (2009) carried out shoot regeneration of Strawberry from shoot tip explants using MS media fortified with 1.0 mg/l BAP produced 5.0 cm length of shoots. This result partially supported present findings.

Table 2. Effect of different concentration of BA on length of shoot at different days after induction (DAI)

BA (mg/l)	Length of shoot (cm)		
	14 DAI	21 DAI	28 DAI
0.0	0.46 e	0.96e	2.04e
1.0	1.14 b	2.18b	2.90b
2.0	0.82c	1.66c	2.58c
3.0	1.42a	2.44a	3.12a
4.0	0.66 d	1.54c	2.36d
5.0	0.60 d	1.34d	2.14e
CV (%)	12.06	7.14	5.32
LSD (0.05)	0.133	0.157	0.175

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

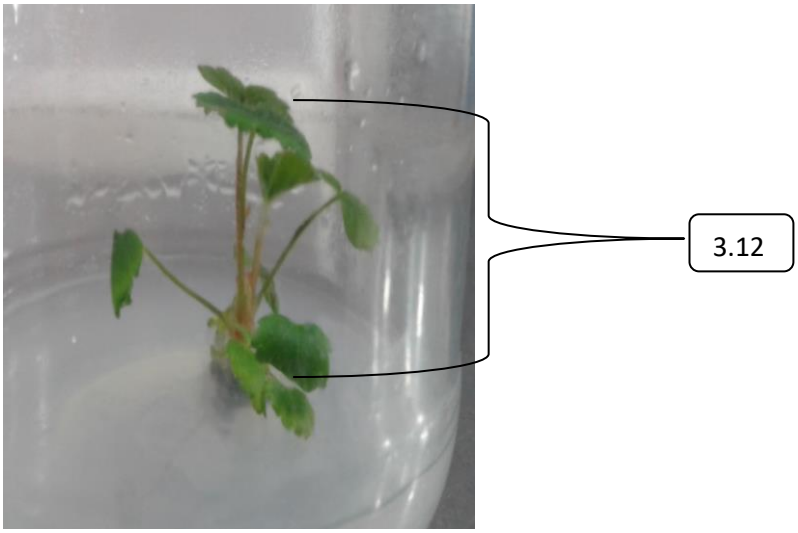

Plate 4. Highest length of shoot induction at 28 DAI in the treatment 3.0 mg/l BA

4.1.5 Number of leaves

The different concentrations of BA showed significant variation on the number of leaves. The highest number of leaves (3.23, 6.20 and 9.60) at 14, 21 and 28 DAI (plate 5), respectively was noticed from the 2.0 mg/l BA which was statistically different from rest of others. Whereas the lowest number of leaves (1.40, 1.80 and 2.40) at 14, 21 and 28 DAI, respectively were noticed in control treatment (Table 3). Waseem *et al.* (2009) carried out an experiment of strawberry from shoot tip explant using MS media supplemented with 1.0 mg/l BAP producing highest number (11.0) of leaves.

Table 3. Effect of different concentration of BA on number of leaves at different days after induction

BA (mg/ l)	Number of leaves		
	14 DAI	21 DAI	28 DAI
0.0	1.40c	1.80c	2.40f
1.0	2.80ab	3.80b	7.42b
2.0	3.23a	6.20a	9.60a
3.0	2.40b	3.20b	6.20c
4.0	2.40b	3.60b	5.00d
5.0	2.21b	3.40b	3.60e
CV (%)	20.38	18.80	15.60
LSD (0.05)	0.533	0.674	1.011

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

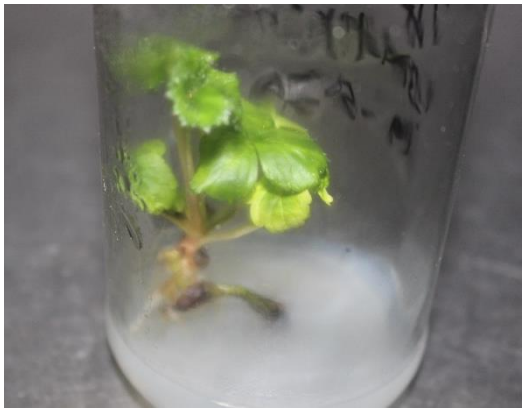

Plate 5. The treatment BA 2.0 mg/l showed the maximum number of leaves at 28 DAI

4.2 Sub-experiment 2. Effect of IBA on root induction potentiality in strawberry

The result of the effect of different concentrations of IBA has been presented under following headings with Figure (2.1-2.2) and Table (4-5).

4.2.1 Days to root induction

Significant variations were observed among different concentrations of IBA on days to root induction. The maximum days (26.60) to root induction were recorded in control treatment followed by 2.5 mg/l (20.60 days) and 2.0 mg/l (17.80 days) of IBA. Besides, minimum 13.00 days were needed in 0.5 mg/l IBA followed by 1.0 mg/l (13.80 days) and 1.5 mg/l (15.80 days) (Figure 2.1). Nalini (2012) revealed that minimum 19.19 days were needed for root initiation through shoot tip explants in MS medium supplemented with IBA 1.0 mg/l. Present results partially supported by the author findings. This variation may be due to explants type, seasonal variations and environmental factors (Sen**,** *et al*., 2002).

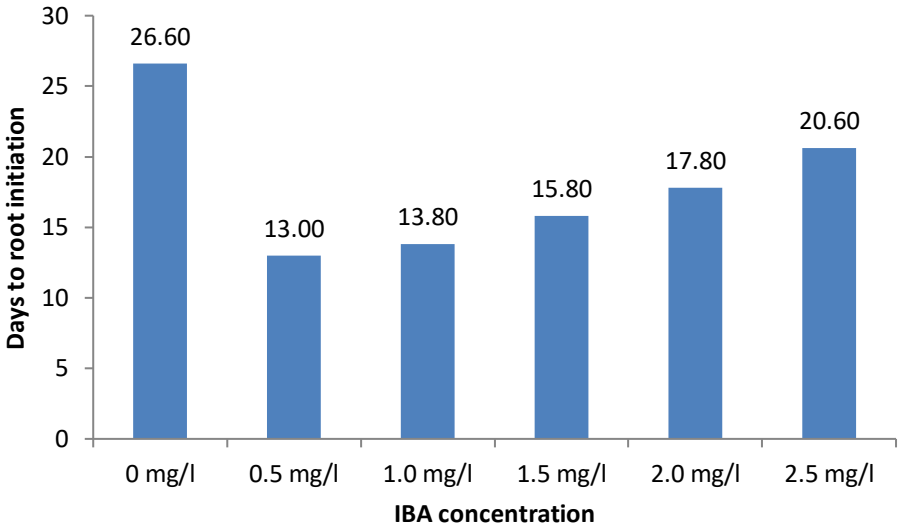

Figure 2.1 Effect of IBA on days to root induction in Strawberry

4.2.2 Percentage of root induction

The maximum percentage of root (80.00%) was obtained in 0.5 mg/l IBA treatment, while the minimum percentage (44.00%) of root initiation was in control treatment (Figure 3.2). Besides, it was noticed that with increasing IBA concentration, percent of root were decreased (Figure 2.2). Similar results on strawberry were obtained by Long *et al*. (2006) and Karim *et al*. (2002). Shatanawi *et al.* (2010) also achieved *in vitro* rooting successfully on MS medium supplemented with IBA (0.2 mg/1) in strawberry.

