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Research Article

in vitro PROPAGATION OF STRAWBERRY *Via*. LEAF

Khatik, PC, Saharan, V and Kaushik, RA

RCA Campus, MPUAT-Udaipur

A R T I C L E I N F O

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A B S T R A C T

An investigation was conducted to standardized a protocol in vitro propagation via leaf of strawberry using cv. Chandler and Ofra to find out In vitro propagation, leaf explant found superior over shoot tip explant of both cultivars of strawberry with best treatment i.e. MS medium fortified with TDZ (2.5 mg L-1) along with IBA (0.5 or 1.0 mg L-1) and PVP (0.1 mg L-1) for shoot induction and 1.0 BA + 0.5 IBA + 0.1 PVP (mg L-1) with MS medium is best over commercially available HiMedia (PT 117) for shoot multiplication. Whereas, ½MS medium supplemented with 1.0 IBA + 200 charcoal (mg L-1) found best for root induction and hardening media combinations of Cocopeat + Vermiculite + Vermicompost + Sand (1:1:1:1, v/v) results higher survival in field. These findings are reproducible for in vitro propagation of strawberry cultivar Chandler and Of ra. The plantlets, thus developed were hardened and successfully established in soil medium.

INTRODUCTION

The presently Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most luscious, refreshing and soft fruit of the world after grapes. Globally it is most widely distributed fruit crop from temperate, Mediterranean, sub-tropical and extreme northern region due to its genotypic diversity, highly heterozygous nature and broad range of environmental adoptions.

It is a delicious small fruit widely appreciated mainly for its characteristic aroma and its bright red color. It is a rich source of vitamin C, vitamin B_1, protein and minerals like phosphorous, potassium, calcium and iron (Sharma and Sharma, 2004). And possess anti cancerous, anti-aging with having, ability to improve brain power. Among the fruits, it gives quickest return in shortest possible time. Being a shallow rooted fruit crop, it can also be grown easily in kitchen gardens, roof gardens and pots. As cash crop, it may be boon for small landholders.The major producers of strawberry in the world are USA, followed by China and Spain. In India Maharashtra, Jammu & Kashmir, Himachal Pradesh, Uttrakhand, Uttar Pradesh, West Bengal, Parts of Delhi, Haryana, Punjab and other hilly tracts of the country produce sizeable quantity of strawberry (Tyagi *et al.* 2015).

Quality planting material is major hurdles to the farmers. Generally, this crop propagated through runner and reported to contribute 90% of total strawberry production. In Rajasthan, because of very high temperature (above 40°C) during the summer the runners get killed and cost of procuring from other places is very high whereas continuous planting of runners from old mother plants for five or more years that will more prone to diseases and viruses. The lack of ideal planting materials is the main causes of low productivity of strawberry. Therefore, tissue culture technique can fulfill this demand.

Tissue culture is an inclusive term used for the range of procedures used to maintain and grow plant cells and organs in aseptic (or *In vitro*) culture. The technique is used for rapid propagation of plants to meet demand of particular cultivar. Moreover this provides extra insurance against infections, variations and diseases caused by viruses, nematodes and mycoplasma.

The present investigation on "*In vitro* propagation of strawberry (*Fragaria* x *ananassa* Duch.) *leaf*" were conducted in the Plant tissue culture laboratory, Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur (Rajasthan), India. The *in-vitro* experiments were conducted to find out the most appropriate explants (s) and culture media to obtain high materials as well as standardization of the hardening procedure for *in vitro* propagated strawberry plantlets for better field survival.

The data pertaining to these experiments obtained during the course of investigations were analyzed and pooled subsequently to accomplish the objectives. These results have been presented under suitable sub-headings.

To find out the most Appropriate Explants (s) and Culture Media to Obtain high Frequency of in vitro Strawberry

In this experiment, a reproducible and efficient plant regeneration system for Chandler and Ofra genotypes of strawberry have been described and the effect of MS basal media supplemented with various combinations of PGR's (TDZ, IBA, BA, Kn, GA_3) and PVP on in vitro propagation

Corresponding author:* **Khatik
RCA Campus, MPUAT-Udaipur

have been discussed. The system comprises three culture steps i) Shoot induction ii) Shoot multiplication and elongation iii) Root regeneration in vitro developed shoots. Different sets of media were formulated with different combinations and concentrations of PGRs with the objective to cover all the combinations of PGRs that have been tried in previous research work and included the other potential possible combinations.

