



## **In Vitro, Propagation of Strawberry (*Fragaria × ananassa* Duch.) Through Organogenesis via Runner Tips**

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Received for publication: January 15, 2014; Accepted: January 27, 2014.

**Abstract:** The cultivated strawberry (*Fragaria × ananassa* Duch.), a member of the Rosaceae, is the most important soft fruit worldwide. *In vitro* techniques are important for clonal multiplication. An efficient method for sterilization, browning, shoot regeneration and root formation from runner tips of strawberry plantlets cv. Sweet Charlie was developed. Runner tips of 1-2 cm long were used as source of explants. In sterilization method, when the runner tips were immersed in sodium hypochlorite solution (1.5%) containing two to three drops of Tween 20 per 100 ml for 20 minutes and 0.1% HgCl<sub>2</sub> for 10 minutes gave the maximum aseptic cultures in August, however, in October all the samples were failed to reduce explants contamination. After surface sterilization apical meristems of 3-5 mm long were isolated and used as explants. To study the effect of various pretreatments on explants browning and survival percentage, different sets of sterilized explants were dipped in three different concentrations of antioxidant solution (1, 1.5 and 2% PVPP) for 1-2 h prior to inoculation with addition of (0.2g/100 ml) PVPP in the MS free hormone medium and subjecting cultures to an initial ten days cold treatment before transfer the explants to growth chamber, the results indicated that percentage of survival explants when treatment with 1% and 2% PVPP were 20 and 60% respectively in August season. However, when repeated the same experiment in October season, with addition to another set dipped in sterilized distilled water without antioxidant solution as a control, the lowest percentage of browning were observed in the control and 2% PVPP were 100 and 70 % respectively. At multiplication stage results indicated that the highest auxiliary buds were observed when MS medium supplemented with 1 mg / l BA. At rooting stage, it was clear visually that MS medium supplemented with 0.5 g/l activated charcoal and MS medium at full strength gave better results in enhanced the root number and length and shoot number and length per plantlet. Effect of IBA on the root response, the results indicated that IBA with (0.5, 1.0, 1.5 mg/l) observed the lowest root response compared to all other treatments.

**Keywords:** Propagation, Strawberry, *Fragaria*, Tissue culture, Benzylaminoburine (BA), Polyvinylpyrrolidone (PVPP), Activated charcoal (AC).

### **Introduction**

The strawberry is an herbaceous perennial member of the family rosaceae. The garden strawberry (*Fragaria x ananassa* Duch), originated in Europe around 1750 as a hybrid between the pistillate South American *Fragaria chiloensis* Duch and a North American *Fragaria virginiana* Duch. For many centuries before, 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 and Sagen, 1974). Following further hybridizations, especially since 1850, *Fragaria x ananassa* has developed into the large, fragrant, tasty red fruit that is now cultivated worldwide. The high degree of genetic heterozygosity present in *Fragaria spp.* enabled the development of strawberry cultivars adapted to widely varying environment conditions and resistant to several diseases and pests. Not only the genetic variability, but also a high adaptability

and plasticity of the strawberry plant itself give this crop such a remarkable range of adaptation (Darrow, 1966). Strawberry is cultivated all around the world, not only for its digestive and tonic properties, but because of the nutritional value of its fruits, important source of folate, vitamin C, fiber, potassium, flavonoids, autocyanidin, phytochemicals and antioxidants. Prior experiences with strawberry micropropagation indicate that vitroplants are more uniform, produce higher number of runners, have better survival in the field, and the fruit yield increases in 24% than plants propagated by the traditional method (Kikas *et al.*, 2006). So far, strawberry regeneration has been obtained by several procedures: In 1974, Boxus first described an *in vitro* method for the mass propagation of strawberry through axillary shoot multiplication. The procedure is now used commercially in many countries (Zimmerman, 1981; Boxus, 1989) direct regeneration was produced with stipules (Jemmali *et al.*, 1992; 1994; Monticelli, *et al.*,

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1995), leaf explants (Nehra *et al.*, 1989), runners (Liu and Sandford, 1988), petioles (Jones, *et al.*, 1995), cotyledons (Miller and Chandler, 1990) or callus from leaf blades (Nehra, *et al.*, 1990). In spite of encouraging research reports, *in vitro* propagation of strawberry species from a more diverse range of cultivars still poses problems. Poor response of explants from to *in vitro* manipulation is associated with sterilization and exudation of phenolic compounds produced by injured explant tissues (Compton and Preece, 1986) which results in browning of cultured tissues. The *in vitro* culture of strawberry species is frequently hampered by browning and necrosis of tissue, particularly when cultures are initiated. Various approaches have been suggested to reduce this problem including pretreatment of explants with antioxidants, incorporation of antioxidants with culture medium and frequent subculturing to fresh medium (Hu and Wang, 1983).