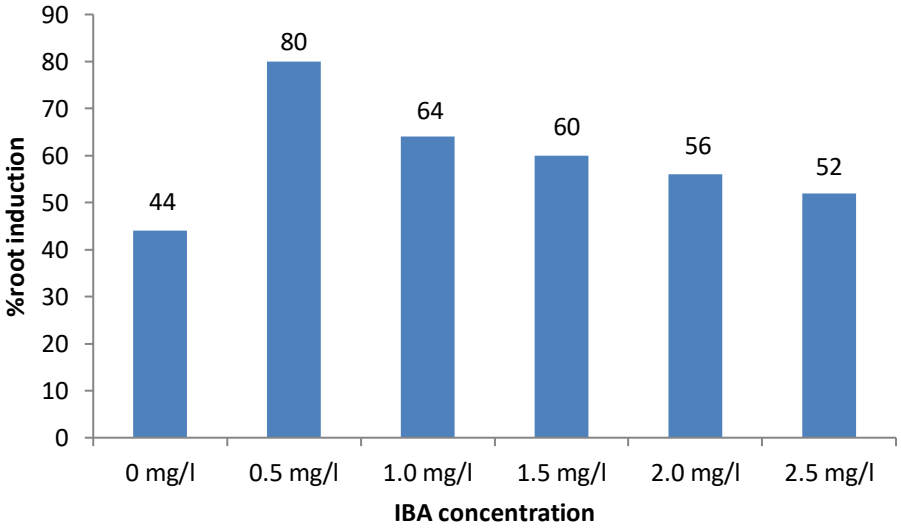

Figure 2.2. Effect of IBA on percentage of root induction in Strawberry

4.2.3 Number of root per explant

There was significant influence of different concentrations of IBA on the number of roots per explant. The treatment IBA 2.0 mg/l gave the highest number of root (2.60, 3.60 and 4.00) at 14, 21 and 28 DAI, respectively (Plate 6 and Table 4) and the control treatment showed the lowest number of root (1.60, 1.80 and 2.00) at 14, 21 and 28 DAI, respectively (Table 4).

Table 4. Effect of different concentration of IBA on number of root at different DAI

IBA (mg/l)	Number of root/explants		
	14 DAI	21 DAI	28 DAI
0.0	1.60bc	1.80c	2.00d
0.5	1.60bc	2.40bc	3.60bc
1.0	2.20ab	2.60b	3.80ab
1.5	1.40c	1.80c	2.80cd
2.0	2.60a	3.60a	4.00a
2.5	1.60bc	1.82c	2.40d
CV (%)	29.30	21.43	21.21
LSD (0.05)	0.6948	0.6527	0.923

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 6. Maximum number of root formation at 28 DAI in the treatment 2.0 mg/l of IBA in Strawberry

4.2.4 Length of root

There was meaningful influence of different concentrations of IBA on the length of root at 5% level of significance at laboratory condition. The highest length of root (2.24 cm, 2.80 cm and 3.34 cm) at 14, 21 and 28 DAI, respectively was found in 1.50 mg/l IBA (Plate 7 and Table 5). The control treatment found the lowest number of root (0.44 cm, 0.80 cm and 1.20 cm) at 14, 21 and 28 DAI, respectively (Table 5 and Plate 7). Labade *et al.* (2016) found best result at 2 mg/l IBA which produced largest roots (5±0.19 cm) of Strawberry.

Table 5. Effect of different concentration of IBA on length of root at different days after Induction

IBA (mg/l)	Length of root (cm)		
	14 DAI	21 DAI	28 DAI
0.0	0.44e	0.80f	1.20e
0.5	1.14c	2.12c	2.72b
1.0	1.68b	2.56b	2.84b
1.5	2.24a	2.80a	3.34a
2.0	0.78d	1.74d	2.22c
2.5	0.66d	1.34e	1.62d
CV (%)	8.35	6.50	6.26
LSD (0.05)	0.1261	0.1608	0.1899

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LS= Level of significance, $LSD_{(0.05)}$= Least significant difference.

Plate 7. Effect of IBA 1.50 mg/l on highest length of root measured at 28 DAI

4.3 Sub-experiment 3. Combined effect of BA and IBA on shoot and root induction Potentiality in Strawberry

The results of the combined effect of different concentrations of BA + IBA have been presented under following headings with Table 6-12.

4.3.1 Days to shoot induction

Variations were observed among different concentrations of BA+IBA on days to shoot initiation. The maximum days (15.80 days) to shoot initiation was recorded in control and BA 2.0 mg/l+ IBA 1.0 mg/l required minimum (7.60 days) which was statistically similar with BA 2.0 mg/l+ IBA 0.5 mg/l (8.20 days) (Table 6).

4.3.2 Percentage of shoot induction

Significant variation was observed for the combine used of BA+IBA on the percent of shoot initiation per explants. Maximum percentage (76.00%) of shoot induction was noticed in the treatment BA 2.0 mg/l + IBA 1.0 mg/l which is statistically similar (76.00%) with BA 2.0 mg/l + IBA 0.5 mg/l treatment whereas minimum percentage (48.00%) initiation was recorded in control (hormone free media) (Table 6). This result was not fully supported by Bo *et al.* (2005). They obtained adventitious shoots of Strawberry of 93.8 % on MS media mixed with 2.0 mg/1 BA and 2.0 mg/1 NAA. This variation may be occurred due to plant internal physiology and hormonal effect.

Table 6. Combined effect of different concentration of BA and IBA on days to shoot initiation and percent of shoot initiation

Treatment	Days to shoot initiation	Percent of shoot initiation
BA 1.0 mg/l+ IBA 0.5 mg/l	10.80ij	68.00a-c
BA 1.0 mg/l+ IBA 1.0 mg/l	10.80ij	60.00c-e
BA 1.0 mg/l+ IBA 1.5 mg/l	11.00ij	52.00ef
BA 1.0 mg/l+ IBA 2.0 mg/l	11.60hi	64.00b-d
BA 1.0 mg/l+ IBA 2.5 mg/l	11.20i	68.00b-d
BA 2.0 mg/l+ IBA 0.5 mg/l	8.20l	76.00a
BA 2.0 mg/l+ IBA 1.0 mg/l	7.60l	76.00a
BA 2.0 mg/l+ IBA 1.5 mg/l	9.40k	72.00ab
BA 2.0 mg/l+ IBA 2.0 mg/l	9.80jk	68.00a-c
BA 2.0 mg/l+ IBA 2.5 mg/l	11.20i	64.00b-d
BA 3.0 mg/l+ IBA 0.5 mg/l	12.60f-h	60.00c-e
BA 3.0 mg/l+ IBA 1.0 mg/l	12.60f-h	68.00a-c
BA 3.0 mg/l+ IBA 1.5 mg/l	12.80f-h	72.00ab
BA 3.0 mg/l+ IBA 2.0 mg/l	13.20d-g	64.00b-d
BA 3.0 mg/l+ IBA 2.5 mg/l	13.40c-f	60.00c-e
BA 4.0 mg/l+ IBA 0.5 mg/l	13.80b-f	60.00c-e
BA 4.0 mg/l+ IBA 1.0 mg/l	12.00g-i	60.00c-e
BA 4.0 mg/l+ IBA 1.5 mg/l	13.00e-g	52.00ef
BA 4.0 mg/l+ IBA 2.0 mg/l	13.60b-f	56.00d-f
BA 4.0 mg/l+ IBA 2.5 mg/l	14.80ab	56.00d-f
BA 5.0 mg/l+ IBA 0.5 mg/l	14.60a-c	48.00f
BA 5.0 mg/l+ IBA 1.0 mg/l	13.80b-f	48.00f
BA 5.0 mg/l+ IBA 1.5 mg/l	14.20b-e	48.00f
BA 5.0 mg/l+ IBA 2.0 mg/l	14.60a-c	48.00f
BA 5.0 mg/l+ IBA 2.5 mg/l	14.40b-d	48.00f
CV (%)	7.14	19.67
$LSD_{(0.05)}$	1.156	7.932

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

4.3.3 Number of shoot per explant

There was significant influence of combined concentrations of BA and IBA on the number of shoot per explant at 14, 21 and 28 days after induction. The treatment BA 2.0 mg/l+ IBA 1.0 mg/l gave the highest number of shoot (2.20, 2.80 and 4.40) at 14, 21 and 28 DAI respectively (Plate 11) whereas the lowest number of shoot (1.00, 1.60 and 1.82) at 14, 21 and 28 DAI, respectively was found with hormone free media (Table 7). Similarly, when the combination of different concentrations of IBA and BAP were used, significant results regarding to the number of shoots regeneration of strawberry plantlets from nodal segments were achieved by Waseem *et al.* (2009).