Surface Sterilization and Establishment of shoot tip and leaf culture

Protocols were standardized for surface sterilization of leaf and shoot tip explants in two strawberry genotypes *i.e.* Ofra and Chandler. It was quite difficult to get rid of contamination as the mother plants were polyhouse and field grown. The disinfection procedures followed were similar in Chandler and Ofra strawberry genotypes except type of explants *i.e.* shoot tip and leaf. Different disinfection treatments were used for surface sterilization of explants, which involved teepol, bavistin, mercuric chloride and physical parameters *i.e.* swirling and dipping time of explants in the solution of bavistin and mercuric chloride. Anti-phenolics like PVP to avoid the release of the phenolic compounds and browning of the cultures. Shoot tips were disinfected with dipping in 0.4% bavistin solution for 15 min. with constant swirling at 110 rpm on shaker and freshly prepared 0.1% mercuric chloride ($HgCl_2$) solution for 10 min. with constant swirls at 110 rpm on shaker. For leaf explants, 0.4% bavistin solution for 10 min. with constant swirling at 110 rpm on shaker, the solution was drained and freshly prepared 0.1% mercuric chloride ($HgCl_2$) solution for 3 min. with constant swirling at 110 rpm on shaker. Then fresh leaf and shoot tip rinsed 3-4 times with distilled sterilized water under laminar air flow cabinet. In both procedures, contamination was observed less than 10%.

Shoot Induction on leaf Explants

The MS basal media supplemented with various combinations and concentrations of PGRs were studied for plant regeneration from leaf explants of strawberry cv. Chandler and Ofra. Different sets of media were formulated with different combinations and concentrations of PGRs with the objective to cover all the combinations of PGRs that have been tried in previous reports and included the other potential possible combinations. All the set of media for leaf explants, MS basal supplemented with TDZ, IBA and PVP alone as well as in combinations were evaluated.

The per cent shoot induction in leaf explants of cv. Chandler were 81.34 and 81.33 per cent on MS medium having 2.5 TDZ + 1.0 IBA + 0.1 PVP (mg l^{-1}) and 2.5 TDZ + 0.5 IBA + 0.1 PVP (mg l^{-1}), respectively. Maximum number of shoots explant^{-1} (3.50) were found in media containing 1.5 TDZ+ 0.1 PVP (mg l^{-1}), whereas maximum mean shoot length (1.43 cm) found 3.5 TDZ + 0.1 PVP (mg l^{-1}).

Per cent shoot proliferation in leaf explants of cv. Ofra were 79.34 and 79.33 per cent on medium having 2.5 TDZ + 1.0 IBA + 0.1 PVP (mg l^{-1}) and 2.5 TDZ + 0.5 IBA + 0.1 PVP (mg l^{-1}) respectively. Maximum number of shoots explant^{-1} (2.97) were found in media containing 0.5 TDZ + 0.1 PVP (mg l^{-1}), whereas maximum shoot length (1.66 cm) was found on 1.5 TDZ + 0.1 PVP (mg l^{-1}) media.

The days taken to emergence of micro shoots of leaf explants from 11-32 days in cv. Chandler and 14-37 days for cv. Ofra on various shoot induction media.

Shoot Induction on shoot tip Explants

The MS basal media supplemented with various combinations and concentrations of PGRs were studied for plant regeneration from shoot tip explants of strawberry cvs. Chandler and Ofra.

The per cent shoot induction in shoot tip explants of cv. Chandler were 76.80 and 76.67 per cent found on medium having 2.5 TDZ + 0.5 IBA + 0.1 PVP (mg l^{-1}) and 2.5 TDZ + 1.0 IBA + 0.1 PVP (mg l^{-1}), respectively. Maximum number of shoots explant^{-1} (2.20) were found in media containing 1.5 TDZ + 0.5 IBA + 0.1 PVP (mg l^{-1}), whereas maximum mean shoot length (1.39 cm) found in media 3.5 TDZ + 0.1 PVP (mg l^{-1}).