Various factors such as sterilization, browning, season, media type and plant growth regulators influence the micropropagation of strawberry plant. These factors have successfully been addressed after addition of some media additives, collection of the explant at right season, standardizing the media formulation and use of plant growth regulators at varying concentrations.

The importance of stock plant treatment in plant tissue culture is critical point, however, its role in controlling oxidative browning in tissue culture of strawberry species has not been reported.

Gaza Strip lies on the eastern coastal of the Mediterranean Sea. Planting strawberry was mainly planted in the north Gaza. Despite the relatively small area of farmland used for sector, it is considered that enjoy high economic and social value. Strawberries annual income is estimated at \$US 10-12 million. However, there are some problems faced planting strawberry in Palestine a) Inability to grow high quality strawberry mother plants in local greenhouse. It is totally relayed on Israeli companies to do that. B) Strawberry mother plants which are got from Israeli companies are expensive. C) Strawberry is planted in one governorate in Gaza Strip. And D) Strawberry is exported

through Israeli companies since Palestinian companies are not able to do that.

The strawberry plant used in this investigation were chosen on the basis of being the most commercially successful varieties currently grown in the Gaza strip, In addition cv. Sweet Charlie was selected for two reasons: it is the most economically important cultivar currently grown in Israel and Gaza strip and it has many characteristics that could be improved in a genetic modification programme. It is generally agreed that plants regenerated from shoot tips or buds are genetically stable and free from somaclonal variations associated with plants differentiated from callus (Abdullah *et al.*, 1989).

The present study was initiated to examine stock plant treatment and mode of culture initiation as a means of controlling contamination and oxidative browning and its consequences on the *in vitro* survival of explants in the course of establishment and the effect of certain plant hormones to produce seedlings to be economically acceptable commensurate with the needs of the geographical reality of the Gaza Strip.

## Materials and Methods

### Source of Explants

Mother plants of strawberry (*Fragaria x ananassa* Duch.) cv. Sweet Charlie grown in the farm land of Bith Lahia city, Gaza Strip, Palestine, were used as source of explants during this study. Runner tips of 1-2 cm long were taken from mother plants as that long was suitable for sterilization procedures.

### Surface Sterilization of Explants

Explants were rinsed under running tap water for 30 minutes. For surface sterilization, explants were immersed in different concentrations (0.5, 1, 1.5, 2.0 and 2.5%) of sodium hypochlorite solution containing two to three drops of Tween 20 per 100ml for 20 minutes. The plant materials were then, rinsed three times with distilled water and were treated with 0.1% mercuric chloride solution for 10 minutes. The explants were then thoroughly washed (4-5 washings) with sterilized distilled water to remove the traces of HgCl<sub>2</sub>. After sterilization, explants were shortened to remove the surfaces of explants and meristems of 3-5 mm long were isolated as final explants. These explants

were cultured with good contact on the surface of the medium.

### **Culture Medium**

The media was formulated in the lab according to the composition of Murashige and Skoog (1962) referred as MS medium was selected as the optimal culture medium including (0.2%) PVPP.

### **Sterilization of the Nutrient Media**

Following preparation of the medium and prior to addition of agar, the pH was adjusted to 5.8. The medium was poured into culture jars of size 350ml where each jar contained 30 ml of the medium and capped with plastic caps. The culture jars were autoclaved at 121°C and 1.2kg/cm<sup>2</sup> air pressure for 20 minutes.