Table 7. Combined effect of BA and IBA on number of shoot

Treatment	Number of Shoot/explants		
	14 DAI	**21 DAI**	**28 DAI**
BA 1.0 mg/l+ IBA 0.5 mg/l	1.60abc	2.40ab	2.62b-e
BA 1.0 mg/l+ IBA 1.0 mg/l	1.80ab	2.40ab	3.20a-c
BA 1.0 mg/l+ IBA 1.5 mg/l	1.40bc	2.10b-d	2.80a-d
BA 1.0 mg/l+ IBA 2.0 mg/l	1.60a-c	2.20a-c	3.20ab
BA 1.0 mg/l+ IBA 2.5 mg/l	1.42bc	2.20a-c	3.20ab
BA 2.0 mg/l+ IBA 0.5 mg/l	1.82ab	2.40ab	3.20a-c
BA 2.0 mg/l+ IBA 1.0 mg/l	2.20a	2.80a	4.40a
BA 2.0 mg/l+ IBA 1.5 mg/l	1.60a-c	2.40ab	3.20a-c
BA 2.0 mg/l+ IBA 2.0 mg/l	1.42bc	2.20a-c	2.60b-e
BA 2.0 mg/l+ IBA 2.5 mg/l	1.40bc	1.60cd	2.20d-g
BA 3.0 mg/l+ IBA 0.5 mg/l	1.42bc	1.60cd	2.20d-g
BA 3.0 mg/l+ IBA 1.0 mg/l	1.20bc	1.60cd	2.20d-g
BA 3.0 mg/l+ IBA 1.5 mg/l	1.40bc	1.60cd	2.40c-f
BA 3.0 mg/l+ IBA 2.0 mg/l	1.60abc	1.80b-d	2.20e-g
BA 3.0 mg/l+ IBA 2.5 mg/l	1.40bc	1.40d	2.20e-h
BA 4.0 mg/l+ IBA 0.5 mg/l	1.20bc	1.60cd	1.80fg
BA 4.0 mg/l+ IBA 1.0 mg/l	1.40bc	1.60cd	2.20d-g
BA 4.0 mg/l+ IBA 1.5 mg/l	1.20cd	1.40d	2.20d-g
BA 4.0 mg/l+ IBA 2.0 mg/l	1.40bc	1.80b-d	2.20d-g
BA 4.0 mg/l+ IBA 2.5 mg/l	1.40bc	2.10b-d	2.20e-g
BA 5.0 mg/l+ IBA 0.5 mg/l	1.20bc	1.42d	1.80fg
BA 5.0 mg/l+ IBA 1.0 mg/l	1.40bc	1.42d	2.00e-g
BA 5.0 mg/l+ IBA 1.5 mg/l	1.40bc	1.60cd	1.60g
BA 5.0 mg/l+ IBA 2.0 mg/l	1.20bc	1.60cd	1.60g
BA 5.0 mg/l+ IBA 2.5 mg/l	1.40bc	1.40d	1.80fg
CV (%)	36.59	31.15	25.22
$LSD_{(0.05)}$	0.66	0.7213	0.742

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 8. Maximum number of shoot observed at 28 DAI in the treatment BA 2.0 mg/l + IBA 1.0 mg/l

4.3.4 Length of shoot (cm)

With different concentrations of BA and IBA, significant influence was found on the average length of shoot. The maximum length of shoot (2.36 cm, 2.58 cm and 3.66 cm) at 14, 21 and 28 DAI was noticed from the BA 2.0 mg/l + IBA 2.0 mg/l which were statistically different from rest of others (Plate 9). It was the minimum (0.40 cm, 0.68 cm and 1.08 cm) at 14, 21 and 28 DAI in control (Table 8). Labade *et al.* (2016) found that MS medium containing 1.0 mg/l BAP + 0.1 mg/l IBA, shown 90% shoot initiation and 5.5 ± 0.51 cm average length of shoot per explants in Strawberry. The results of our experiment were varied may be due to genotype and culture environment. (Sen, *et al.*, 2002).

Table 8. Combined effect of different concentration of BA and IBA on length of shoot

Treatments	Length of shoot (cm)		
	14 DAI	21 DAI	28 DAI
BA 1.0 mg/l+ IBA 0.5 mg/l	2.10b	2.42b	2.56c
BA 1.0 mg/l+ IBA 1.0 mg/l	1.84de	2.36c	2.48cd
BA 1.0 mg/l+ IBA 1.5 mg/l	1.70ef	2.32c	2.32de
BA 1.0 mg/l+ IBA 2.0 mg/l	1.58fg	2.28cd	2.30ef
BA 1.0 mg/l+ IBA 2.5 mg/l	1.50gh	2.24cd	2.12f
BA 2.0 mg/l+ IBA 0.5 mg/l	2.22a	2.880c-e	3.14b
BA 2.0 mg/l+ IBA 1.0 mg/l	2.02bc	2.84a	2.54c
BA 2.0 mg/l+ IBA 1.5 mg/l	2.10b	2.18de	3.12b
BA 2.0 mg/l+ IBA 2.0 mg/l	2.36a	2.58a	3.66a
BA 2.0 mg/l+ IBA 2.5 mg/l	1.92cd	1.86fg	2.44cd
BA 3.0 mg/l+ IBA 0.5 mg/l	1.52gh	1.74gh	2.16fg
BA 3.0 mg/l+ IBA 1.0 mg/l	1.41gh	1.56hi	1.90gh
BA 3.0 mg/l+ IBA 1.5 mg/l	1.40h	1.38ij	1.74hi
BA 3.0 mg/l+ IBA 2.0 mg/l	0.98i	1.34jk	1.56ij
BA 3.0 mg/l+ IBA 2.5 mg/l	0.82i-l	1.22j-l	1.46jk
BA 4.0 mg/l+ IBA 0.5 mg/l	0.68k-m	1.16k-m	1.32k-m
BA 4.0 mg/l+ IBA 1.0 mg/l	0.72k-m	1.14k-n	1.32k-m
BA 4.0 mg/l+ IBA 1.5 mg/l	0.60m	1.06l-o	1.22l-n
BA 4.0 mg/l+ IBA 2.0 mg/l	0.84i-k	1.04l-o	1.40j-l
BA 4.0 mg/l+ IBA 2.5 mg/l	0.92ij	1.00m-o	1.50jk
BA 5.0 mg/l+ IBA 0.5 mg/l	0.74k-m	1.00m-o	1.34k-m
BA 5.0 mg/l+ IBA 1.0 mg/l	0.76j-m	0.96m-0	1.16mn
BA 5.0 mg/l+ IBA 1.5 mg/l	0.67k-m	0.94no	1.16mn
BA 5.0 mg/l+ IBA 2.0 mg/l	0.65lm	0.92o	1.22lmn
BA 5.0 mg/l+ IBA 2.5 mg/l	0.60m	0.66p	1.10n
CV (%)	11.16	9.99	7.85
$LSD_{(0.05)}$	0.1794	0.203	0.1855

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 9. Effect of BA 2.0 mg/l+ IBA 2.0 mg/l on length of shoot observed at 28 DAI

4.3.5 Number of leaves

There was significant influence of different concentrations of BA on the number of leaves. The highest number of leaves (6.20, 8.20 and 11.60) at 14, 21 and 28 days after induction, respectively was noticed from the BA 2.0 mg/l + IBA 0.5 mg/l which showed better performance from rest of others (Plate 10). Whereas the lowest average number of leaves (1.20, 1.60 and 2.21) at 14, 21 and 28 DAI, respectively were noticed in control treatment (Table 9).