Per cent shoot proliferation in shoot tip explants of cv. Ofra were 73.34 and 73.33 per cent on medium having 2.5 TDZ + 0.5 IBA + 0.1 PVP (mg l^{-1}) and 2.5 TDZ + 1.0 IBA + 0.1 PVP (mg l^{-1}) respectively. Maximum number of shoots explant^{-1} (3.00) were found in media containing 1.5 TDZ + 0.1 PVP (mg l^{-1}), whereas maximum mean shoot length (1.65 cm) found in media 2.5 TDZ + 1.0 IBA 0.1 PVP (mg l^{-1}).

The days taken to emergence of micro shoots of shoot tip explants from 8-31 days in cv. Chandler and 9-37 days for cv. Ofra on various shoot induction media.

Optimization of plant Growth Regulators for shoot Multiplication of shoots Regenerated From Leaf Explants

After shoot induction of leaf explants of strawberry *cvs.* Chandler and Ofra, on shoot induction media, the shoots were multiplied and regenerated *in vitro* by repeated transfer of micro shoots. For this, culture were repeatedly transfered to MS basal medium containing different concentrations of BA, Kn, GA_3, IBA, PVP combinations and ready made available commercial strawberry multiplication medium *i.e.* HiMedia.

HiMedia and MS basal media supplemented with various combinations and concentrations of PGRs were studied for shoot multiplication, proliferated from leaf explants of cv. Chandler. After three weeks of culture number of shoots explant^{-1}, number of leaves shoot^{-1} and mean shoot length (cm) were recorded. Significant differences were observed in maximum number of shoots explant^{-1} (19.60 and 19.20) on MS medium fortified with 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) and HiMedia, respectively. Similar results were found in numbers of leaves shoot^{-1} (4.07 and 4.03) and mean shoot length (5.20 and 4.80 cm) in MS media supplemented with 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) and HiMedia, respectively.

HiMedia and MS basal media supplemented with various combinations and concentrations of PGRs were studied for shoot multiplication, proliferated from leaf explants of cv. Ofra. After three weeks of culture number of shoots explant^{-1}, number of leaves shoot^{-1} and mean shoot length (cm) were recorded. Significant differences were observed in maximum number of shoots explant^{-1} (19.60 and 19.27) on MS medium fortified with 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) and HiMedia, respectively. Maximum numbers of leaves shoot^{-1} (4.57 and 4.47) were found in HiMedia and MS media supplemented with 1.5 BA + 0.5 IBA + 0.1 PVP (mgL^{-1}) and

similar results were recorded for higher mean shoot length (3.93 and 3.60 cm) in above medium.

Optimization of Plant growth Regulators for shoot Multiplication of Shoots Regenerated from Shoot tip Explants

Results obtained for shoot multiplication of shoots regenerated from shoot tip explants of both the strawberry genotypes (Ofra and Chandler) on eight MS based media (control and fortified with BA, Kn, IBA, GA_3 , 0.1 PVP) and commercial HiMedia (PT 117) were used for shoot multiplication in both genotypes.

After three weeks of culture shoot multiplication response in terms of number of shoots, leaves and mean shoot length (cm) per explant were observed on shoots regenerated from shoot tip explants cultivar Chandler. The maximum number of shoots explant^{-1} (16.27 and 15.53) on MS medium fortified with 1.0 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) and 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}), respectively. Maximum numbers of leaves shoot^{-1} (4.53 and 4.13) were found on media supplemented with 0.5 BA + 0.5 Kn + 2.0 GA_3 + 0.1 PVP (mgL^{-1}) and 1.5 BA + 0.5 Kn + 0.1 PVP (mgL^{-1}) and in respect of mean shoot length (4.83 and 4.57 cm) were observed on MS media supplemented with 2.0 BA + 0.5 IBA + 0.1 PVP (mg l^{1}) and followed by HiMedia (PT 117).