### **Effect of Pretreatments on Explant Browning**

To study the effect of various pretreatments on explant browning and survival percentage, different sets of sterilized explants were dipped in three different concentrations of antioxidant solution (1, 1.5 and 2% PVPP) and another set dipped in sterilized distilled water without antioxidant solution as a control. Meristem tips were cultured on hormone-free MS solid medium to establish clean and healthy materials for next experiments. Cultures were kept for chilling treatment at 4°C for 10 days and then incubated for 4 weeks in the growth room. All cultures of this study were incubated at 25°C ± 2 day and night. Light was provided by fluorescent tubes giving an intensity of 1500 lux at the level of culture vessels for 16 hours per day.

### **Multiplication Stage**

Meristem - derived plantlets were multiplied by sequential subculturing into fresh medium in 300 mL jars each with 30 mL of culture medium supplemented with 1.0 mg/L BA hormone and closed with plastic caps. At this stage of culture, the shoots proliferated rapidly within one month. Two subcultures were then conducted.

### **Rooting Stage**

**Effect of Different Strengths of MS Medium:** Shoots of strawberry obtained from multiplication stage were cultured on different strengths of MS, 1/8, 1/4, 1/2, and full strength of MS medium. All treatments of this set of experiment contained hormone free

media. After 4 weeks, data were recorded as, root number/plant, root length, shoot length and fresh / plant.

**Effect of Different Concentrations of IBA:** Shoots of strawberry obtained from multiplication stage were cultured on different concentration of IBA, 0.5, 1.0, and 1.5mg/L. After 4 weeks, data were recorded as, root number/plant, root length and shoot length and fresh /plant.

**Effect of Different Concentrations of Activated Charcoal:** Shoots of strawberry obtained from multiplication stage were cultured on different concentration of activated charcoal, 0.2, 0.5 and 1.0 mg/L. All treatments of this experiment contained hormone free media. After 4 weeks, data were recorded as, root number/plant, root length and shoot length and fresh /plant.

## **Results and Discussions**

### **Source of Explant**

*In vitro* culture of strawberry greatly affects the quality of material obtained and its field performance (Lopez *et al.*, 1994). Generally, cultures are most easily established from explants harvested from the active growth of the mother plant. The best time for collecting explants is usually when stock plants are producing new vigorous shoots (i.e. in temperate regions, in spring to early summer), but many exceptions have been observed. The optimum time for collecting explants may depend on the kind of culture, on the particular genotype to be used, on the relative amounts of contamination, and browning of the explant occurring at each season (George, 2008). However, in our experiment it was observed that establishment stage *in vitro* culture of strawberry depends on several factors such as: the quality of material obtained, shoot collecting season of explants, contamination treatment without any effect on the explants and reduction browning due to reaction between the phenol exudation secretion from the explants and some chemical reagent such as sodium hypochlorite.

### **Surface Sterilization of Explants**

In the experiments reported here, both chemicals gave good reduction of bacterial contaminants and gave the maximum aseptic cultures and maximum explant browning in cv. Sweet Charlie except the treatment with 1.5% Sodium hypochlorite

which gave the best results, especially, when the explant treatment with 1 and 2% PVPP for 1-2 h before culture, the browning percentage were 80 and 40% respectively, and survival percentage were 20 and 60% respectively in August season (Table 1).

Moutio *et al.*, (1999) explain that none of the surface-sterilizing agents used achieved total elimination of bacterial contaminants when using sodium hypochlorite alone and mercuric chloride alone, and they demonstrated that the disinfection with 1% mercuric chloride gave 56.3% clean cultures as opposed to 46.8% with 2.7% sodium hypochlorite. However, in our experiment we think that the combination with two treatments increase the reduction of contamination. According to these notes in our experiment is acceptable that we could reach the suitable concentrations in sterilization and browning methods. By this way we have got survival explants. While the same experiment was applied on the explants but was collected in October season and from other field, we observed that all the samples were failed to reduce explants contamination. We believed that these results depend on the selection of the material in the field and choice a good farm land which used better bacterial and fungal treatment before and after culture, or that the shoot collection season proved to play important role in reducing contamination. Boxus (1999) reported some ways to select the best mother plants in the field. The very young runner tips are the best plant material, and must be collected in a fruit production field in June, before the runners have formed leaves and roots. This very young material has fewer hairs surrounding the meristematic zone and results in little contamination. Swartz *et al.*, (1981) noted that the contamination problems occurred with cultures initiated during late Summer and Autumn, or some laboratories keep the mother plants in screen houses for several years, and always use the same plants for the meristem excision. Another investigation, in our study the shoot collection season plays an important role in reduction of browning and bacterial contaminants. Indra *et al.*, (2004) reported that shoot collection season proved to play important role in reducing percent browning and inducing bud break. Explants collected during winter (November - December) gave the maximum response as measured by percentage of explants with axillary bud break