Table 9. Combined effect of different concentration of BA and IBA on number of leaves

Treatments	Number of leaves		
	14 DAI	21 DAI	28 DAI
BA 1.0 mg/l+ IBA 0.5 mg/l	4.00b-d	6.00b	7.60cd
BA 1.0 mg/l+ IBA 1.0 mg/l	4.00b-d	5.82b	7.10de
BA 1.0 mg/l+ IBA 1.5 mg/l	3.62c-e	5.20b-d	6.42ef
BA 1.0 mg/l+ IBA 2.0 mg/l	3.62c-e	4.40d-f	6.20ef
BA 1.0 mg/l+ IBA 2.5 mg/l	3.20e-g	5.20b-d	6.42ef
BA 2.0 mg/l+ IBA 0.5 mg/l	6.2a	8.2a	11.6a
BA 2.0 mg/l+ IBA 1.0 mg/l	4.40b	6.00b	9.80b
BA 2.0 mg/l+ IBA 1.5 mg/l	4.20bc	5.40bc	8.40c
BA 2.0 mg/l+ IBA 2.0 mg/l	3.40d-f	4.62c-e	6.40ef
BA 2.0 mg/l+ IBA 2.5 mg/l	2.82f-h	3.80e-h	5.60fg
BA 3.0 mg/l+ IBA 0.5 mg/l	2.62g-h	4.20e-g	4.80gh
BA 3.0 mg/l+ IBA 1.0 mg/l	2.40hi	3.60f-i	4.6g-i
BA 3.0 mg/l+ IBA 1.5 mg/l	2.60gh	3.40g-i	4.00h-j
BA 3.0 mg/l+ IBA 2.0 mg/l	2.80f-h	3.20h-k	4.00h-j
BA 3.0 mg/l+ IBA 2.5 mg/l	2.40hi	2.80i-k	3.60ij
BA 4.0 mg/l+ IBA 0.5 mg/l	2.22h-j	2.82i-k	3.22j-l
BA 4.0 mg/l+ IBA 1.0 mg/l	2.22h-j	2.80i-k	3.40jk
BA 4.0 mg/l+ IBA 1.5 mg/l	2.20h-j	2.60jk	3.40jk
BA 4.0 mg/l+ IBA 2.0 mg/l	1.80i-k	3.20h-k	4.22h-j
BA 4.0 mg/l+ IBA 2.5 mg/l	1.80i-k	2.80i-k	3.40jk
BA 5.0 mg/l+ IBA 0.5 mg/l	1.82i-k	2.60jk	3.20j-l
BA 5.0 mg/l+ IBA 1.0 mg/l	1.62jk	2.40kl	2.40k-m
BA 5.0 mg/l+ IBA 1.5 mg/l	1.60jk	2.40kl	2.40k-m
BA 5.0 mg/l+ IBA 2.0 mg/l	1.40k	1.60lm	1.80m
BA 5.0 mg/l+ IBA 2.5 mg/l	1.20k	1.20m	1.40m
CV (%)	21.96	20.52	18.27
LSD (0.05)	0.455	0.673	1.119

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 10. Effect of BA 2.0 mg/l + IBA 0.5 mg/l showing number of leaves observed at 28 DAI

4.3.6 Days to root induction

Significant variation was observed among different concentrations of BA and IBA on days to root induction. The maximum days (29.80 days) to root induction was recorded in control treatment and minimum days (12.20 days) was required in BA 2.0 mg/l + IBA 1.0 mg/l concentration (Table 10). Nalini (2012) cultured shoot tip explants in MS medium containing kinetin 3.0 mg/1 + IBA 2.0 mg/1 and found good percentage of root in 17.91 days (minimum).

4.3.7 Percentage of root induction

Variations were observed among different concentrations of BA and IBA on percent of explants showing root induction. The highest percentage (76.00%) of root induction was recorded with BA 3.0 mg/l+ IBA 1.5 mg/l. The treatment BA 2.0 mg/l + IBA 0.5 mg/l, BA 2.0 mg/l+ IBA 1.0 mg/l and BA 2.0 mg/l+ IBA 2.5 mg/l showed statistically similar percent (68.00%) followed by best result whereas the lowest percentage (24.00%) of root induction was recorded in control condition (Table 10). Nalini (2012) cultured shoot tip explants in MS medium containing kinetin 3.0 mg/1 + IBA 2.0 mg/1 and reported it to be the best treatment combination as it produced 67.82 per cent.

Table 10. Combined effect of BA and IBA on Days to root induction and percent of root initiation of roots

Treatments	Days to root induction	Percentage of root initiation
BA1.0 mg/l+ IBA 0.5 mg/l	17.60jk	64.00bc
BA 1.0 mg/l+ IBA 1.0 mg/l	18.00ij	64.00bc
BA 1.0 mg/l+ IBA 1.5 mg/l	17.60jk	56.00c-e
BA 1.0 mg/l+ IBA 2.0 mg/l	17.40jk	48.00ef
BA 1.0 mg/l+ IBA 2.5 mg/l	18.20h-j	56.00c-e
BA 2.0 mg/l+ IBA 0.5 mg/l	13.60m	68.00ab
BA 2.0 mg/l+ IBA 1.0 mg/l	12.20n	68.00ab
BA 2.0 mg/l+ IBA 1.5 mg/l	15.20l	60.00b-d
BA 2.0 mg/l+ IBA 2.0 mg/l	16.40kl	60.00b-d
BA 2.0 mg/l+ IBA 2.5 mg/l	17.00jk	68.00ab
BA 3.0 mg/l+ IBA 0.5 mg/l	19.20g-i	64.00bc
BA 3.0 mg/l+ IBA 1.0 mg/l	19.60f-h	60.00b-d
BA 3.0 mg/l+ IBA 1.5 mg/l	19.60gh	76.00a
BA 3.0 mg/l+ IBA 2.0 mg/l	19.40gh	60.00b-d
BA 3.0 mg/l+ IBA 2.5 mg/l	19.60gh	64.00bc
BA 4.0 mg/l+ IBA 0.5 mg/l	21.00ef	64.00bc
BA 4.0 mg/l+ IBA 1.0 mg/l	20.00e-g	60.00b-d
BA 4.0 mg/l+ IBA 1.5 mg/l	21.20e	60.00b-d
BA 4.0 mg/l+ IBA 2.0 mg/l	22.80d	52.00d-f
BA 4.0 mg/l+ IBA 2.5 mg/l	24.00cd	48.00ef
BA 5.0 mg/l+ IBA 0.5 mg/l	23.60cd	48.00ef
BA 5.0 mg/l+ IBA 1.0 mg/l	24.20c	48.00ef
BA 5.0 mg/l+ IBA 1.5 mg/l	24.80bc	44.00f
BA 5.0 mg/l+ IBA 2.0 mg/l	26.00b	44.00f
BA 5.0 mg/l+ IBA 2.5 mg/l	26.00b	44.00f
CV (%)	12.4	21.13
LSD (0.05)	1.273	8.231

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

4.3.8 Number of root per explant

There was significant influence of different concentrations of BA and IBA on the number of root per explant. The treatment BA 2.0 mg/l + IBA 1.50 mg/l gave the highest number of root (2.40, 3.20 and 4.20) at 14, 21 and 28 DAI (Plate 11), respectively whereas the lowest number of root (1.20, 1.60 and 2.00) at 14, 21 and 28 DAI, respectively was found with hormone free media (Table 11).