Response of cultivar Ofra, Significant differences were observed in maximum number of shoots explant^{-1} (16.23 and 15.27) on MS medium fortified with 1.0 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) followed by 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}). Maximum numbers of leaves shoot^{-1} (4.33 and 4.13) were found in HiMedia (117) and MS media supplemented with 0.5 BA + 0.5 Kn + 2.0 GA_3 + 0.1 PVP (mg l^{-1}) and higher mean shoot length (4.37 and 3.97 cm) were observed in MS media supplemented with 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) and HiMedia.

***In vitro* root induction**

Successful in vitro propagation depends on profuse, sturdy, healthy and strong root system of the plantlets. A protocol for induction of in vitro rooting was developed by using HiMedia (PT 118) and ½MS basal medium of different strength supplemented with IBA (mg l^{-1}) and with or without charcoal. Multiple shoots regenerated from leaf explant of strawberry genotypes Chandler and Ofra were excised and cultured.
Microshoots regenerated from leaf explant of cultivars Chandler and Ofra responded rooting after 10-67 and 9-63 days, respectively of inoculation on different medium. After 3 weeks of culture on the rooting medium the per cent of rooted shoots, total number of roots and root length for each explant were recorded. ½MS medium supplemented with 1.0 IBA + 200 charcoal (mg l^{-1}) was found most effective in present study where optimal rooting response in terms of per cent root induction (99.02 %, 98.44 %), number of roots (7.20, 6.60) and mean root length (6.80, 6.07 cm) per micro shoots these were followed by HiMedia + 200 mg l^{-1} charcoal for cultivar chandler and Ofra, respectively. Lowest number of roots was noticed with increased concentration of IBA alone and in combinations with charcoal at 0.5 and 1.0 mg/L. The longest shoot length was recorded on HiMedia and ½MS basal supplemented with 1.0 mg l^{-1} IBA which were statistically similar.

Whereas microshoots regenerated from shoot tip explant of cultivars Chandler and Ofra rooting was initiated after 10-62 and 9-63 days, respectively of inoculation on different medium used in present investigations. After 3 weeks of culture on the rooting medium the per cent of rooted shoots, total number of roots and root length for each explant were observed in ½MS medium supplemented with 1.0 IBA + 200 charcoal (mg l^{-1}) was found optimal rooting response in terms of per cent root induction (99.67%, 98.62%), number of roots (6.67, 6.83) and mean root length (6.10, 5.73 cm) per shoots these were followed by HiMedia + 200 mg l^{-1} charcoal for cultivar chandler and Ofra, respectively. No responses were observed on ½MS medium without IBA and charcoal. Lowest number of roots was noticed with increased concentration of IBA alone and in combinations with charcoal at 0.5 and 1.0 mg/L. The longest shoot length was recorded on HiMedia and ½MS basal supplemented with 1.0 mg l^{-1} IBA which were statistically similar.

Standardization of the Hardening Procedure for in vitro Propagated Strawberry Plantlets for Better field Survival

Hardening media play an important role for establishment of successful plant material. Therefore, the rooted plants were taken out from the culture vessels, washed thoroughly with sterile water in order to remove the adhered nutrient agar, and potted in bottles containing hardening media. Initially plants were kept in plastic boxes and covered to maintain high humidity and supplied with ½MS nutrient solution at 3-4 days interval. Hardening media containing different combination (Cocopeat, vermicompost, vermiculite, sand and perlite) were used in persent investigation. the different growing media had significantly affected the survival percentage of plantlets of strawberry *cv.* Chandler and Ofra. The maximum survival percentage (98.33 %) was found in plantlets regenerated from shoot tip explant of strawberry cultivar Chandler on hardening media combinations of Cocopeat + Vermiculite + Vermicompost + Sand (1:1:1:1, v/v) and was closely followed by media containing Cocopeat + Vermiculite + Vermicompost (1:1:1, v/v). Minimum survival percentage (40.50 %) was recorded on Vermiculite + Perlite (1:1, v/v) hardening media.

In strawberry cultivar Ofra the same hardening media mixture of Cocopeat + Vermiculite + Vermicompost + Sand (1:1:1:1, v/v) found appropriate. It recorded maximum survival percentage (98.0 %) on plantlets regenerated from shoot tip and leaf explants. Vermiculite + Perlite (1:1, v/v) hardening media was recorded minimum survival percentage (38.60 %) on leaf regenerated plantlets of *cv.* Ofra. No significant differences were observed with respect of genotypes and explant.