*in vitro*, and they observed that seasonal changes greatly influence explant establishment and cultures could not establish in rainy season due to heavy fungal and bacterial contamination. The effect of tissue culture on leaf formation was studied by Zebrowska *et al.*, (2003) who revealed that number of leaves per microplant in the Autumn after planting and in the next year of cultivation was significantly higher in comparison to the standard propagate seedlings.

**Table 1:** Surface sterilization treatments for the elimination of bacterial contaminants in *in vitro* bud culture of strawberry cv. Sweet Charlie

Sterilizing agent	Percentage of explant contamination	Percentage of explant browning (1% PVPP)	Percentage of explant browning (2% PVPP)
Sodium hypochlorite 1.0%	0	100	100
Sodium hypochlorite 1.5%	0	80	40
Sodium hypochlorite 2.0%	0	100	100
Sodium hypochlorite 2.5%	0	100	100

#### **Effect of Pretreatment on Explant Browning**

The results of the present study demonstrated that the concentration of PVPP was beneficial to reduce exudates and inhibit the browning in the October season, the highest percentage were observed in the control and 2% PVPP compared with 1.5% and 1% PVPP respectively (Table 2) and low survival percentage for all treatments, these results are considered abnormal. However, the results can be explained on the basis that PVPP 0.2g/100ml when added into medium and subjecting cultures for ten days to cold treatment before transfer the explants to growth chamber was found most effective resulting in 100% browning inhibition in control. This finding is consistent with the fact that addition of PVPP into the media alone able to eliminate and prevent the browning. It is known that cell death is correlated with elevated levels of peroxides during tissue browning; in addition there are many factors able to induce browning after the explants treatment such as temperature and light intensity through the experiment.

**Table 2:** The effect of PVPP in the browning inhibition. Clear = (without browning), moderate = (moderate browning), high = (high browning).

Time	1% PVPP	1.5% PVPP	2% PVPP	Control
6 days	3 clear	5 clear	7 clear	10 clear
	7	3	2	0
	moderate	moderate	moderate	moderate
	0 high	2 high	1 high	0 high
12 days	2 clear	4 clear	7 clear	10 clear
	7	4	2	0
	moderate	moderate	moderate	moderate
	1 high	2 high	1 high	0 high

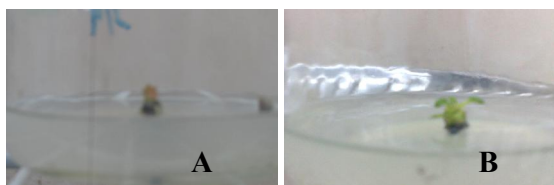
In some plants higher peroxidase activity is induced by mechanical injury, environmental stress, H<sub>2</sub>O<sub>2</sub>, and polyphenols (Wei *et al.*, 2004). We speculate that wounding derived from these stress responses may be associated with the ability of peroxidase to detoxify oxidative damage and cause cell death, as well as subsequent tissue browning. When antioxidants were added to the plant tissue-culture medium, they reacted with polyphenols and decreased the concentration of harmful compounds. Antioxidants reduced the accumulation of peroxidase in strawberry tissue and protected cells from oxidative damage. Antioxidants also reduced the inhibition of polyphenols to cell growth and shoot formation; therefore, the frequencies of runner tips induction, shoot differentiation, and rooting were increased. The results from this study indicate the browning phenomenon in strawberry runner tissue can be greatly reduced by addition of 0.2g/100ml PVPP into the medium culture. Also, incubation in the cold at 4°C for the first 10 days may arrest the rate of tissue browning by slowing the enzymatic activity responsible for tissue oxidation (Table 1). Frequent subculturing to fresh medium may also assist so that toxic phenolic compounds do not hinder the activity of plant growth regulators on tissues. Madany *et al.*, (2007) show that addition of 0.2 % activated charcoal was better than 2% PVPP to eliminate inhibitory substances from *in vitro* cultures of (*Fragaria x ananassa* Duch.) cv. Camorosa and Selva. PVPP added in the medium was also found effective in explant browning prevention from earlier reports on guava and *Cleistanthus collinus* (Indra, *et al.*, 2004). Media containing only nitrogen as nitrate are used for the shoot culture of some plants, for example strawberry (Boxus, 1974), which can be cultured with 10.9 mM NO<sub>3</sub> alone; supplementing the medium with 6 mM NH<sub>4</sub><sup>+</sup> causes phytotoxicity. However,