Table 11. Combined effects of BA and IBA on number of roots at different DAI

Treatments	Number of root		
	14 DAI	21 DAI	28 DAI
BA 1.0 mg/l+ IBA 0.5 mg/l	2a-c	2.6ab	4.00ab
BA 1.0 mg/l+ IBA 1.0 mg/l	2.2ab	2.6ab	3.4b-d
BA 1.0 mg/l+ IBA 1.5 mg/l	2.4a	2.2bc	3.4b-d
BA 1.0 mg/l+ IBA 2.0 mg/l	2.2ab	2.4b	2.8d-f
BA 1.0 mg/l+ IBA 2.5 mg/l	1.6b-d	2.2bc	2.8d-f
BA 2.0 mg/l+ IBA 0.5 mg/l	1.4cd	2.00b-d	2.4e-h
BA 2.0 mg/l+ IBA 1.0 mg/l	1.6b-d	2.4b	3.00c-d
BA 2.0 mg/l+ IBA 1.5 mg/l	2.4a	3.2a	4.2a
BA 2.0 mg/l+ IBA 2.0 mg/l	1.4cd	1.6c-e	2.6e-g
BA 2.0 mg/l+ IBA 2.5 mg/l	1.6b-d	2.2bc	2.2f-i
BA 3.0 mg/l+ IBA 0.5 mg/l	1.4cd	2.2bc	2.4e-h
BA 3.0 mg/l+ IBA 1.0 mg/l	2.00a-c	2.6ab	3.6a-c
BA 3.0 mg/l+ IBA 1.5 mg/l	1.4cd	1.6c-e	2.4e-h
BA 3.0 mg/l+ IBA 2.0 mg/l	1.4cd	1.6c-e	2.2f-i
BA 3.0 mg/l+ IBA 2.5 mg/l	1.4cd	1.6c-e	2.2f-i
BA 4.0 mg/l+ IBA 0.5 mg/l	1.4cd	1.2e	1.6ij
BA 4.0 mg/l+ IBA 1.0 mg/l	1.4cd	1.4de	2.00g-j
BA 4.0 mg/l+ IBA 1.5 mg/l	1.2d	1.6c-e	2.00g-j
BA 4.0 mg/l+ IBA 2.0 mg/l	1.2d	1.4de	1.6ij
BA 4.0 mg/l+ IBA 2.5 mg/l	1.4cd	1.6c-e	1.8h-j
BA 5.0 mg/l+ IBA 0.5 mg/l	1.4cd	1.4de	1.4j
BA 5.0 mg/l+ IBA 1.0 mg/l	1.2d	1.2e	1.4j
BA 5.0 mg/l+ IBA 1.5 mg/l	1.00d	1.2e	1.4j
BA 5.0 mg/l+ IBA 2.0 mg/l	1.2d	1.4de	1.4j
BA 5.0 mg/l+ IBA 2.5 mg/l	1.2d	1.4de	1.4j
CV (%)	33.8	29.23	26.31
$LSD_{(0.05)}$	0.6554	0.6825	0.7817

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 11. Effect of BA 2.0 mg/l+ IBA 1.50 mg/l showing maximum number of root observed At 28 DAI

4.3.9 Length of root (cm)

There was significant influence of different combined concentrations of BA and IBA on the length of root. The highest length of root (2.50 cm, 3.60 cm and 4.40 cm) at 14, 21 and 28 DAI (Plate 15), respectively was found in BA 2.0 mg/l+ IBA 1.0 mg/l (Table 14). The control treatment found the lowest number of root (0.38 cm, 0.62 cm and 1.04 cm) at 14, 21 and 28 DAI, respectively (Table 12).

Table 12. Combined effect of BA and IBA on length of root at different DAI

Treatments	Length of root		
	14 DAI	21 DAI	28 DAI
BA 1.0 mg/l+ IBA 0.5 mg/l	2.14cd	3.16b	3.34d
BA 1.0 mg/l+ IBA 1.0 mg/l	1.74e	3.14bc	3.26de
BA 1.0 mg/l+ IBA 1.5 mg/l	1.73e	3.06b-d	3.18d-f
BA 1.0 mg/l+ IBA 2.0 mg/l	1.65ef	2.94c-e	3.08e-f
BA 1.0 mg/l+ IBA 2.5 mg/l	1.52f	2.80ef	3.10f
BA 2.0 mg/l+ IBA 0.5 mg/l	2.24bc	3.44a	4.08b
BA 2.0 mg/l+ IBA 1.0 mg/l	2.50a	3.60a	4.40a
BA 2.0 mg/l+ IBA 1.5 mg/l	2.33ab	3.48a	4.10b
BA 2.0 mg/l+ IBA 2.0 mg/l	1.98d	2.90de	3.74c
BA 2.0 mg/l+ IBA 2.5 mg/l	1.65ef	2.64f	3.18d-f
BA 3.0 mg/l+ IBA 0.5 mg/l	1.30g	2.32g	2.72c-f
BA 3.0 mg/l+ IBA 1.0 mg/l	1.12g	2.28gh	2.50gh
BA 3.0 mg/l+ IBA 1.5 mg/l	1.00h	2.10hi	2.29f-h
BA 3.0 mg/l+ IBA 2.0 mg/l	0.88h-j	2.02ij	2.45hi
BA 3.0 mg/l+ IBA 2.5 mg/l	1.00h	1.88jk	2.21i-k
BA 4.0 mg/l+ IBA 0.5 mg/l	0.84h-j	1.48mn	1.86lm
BA 4.0 mg/l+ IBA 1.0 mg/l	0.90hi	1.53lm	2.06j-l
BA 4.0 mg/l+ IBA 1.5 mg/l	0.72j-l	1.48mn	1.98kl
BA 4.0 mg/l+ IBA 2.0 mg/l	0.72j-l	1.70kl	2.14jk
BA 4.0 mg/l+ IBA 2.5 mg/l	0.68k-m	1.55lm	2.10kl
BA 5.0 mg/l+ IBA 0.5 mg/l	0.76i-l	1.50ln	1.84lm
BA 5.0 mg/l+ IBA 1.0 mg/l	0.62l-n	1.30no	1.58n
BA 5.0 mg/l+ IBA 1.5 mg/l	0.54m-o	1.17op	1.64mn
BA 5.0 mg/l+ IBA 2.0 mg/l	0.42o	1.00p	1.26o
BA 5.0 mg/l+ IBA 2.5 mg/l	0.46no	1.02p	1.14o
CV (%)	11.69	7.5	7.93
$LSD_{(0.05)}$	0.1779	0.2034	0.2534

Figures in a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, $LSD_{(0.05)}$= Least significant difference.

Plate 12. Effect of BA 2.0 mg/l+ IBA 1.0 mg/l showing highest length of root observed at 28 DAI

4.4 The comparative performance of growth hormone on shoot and root development are shown as following discussion (Table 13 and Table 14):

Table 13. Comparative performance of growth hormone in case of shoot morphology

Growth Hormone	**Percent of shoot (%)**	**No. of shoot**	**Length of shoot (cm)**
BA	80 (2.0 mg/l)	3.60 (2.0 mg/l)	3.12 (3.0 mg/l)
BA and IBA	76 (2.0 mg/l+ 1.0 mg/l)	4.40 (2.0 mg/l+ 1.0mg/l)	3.66 (2.0 mg/l+ 2.0 mg/l)

The results showed that combination of BA and IBA was better than BA alone in the basal MS medium in terms of number and length of shoot. But BA showed the better performance for percentage of shoot than the combine dose of BA and IBA (Table 13).

Table 14. Comparative performance of growth hormone in case of root morphology

Growth Hormone	Percent of root (%)	No. of root	Length of root (cm)
IBA	80 (0.5 mg/l)	4.00(2.0 mg/l)	3.34(1.50 mg/l)
BA and IBA	76 (3.0 mg/l+ 1.5 mg/l)	4.20 (2.0 mg/l+ 1.50 mg/l)	4.40 (2.0 mg/l+ 1.0 mg/l)

The treatment IBA 0.5 mg/l gave the best result than combine dose of BA and IBA with (3.0 mg/l + 1.5 mg/l) in case of percentage of root. On the other hand, combination of BA and IBA with 2.0 mg/l + 1.50 mg/l and 2.0 mg/l + 1 mg/l showed the good performance than BA 2.0 mg/l and 1.5 mg/l for no. of root and length of root (Table 14).

Sub-experiment 4. Acclimatization and establishment of plantlets on soil

After a satisfactory number of shoot and root development at 6-8 weeks of culture the individual plantlets were moved from vial carefully without any root damage. The roots were washed with running tap water for removing surplus media. The plantlets were then transplanted into small plastic pot prepared with a standard ratio of cow dung and soil in a shade condition. The plantlets were sprayed occasionally with water for maintaining humidity. At first 25 plants were transplanted, 20 survived in shade condition and survival rate was 80%. Finally in open atmospheric condition 20 plants were transplanted 15 survived (Table 15 and Plate 16) and survival rate was 75%. Sarkar and Shaheen (2009) carried out *in vitro* regeneration of strawberry and plantlets were successfully established in soil after rooting (100%). So, analyzing the survival rate it can be said that acclimatization potentiality of Strawberry was satisfactory.

Table 15. Survival rate of *in vitro* regenerated plantlets of Strawberry

Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate
In shade house with controlled atmosphere	25	20	80
In open atmospheric area	20	15	75

Plate 13. Hardening of strawberry plantlet in (a) controlled condition; and (b) in open condition.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present research was carried out in Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka-1207 from the period of May 2019 to November 2019. The shoot tip of *Fragaria×ananassa* were used as experimental materials in the present investigation. Five levels of BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) were used to shoot induction. BA 2.0 mg/l were used to study no. of shoot induction and no. of leaves potentiality. Five levels of IBA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) were used for root induction. Combined effect of BA and IBA on shoot and root induction potentiality were also investigated in strawberry regeneration. The experiments were arranged in Completely Randomized Design (CRD) with 5 replications.

BA 2.0 mg/l had produced the highest frequency of shoot (80.00%) with minimum (7.60) days. BA 2.0 mg/l gave the highest number of shoot (2.40, 3.20 and 3.60) at 14, 21 and 28 DAI and the highest number of leaves (3.23, 6.20 and 9.60) at 14, 21 and 28 DAI, respectively. The highest length of shoot (1.42 cm, 2.44 cm and 3.12 cm) at 14, 21 and 28 DAI, respectively was found in 3.0 mg/l BA.

IBA 0.5 mg/l had produced the highest percentage of root initiation (80.00%) with in minimum 13.00 days. The highest number of root (2.60, 3.60 and 4.00) at 14, 21 and 28 DAI, respectively found in 2.0 mg/l IBA and the control treatment found the lowest number of root at all DAI. The highest length of root (2.24 cm, 2.80 cm and 3.34 cm) at 14, 21 and 28 DAI, respectively) was found in 1.50 mg/l IBA.

The maximum percentage (76.00%) of shoot induction was noticed in treatment BA 2.0 mg/l+ IBA 1.0 mg/l in minimum (7.60 days) and minimum percentage (48.00%) was noticed in control hormone free medias in maximum (15.80 days). The treatment BA 2.0 mg/l+ IBA 1.0 mg/l gave the highest number of shoot (2.20, 2.80 and 4.40) and BA 2.0 mg/l+ IBA 2.0 mg/l length of shoot (2.36 cm, 2.58cm and 3.66 cm) observed at 14, 21 and 28 DAI, respectively.

The minimum days (12.20) were required for root initiation in BA 2.0 mg/l+ IBA 1.50 mg/l concentration. The highest percentage (76.00%) of root induction was recorded with BA 3.0 mg/l + IBA 1.5 mg/l, whereas the lowest percentage (24.00%) of root induction was recorded in control condition. The treatment BA 2.0 mg/l + IBA 1.50 mg/l gave the highest number of root (2.40, 3.20 and 4.20) and BA 2.0mg/l+ IBA 1.0mg/l gave the highest length of root (2.50 cm, 3.60 cm and 4.40 cm) at 14, 21 and 28 DAI, respectively.

So, finally it can be concluded that, a convenient protocol of rapid regeneration of strawberry is established. From the above summary, the results of the present study indicated that strawberry could be successfully micro propagated with 2.0 mg/l and 3.0 mg/l BA for rapid shoot regeneration and proliferation. BA 2.0 mg/l showed the good performance for shoot induction. IBA 1.5 mg/l and 2.0mg/l gave the best performance in case of root. Considering the finding of the study MS medium supplemented with 2.0 mg/l BA+1.0 mg/l IBA showed the best response for shoot and root formation.

RECOMMENDATIONS

Based on the summary and conclusions following recommendations can be made:

i. For further study BAP, KIN, NAA and IBA etc. types of cytokinin and auxin can be used for more trial.
ii. Except nodal segment other explants like shoot tip, petiole, leaf and root portion could be practiced for culture.
iii. For callus induction, 2, 4-D or other callus induction hormone could be used individually or in combine dose for large number of shoot induction.
iv. Furthermore research can be done with more genotypes of Strawberry.

REFERENCES

Anonymous (2016). Commercial strawberry cultivation on the rise in Rajshahi. https://www.observerbd.com/2016/02/10/135849.php

Bajaj, Y.P.S., Springer, V., Berlin and Tliedelberg, (1991). The effect of nature and origin of explants on micropropagation. Biotec in Agri and Forestry. **17**: 142-167.

Bhandari, A.K., Negi J.S.,Bisht V.K., and Bharti M.K., (2010). *In vitro* propagation of *Aloe vera*-A Plant with Medicinal Properties. *Nature and Science*. **8**(8):174-176.

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

Bickford, P.C., Gould, T., Briederick, L., Chadman, K., Pollock, A., Young, D., Shukitt-Hale, B. and Joseph, J. (2010) Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. *Brain Research* **866**: 211-217

Biswas M, Hossain MB,Ahmed UK, Roy R, Karim MA , Razvy M and Islam R. (2007). Multiple shoots regeneration of strawberry under various colour illuminations, American-Eurasian Sci Res, **2** (2): 133-135.

Biswas, M. K., Roy, U. K., Islam, R. and Hossain, M. (2010). Callus culture from leaf blade, nodal, and runner segments of three strawberries (*Fragaria* sp.) clones. Turk *J. Biol.* **34**:75-80.

Bo, C., Roest, S. and Bokelmann, G.S. (2005). Mutation breeding of *Dendranthema grandiflorm* Snow ball using *in vivo* and *in vitro* technoiques. Euphytica. **25**:11-19.