shoot cultures of strawberry grown without NH<sub>4</sub><sup>+</sup> can become chlorotic: adding a small amount of NH<sub>4</sub>NO<sub>3</sub> (or another source of reduced nitrogen) to the medium at the last proliferation stage, or to the rooting medium, may then give more fully developed plants with green leaves. On some occasions it is necessary to eliminate or reduce NH<sub>4</sub><sup>+</sup> from the medium for shoot cultures to prevent hyperhydricity (George *et al.*, 2008). We believe that incubation the culture in the cold at 4°C for the first 10 days preventing high nitrogen content in the MS medium and alleviated the problem of browning and toxicity. This finding further supports the importance of this treatment in promoting organogenesis in strawberry. In that concern, Passy *et al.*, (2003) focused that pre-chilling of stock material at 4°C enhanced subsequent shoot regeneration.

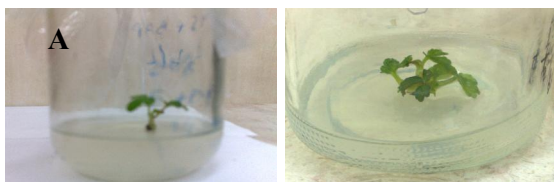
After transfer the samples to the growth room the runner tips starts swelling and about two weeks later, the excised meristem tips have developed into a rosette of leaflets, and has developed to a size of a few millimeters (Figure 1. A, B). Within 3 - 4 weeks after this excision, the shoots reach to 2-3 centimeters (Figure 2. A, B), transfer to MS propagation media supplemented with 1mg/l BA hormones. Within 3 - 4 weeks after this transfer, two or three new buds appeared at the base of the petioles of the oldest leaves. These young axillary buds grow very quickly and, in turn, produce new axillary buds. After 6. 8 weeks the axillary buds invade the base of the petioles of the oldest leaves (Figure 3). The initial plantlet is transformed into a cluster of buds, these clusters may contain more than 15. 25 small buds. No roots are present on these buds.

### **Multiplication Stage**

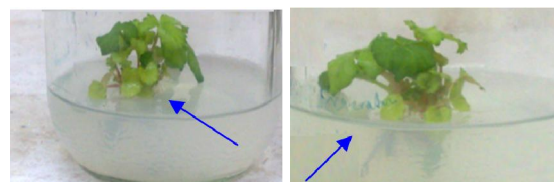
At this stage, the clusters of buds may be aseptically separated and transferred onto a fresh medium to continue the clonal propagation. Every 6. 8 weeks, small portion of the isolated buds produced high number of new axillary buds (Figure 4). During the second division of the clusters, only a small fraction of the isolated buds is transferred to proliferation medium to continue the propagation.



**Figure 1:** (A) Swilling of runner tips explants of cv. Sweet Charlie on MS medium free hormone after 8 days of culture. (B) Development of bud from runner tips explants (arrow) after 15 days of culture.



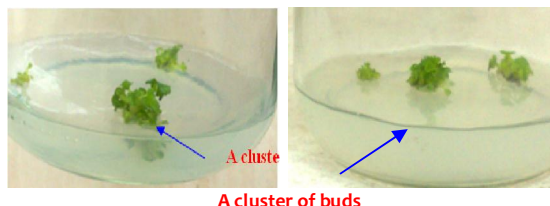
**Figure 2:** (A). Runner tips explants of strawberry in MS media free hormone after 4 weeks culture the leaf reach to 2-3 cm. (B). Explant after 6 weeks in MS media supplemented with 1.0mg/l BA.



**Figure 3:** Explant in multiplication media after 8 weeks, the axillary buds invades the base of the petioles of the oldest leaves (first division), (arrow) A cluster of buds.