Borkowska, B. (2001) Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. *Scientia Horticul- turae* **89**: 195-206

Boxus, P. (1974) The production of strawberry plants by *in vitro* micropropagation. *Journal of Horticultural Sciences* **49**: 209-210

Bozena, B. (2001). Morphological and phylosiological characteristics of micropropagationed strawberry plants rooted in vitro or ex vitro. *Scientia Hort.* **89**: 195-206.

Chao yanjie, (2011). Callus induction and plant regeneration from shoot tip explants of strawberry. *Class 2 of Biotechnology, College of Life Science and Technology, Huazhong Agricultural University,* Wuhan 430070, China

Childers, N.F. (1980) Foreward. In: Childers NF (Ed) *The Strawberry: Cultivars to Marketing*, Horticulture Publishers, Gainesville, FL, p ix

Debnath, S.C. (2003) Micropropagation of small fruits. In: Jain SM, Ishii K (Eds) *Micropropagation of Woody Trees and Fruits*, Kluwer Academic Publishers, Dordrecht, Germany, pp 465-506.

Doliński, R. and Olek, A. (2001) Micropropagation of sweet potato (*ipomoea batatas* (l.) lam.) from node explants. *Acta Sci. Pol., Hortorum Cultus* **12**(4): 117-127.

Flick, C. E., Evans, D. A.(1998), and Sharp, W.R. (1983). Organogenesis, in Handbook of Plant Cell Culture, vol. I (Evans, D. A., Sharp, W. R., Ammitato, P. V., and Yamada, Y. eds.), MacMillan, New York, pp. 13-81.

Geethalakshmi, S., Hemalatha, B., Saranya, N Optimization of Media Formulations for Callus Induction, Shoot Regeneration and Root Induction in *Nicotiana benthamiana*. *J Plant Sci Res*. **3**(1): 150.

Giampieri F, Alvarez-Suarez JM, Gasparrini M, Forbes-Hernandez TY, Afrin S, Bompadre S, et al.(2012) Strawberry consumption alleviates doxorubicin-induced toxicity by suppressing oxidative stress. Food Chem Toxicol. (2016); **94**: 128–37.

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

Hannum, S. M. (2004). Potential impact of strawberries on human health: a review of the science. *Crit Rev Food Sci Nutr***. 44**(1):1-17.

Hayashi, M., Kogami, N., Takatuji, M. and Honjo, T. (1997). Growth of strawberry plantlets rooted by the direct *ex vitro* rooting method in environmental con- trol unit. *Society High Tech in Agri* **9**: 67-71

Heinonen, M.I., Meyer, A.S. and Frankel, E.N. (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry* **46**: 4107-4112

Hesami, M. Najafabadi, M. Y. Alizadeh, M. 2018. Effect of plant growth regulators on indirect shoot organogenesis of *Ficus religiosa* through seedling derived petiole segments. *J. Genetic Engineering and Biotechnology*. **16**(1): 175-180

Himstedt, J.P., Jacobsen, H.J., Fisher-Klaver, G., Sorvani, S. and Karshu, S., Kanervo, E. and Pihakaski. (2001). Shoot regeneration from stem and leaf explants of strawberry. Acta-Hort. 421-424 pp.

http://www.wikipedia.org

Ilahi, H and Jabeen, N. 1987. Micropropagation of Zingiber officinale. Pakistan Journal of Botany **19**: 61-65.

Jiajun, L., Yuhua, L., Guodong, D., Hanping, D. and Mingqin, D. (2005) A natural pentaploid strawberry genotype from the Changbai mountains in Northeast China. *Horti Sci* **40**: 1194-1195

Karim M.Z., Amin M.N., Azad M.A.K., Begum F., Islam M.M. and Alam R. (2002). Effect of different plant growth regulators on in vitro Shoot multiplication of *Fragaria*× *ananassa*. Online Journal of Biological Science. **3**(6): 553-560.

Knop, W. (1965) Quantitative Untersuchungen über die Ernahrungsprozesse der Pflanzen. *Landwirtschaftlichen Versuchsstationen* **7**: 93-107

Kozai, T. (1991) Micropropagation under photoautotrophic conditions. In: De- berg PC, Zimmerman RH (Eds) *Micropropagation: Tech and Applica- tions*, Kluwer Academic Publishers, Dordrecht, pp 447-469

Labade, G.B., Dale, N.S., Umbarkar, R.B., Gadhe, S.K. and Rote, Y.N. (2016). *In Vitro* Regeneration of Strawberry *(Fragaria ananassaD.)*. International Information Research and Review. **03**(11): 3043-3045.

Lal, M., Sharma S. and Hegde, M.V. (2003). Micropropagation of strawberry (*Fragaria ananassa* L,). *Indian J.Agric. Res.* **37**: 231-234.

Lines, R., Kelly, G., Milinkovic, M. and Rodoni, B. (2006) Runner certification and virus elimination in commercial strawberry cultivars in Australia. *Acta Horticulturae* **708**: 253-254

Ling, A.P.K., Tan, K. P. and Hussein, S. 2013. Comparative effects of plant growth regulators on leaf and stem explants of *Labisia pumila* var. *alata* . J Zhejiang Univ Sci B. **14**(7): 621–631.

Long V., Vinh B., Don N., Thuy D. and Nhut D. (2006). Microponic technology in disease free chrysanthemum production. Appli Horti. **7**: 67-73.

Martin RR, Tzanetakis IE. High risk strawberry viruses by region in the United States and Canada: implications for certification, nurseries, and fruit production. Plant Dis. 2013; **97**: 1358–62

Mohammed, A and Quraishi. (1999). Clonal propagation of ginger through shoot tissue culture. Pakistan Biolo Sci **2**: 145-147.

Mohan, R., Chui, E.A., Biasi, L.A. and Soccol, C.R. (2005) Alternative *in vitro* propagation: use of sugarcane bagasse as a low cost support material during rooting stage of strawberry cv. Dover. *Brazilian Archives Bio Tech* 48, 37-42

Mohebodini, M. Javaran, M. J. Mahboudi, F. and Alizadeh, H. 2011. Effects of genotype, explant age and growth regulators on callus induction and direct shoot regeneration of Lettuce (*Lactuca sativa* L.). AJCS **5**(1):92.

Molot, P.M., Leroux, J.P. and Nourrisseau, J.G. (1972) Régéneration par culture d'apex de clones de fraisiers infectés de façon chronique par le *Phytophthora cactorum*. In: Actas do III Congresso da Uniao Fitopatologica Mediterranea Oeiras, Portugal, 1972, pp 415-419.

Morel, G. (1960) Producing virus free cymbidiums. *American Orchid Society Bulletin* **29**, 495-497.

Morozova, T. (2002). Genetic stability of Pure lines of *Fragaria vesca* L. in micro propagation and long-term storage *in vitro*. *Acta Hort*. **567**: 85-87.

Mullin, R.H., Smith, S.H., Frazier, N.W., Schlegel, D.E. and McCall, S.R. (1974) Meristem culture of strawberries free of mild edge, pallidosis and mottle disease. *Phytopathology* **64**, 1425-1429

Murashige, T. and Skoog, F**.** (1962) A revised medium for rapid growth and bioas- says with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473-497

Nalini R. (2012). Microprogation on Strawberry (*Fragaria ananassa D*) using shoot tip as explant. International Food Agri and Veterinary Sci. **2**(2): 62-66.

Nhut DT, Takamura T, Watanabe H, Tanaka M (2000) Light-emitting diodes (LEDs) as a radiation source for micropropagation of strawberry. In: Kubota C, Chun C (Eds) Transplant Production in the 21st Century, Kluwer Academic Publisher, The Netherlands, pp 114-118.

Nhut, D.T., Don, N.T., Vu, N.H., Thien, N.Q., Thuy, D.T.T., Duy, N. and Teixeira da Silva, J.A. (2006) Advanced technology in micropropagation of some important plants. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Bio- tech: Advan and Topical Iss* (1st Edn, Vol II) Global Science Books, London, pp 325-335.