In our experiments the multiplication stage play an important role in micropropagation process, our results indicated that medium contained 1.0 mg/l BA alone proved to be the best for shoot formation, shoot number and number of buds. The results are in agreement with those of Emara (2008). WHO reported that BA alone at 1.0 mg/l significantly showed the highest record of shoot number followed by the treatment contained 1.0 mg/l BA and 0.1 IBA that a significant similar response. Boxus (1999) revealed that when shoot tips of strawberry were cultured on a medium supplemented with 0.5 mg dm<sup>-3</sup> BA, 0.1 mg dm<sup>-3</sup> GA3 and 6.4 g dm<sup>-3</sup> agar, shoots were multiplied, while Marcotrigiano *et al.*, (1984) found that BA at 0.3 mg/l was as effective for shoot proliferation as 1 and 3 mg/l. The importance of BA, in strawberry shoot formation, was also reported by Lal *et al.*, (2003) who found that the maximum number of shoots per explant was observed in MS medium supplemented with BAP at 4.0 mg/l. The results can be explained on the basis that different plants and even different organs of

the same plant are characterized not only by their unique intrinsic biochemical make-up but also by the sensitivity of the endogenously supplied chemical stimuli.



**Figure 4:** Induction of multiple shoots from Runner explants on MS medium supplemented with 1.0 mg/L BAP, after 12 weeks of culture.

Economically, the division within isolated buds is not very easy; it takes a lot of time. Therefore, after one or two transfers on proliferation medium, the cluster are now divided more roughly into four to five small tufts of buds. To maintain a high proliferation rate, the transfers must be done immediately after the appearance of the first roots. In this way, the proliferation rate is about four- to five fold per month, i.e., several million from one meristem in only one year, the results are in agreement with those of (Anon., 1982). Lopes *et al.*, (1994) explained that the number of subcultures (3-8) did not affect productivity; in accordance with another observation, they pointed out that problems arise when using material with over 10 subcultures, although Sen and Dhawan (2010) concluded that the tissue culture raised plantlets of strawberry are genetically identical and clonally uniform at least up to the 15th culture passage.

#### **Effect of Different Strengths of MS Medium**

The results on the effect of MS strengths on root number and root length indicated that, highest response was obtained with full MS followed by 1/2 MS, but 1/4 and 1/8 MS came in the second order. In case of full MS the root number, root length and shoot elongate was high response, but 1/2 MS the root number was high but root length and shoot elongate was less than full MS. Lower responses were obtained with the 1/4 and 1/8 MS with respect to root number and root length, but the shoots remained too short for rooting.

### **Effect of Different Concentrations of Activated Charcoal**

The effect of different concentration of AC on root number and root length revealed that the highest response was obtained with 0.5 mg/L, similar to full MS, all other concentration showed lower effect in elongation of both the roots and shoots.

### **Effect of Different Concentrations of IBA**

Regarding the effect of IBA on the root response, the results indicated that the IBA with (0.5, 1.0, and 1.5 mg/L) showed the lowest roots response compared to all other treatments. These findings are somewhat similar to those previously reported by Emara (2008). WHO studied the effect of MS strengths on root length, who reveal that the highest significant record was obtained with full MS, all other strengths showed lower effect. In that concern, some investigations did not recommend full MS for strawberry rooting, Kaushal *et al.*, (2006) found that rooting of strawberry was done in MS half strength with 1.0 mg/l IBA and 0.2 mg/l activated charcoal. The same strength was recommended for strawberry rooting by Yonghua *et al.*, (2005) WHO reported that half strength MS containing 1.0 mg/l AgNO<sub>3</sub> was optimum medium for rooting. Kikas *et al.*, (2006) found that high salt concentration of MS promoted active proliferation but the shoots remained too short for rooting, and with low salt concentration of MS, the proliferation rate was lower but shoots elongated enough. Gautam *et al.*, (2001) indicated that the highest root induction frequency obtained was 95.23% on 1/4 MS medium with IBA at 1.0 mg/l and charcoal (200 mg/l). However, Lal *et al.*, (2003) found that maximum rooting was obtained in both full and half strength MS medium supplemented with IBA at 1.0 mg/l. Mereti *et al.*, [160] found that the highest percentages of rooting were achieved in MS medium contained 10 µM IBA (92%) and 10 µM IAA (82%). In that concern, Kikas *et al.*, (2006) found that high salt concentration of MS promoted active proliferation, but the shoots remained too short during rooting period, but with low salt concentration of MS, the proliferation rate was lower but shoots elongated enough. Damiano *et al.*, (1997) found that the addition of 1 or 2 g of activated charcoal per liter to MS medium promotes elongation of both the shoots and roots of strawberry.

Root formation and growth were better in BA-free medium containing activated charcoal than in the medium without activated charcoal. Similar results were observed in bulb formation. This could be due to activated charcoal regulating internal physiological processes. It is also possible that a substance(s), which antagonizes cytokinin activity, was adsorbed by activated charcoal (Pan *et al.*, 1998). Other research has shown that activated charcoal had an effective role in absorption of unknown components, which were produced through chemical processes within the media. Sometimes these unknown components played the role of growth inhibitor at morphogenesis stages. In addition, the activated charcoal, which eliminated light and provided a reasonable physical environment for the rhizosphere, prevents undesirable callusing and helps rooting (Nissen *et al.*, 1990). The poor root stimulation in our experiment may possibly be due to a hormonal imbalance caused by accumulation of auxin in leaf tissues. Contrary to our observation, in other genotypes of strawberry (Borkowska, 2001) who studied the micropropagation of strawberry cultivars Senga sengana, Kent and Kama were rooted by a medium supplemented with 1.0 mg/L IBA. These possibility are somewhat similar to those previously reported by Nehra *et al.*, (1994) whereby it was noted that the calli from *in vitro* strawberry cv. Redcoat leaf explants had very poor shoot regeneration because the hormonal imbalance due to maintained the explant on a high cytokinin medium prior to explant preparation.

It is notable that using a full strength MS media free hormone on establishment and rooting stage of strawberry give us optimal results. We think that the hormones which present in the strawberry plant play an important role in shoot and roots stimulation. These possibilities are somewhat similar to those previously reported by Jammaly *et al.*, (2002) whereby it was noted that the six cytokinines and two auxins were isolated and identified in axillary shoots in strawberry. And they observed that Benzyladenine and riboside were the major cytokinines whereas the free IAA and its conjugate IAA aspartate showed higher concentrations in axillary shoots that means the amount of hormones inside the strawberry tissues gave optimal growth conditions in shooting and rooting response.

### Conclusion

The maximum aseptic cultures was obtained when the runner tips were immersed in sodium hypochlorite solution (1.5%) containing two to three drops of Tween 20. per 100ml for 20 minutes and then in 0.1 % HgCl<sub>2</sub> for 10 min gave the maximum aseptic cultures and maximum explant browning in except the treatment with 1.5% Sodium hypochlorite which gave the better results, especially, when the explant treatment with 1% and 2% PVPP for 1-2 h before culture, the browning percentage were 80 and 40% respectively, and survival percentage were 20 and 60% respectively in August season. However, in October season all the samples were affected with contamination. for reduction of phenolic compounds explants were pre-soaked in antioxidant solution of 1% or 2% PVPP for 1-2 h prior to inoculation with addition of (0.2g/100ml) PVPP in the medium and subjecting cultures cv. Sweet charly during the initial ten days in cold treatment before transfer the explants to growth chamber. The highest percentage were observed in the control and 2% PVPP compared with 1.5% and 1% PVPP respectively, survival percentage were low.

For successful explant establishment, the runner tips apices were cultured for 4 weeks on MS basal medium free hormone, the runner tips starts swelling and about two weeks later, the excised meristem tips have developed into a rosette of leaflets.

For successful explant multiplication, within 3 - 4 weeks after this excision, transfer to MS propagation media supplemented with 1mg/l BA hormone, two or three new buds will appear at the base of the petioles of the oldest leaves (within 3 - 4 weeks after this transfer). After 6.8 weeks the axillary buds invade the base of the petioles of the oldest leaves.

For successful rooted plantlets, the highest response was obtained when transfer the buds to the MS medium full strength and to MS medium supplemented with activated charcoal (0.5g/l), all two media without hormone.

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**Source of support:** Nil

**Conflict of interest:** None Declared