Nhut, D.T., Takamura, T., Watanabe, H., Okamoto, K. and Tanaka, M. (2003) Response of strawberry plantlets cultured *in vitro* under super bright red and blue light-emitting diodes (LEDs). *Plant Cell, Tissue and Organ Culture* **73**: 43-52

Posnette, A.F. (1953) Heat inactivation of strawberry viruses. *Nature* **171**: 312

Rahman, M. A., Alam, M.A., Hossain, M.R., Hossain, A. and Afroz, R. (2010). *In vitro* regeneration of popular tobacco varieties of Bangladesh from leaf disc. *Bangladesh J. Agril. Res*. **35**(1): 125-134.

Rajora, R. K. Sharma, N. K. and Sharma, V. (2013). Effect of plant growth regulators on micropropagation of *Catharanthus roseus.* Intel. J. Advanced Biotech. and Res. **4**(1) :123-130

Sarkar, R.H. and Shaeen, N. (2009). *In vitro* propagation of strawberry (*Fragaria ananassa* Ramat) through callus culture. Plant Tissue Culure. **11**(1): 85-91.

Sen, J., Kalia, S. and Mukherjee, S.G. (2002). Level of endogenous free amino acid during various stages of culture of *Vigna mungo* (L) somatic embryogenesis, organogenesis and plant regeneration. Cun: Sci. **82**(4): 429-433.

Shatnawi M.A. (2006). Micropropagation and germplasm storage of *Prunus amygdalus* by the vitrification method. Jordan Agri Sci. **2**(3): 222-233.

Shukla, M.R., Jones, A.M.P., Sullivan, J.A., Liu, C.Z., Gosling, S., Saxena, P.K. (2012). *In vitro* conservation of American elm (*Ulmus americana*): potential role of auxin metabolism in sustained plant proliferation. *Can J. Forest Res*. **42**:686–697.

Sowik I, Bielenin A, Michalczuk L (2001) *In vitro* testing of strawberry resistance to *Verticillium dahliae* and *Phytophthora cactorum*. *Scientia Horticul- turae* **88**: 31-40

Sowik, I., Wawrzynczak, D. and Michalczuk, L. (2003) *Ex vitro* establishment and greenhouse performance of somaclonal variants of strawberry selected for resistance to *Verticillium dahliae*. *Acta Horticul* **616**: 497-500

Staudt, G. (1999) *Systematics and Geographical Distribution of the American Strawberry Species: Taxonomic Studies in the Genus Fragaria (Rosaceae: Potentilleae)*. University of California Publications in Botany, Berkeley, CA, **81**: 122

Suvalaxmi Palei , Arun Kumar Das1 and Gyana Ranjan Rout (2002) Department of Fruit Science, 2Department of Agriculture, Biotechnology, College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar- 751003, Odisha, India*Email: Pl *Sci Res* **31**(2) 115-131 2015

Wang, H., Cao, G. and Prior, R.L. (1996) Total antioxidant capacity of fruits. *Agri. and Food Chem.* **44**: 701-705

Waseem, K., Jilani, M.S. and Khan, M.S. (2009). Rapid plant regeneration of strawberry (*Fragaria×ananassa D.*) through shoot tip culture. African Biotech. **8**(9): 1871-1877

Yokoya, N. S., West, J.A. and Luchi, A. E. (2006). Effects of plant growth regulators on callus formation, growth and regeneration in axenic tissue cultures of *Gracilaria tenuistipitata* and *Gracilaria perplexa* (Gracilariales, Rhodophyta). Phycological research. 27.

Zhou, R.J. and Liu, M.J. (2009). Effect of plant growth regulators on tissue culture in chinese jujube. Acta Hortic. 840: 309-314.

APPENDICES

Appendix I. Analysis of variance (ANOVA) of effect of different concentration of BA on days to shoot initiation, percent of shoot and number of shoot at different DAI

Source of variance	d.f.	Days to shoot induction	Percent (%) Shoot initiation	Number of Shoot		
				14 DAI	21 DAI	28 DAI
Treatments	5	71.600	981.33	0.993**	1.973**	4.353**
Error	24	0.383	120.00	0.266	0.5	0.816
Total	29					

**= Significant at 1% level of Probability.

Appendix II. Analysis of variance (ANOVA) of effect of different concentration of BA on length of shoot at different DAI

Source of variance	d.f.	Length of Shoot (cm)		
		14 DAI	21 DAI	28 DAI
Treatments	5	0.660**	1.481**	0.908**
Error	24	0.0105	0.014	0.018
Total	29			

**= Significant at 1% level of Probability.

Appendix III. Analysis of variance (ANOVA) of effect of different concentration of BA on number of leaves at different DAI

Source of variance	d.f.	Number of leaves		
		14 DAI	21 DAI	28 DAI
Treatments	5	1.84**	10.213**	34.14**
Error	24	0.333	0.483	0.733
Total	29			

**= Significant at 1% level of Probability.

Appendix IV. Analysis of variance (ANOVA) of effect of different concentration of IBA on days to root initiation, percent of root and number of root at different DAI

Source of variance	d.f.	Days to root initiation	Percent of root initiation	Number of root		
				14 DAI	21 DAI	28 DAI
Treatments	5	128.213**	67.267**	1.073**	2.533**	6.533**
Error	24	1.033	13.432	0.283	0.25	0.5
Total	29					

**= Significant at 1% level of Probability.

Appendix V. Analysis of variance (ANOVA) of effect of different concentration of IBA on length of root at different DAI

Source of variance	d.f.	Length of root (cm)		
		14 DAI	21 DAI	28 DAI
Treatments	5	2.349**	2.842**	3.225**
Error	24	0.009	0.015	0.021
Total	29			

**= Significant at 1% level of Probability

Appendix VI. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to shoot initiation, % of shoot initiation and number of shoot at different DAI

Source of variance	d.f.	Days to shoot initiation	Percent of shoot initiation	Number of Shoot		
				14 DAI	21 DAI	28 DAI
Treatments	25	21.788**	425.477**	0.288**	0.820**	1.40**
Error	104	0.850	140.000	0.276	0.330	0.35
Total	129					

**= Significant at 1% level of Probability.

Appendix VII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on length of shoot at different DAI

Source of variance	d.f.	Length of shoot (cm)		
		14 DAI	21 DAI	28 DAI
Treatments	25	1.986**	1.977**	2.552**
Error	104	0.0204	0.0261	0.0218
Total	129			

**= Significant at 1% level of Probability.

Appendix VIII. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on number of leaves at different DAI

Source of variance	d.f.	Number of leaves		
		14 DAI	21 DAI	28 DAI
Treatments	25	7.020**	13.836**	32.178**
Error	104	0.361	0.588	0.796
Total	129			

**= Significant at 1% level of Probability.

Appendix IX. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on days to root initiation, percent of root and number of roots at different DAI

Source of variance	d.f	Days to root initiation	% Root initiation	Number of root		
				14 DAI	21 DAI	28 DAI
Treatments	25	84.229**	593.231**	0.792**	1.468**	3.435**
Error	104	1.031	43.077	0.2730	0.296	0.388
Total	129					

**= Significant at 1% level of Probability.

Appendix X. Analysis of variance (ANOVA) of combined effect of different concentration of BA and IBA on length of root at different DAI

Source of variance	d.f.	Length of root		
		14 DAI	21 DAI	28 DAI
Treatments	25	2.115**	3.828**	4.294**
Error	104	0.0205	0.026	0.0408
Total	129			

**= Significant at 1% level of Probability.