DISCUSSION

Strawberry (*Fragaria* x *ananassa* Duch.) is a natural hybrid of *Fragaria chiloensis* L.P. Mill. and *Fragaria virginiana* Duch. The domestication of this fruit was coincident with the introduction into Europe of American type. It is a perennial, stoloniferous herb belonging to the Rosacea family, genus *Fragaria* and most widely consumed fruits throughout the world. It is one of the most popular fruits growing in the Northern hemisphere in temperate and sub temperate environment. It is traditionally popular delicious fruit for flavor and taste. Strawberry is commercially grown in temperate regions of the world. In india, the principle strawberry growing areas fall in the hilly region of Kashmir Himachal Pradesh. Dehradun and Mahabaleshwar.

These days *in vitro* propagation technique has been widely used in the strawberry industry. Million of plants produced in a year from a single meristem tip (Damiano, 1980). The first commercial application of strawberry micropropagation was reported in Germany followed by Italy and France. Micropropagation of strawberry is now an accepted method for mass multiplication of strawberry (Zimmerman, 1991). An attempt has been made to discuss these results using cause and effect analysis and explain in the light of available information/literature by earlier researchers in order to support the findings of the present investigation under following heads.

In vitro is a powerful and well practiced tool for large scale propagation of horticultural crops. It is a rapid technique where mass multiplication of ornamental, timber and fruit tree can be achieved in relatively short period of time with high fidelity index. It is especially important where no other mean of propagation available or conventional method is time consuming, rate of multiplication is very slow or diseases also got transferred with planting material (as in case of strawberry).

In vitro propagation has several other advantages over conventional propagation like elimination of pathogen, preservation of breeding stock as juvenile plants and removal of quarantine problems (Maretaki, 1987). The entire sequence of clonal propagation of a species through tissue culture can be divided in to four stage *viz*. stages i) Culture establishment (Regeneration of shoots), ii) Multiplication of propagules (Multiplication of shoots), iii) Induction of roots in regenerated shoots (Roots), Vi) establishment of plantlets in soil (transplantation) (Singh, 2002). *In vitro* propagation has specific set of requirement and the technique in a particular species is dependent upon fulfilling these conditions. The best explants and PGR's for *in vitro* propagation of two cultivars (Ofra and Chandler) of strawberry have been standardized in this investigation and attempts have been made to develop an efficient *in vitro* multiplication of the above cultivars. Various steps involved and their results are discussed below.

Surface Sterilization and Establishment of shoot tip and leaf Cultures

In present study surface sterilization of shoot tip with dipping in 0.4% bavistin solution for 15 minute with constant swirling at 110 rpm on shaker and freshly prepared 0.1% mercuric chloride ($HgCl_2$) solution for 10 minute with constant swirling at 110 rpm on shaker. While, leaf explants were sterilized with dipping in 0.4% bavistin solution about 10 minute with constant swirling at 110 rpm on shaker, the solution was drained and freshly prepared 0.1% mercuric chloride ($HgCl_2$) solution for 3 minute with constant swirling at 110 rpm on shaker, the solution was drained. Then fresh leaf and shoot tip rinsed 3-4 times with distilled sterilized water under laminar air flow cabinet. Sterilization procedure was adjudged best in the strawberry cultivars Chandler and Ofra. This indicates that bavistin and $HgCl_2$ with constant shaking time in necessary for getting high frequency of contamination free cultures for the experiments.

Proper surface sterilization of explants is necessary for establishing contamination free axenic culture for *In vitro* propagation in plants (Pierik, 1987). The results and visual observations recorded in both strawberry cultivars in the present investigation showed that fungal contamination was the major problem in establishing contamination free axenic shoot tip and leaf cultures. Alcohol (70%) is not sufficient to kill all micro-organisms, thus explants require to be treated with mercuric chloride (Pierik, 1987). Yeoman (1977) recommended concentration and time of treatment of explants surface sterilization. Thakur, *et al.* (2002) also used mercuric chloride and bavistin to control fungal contamination in carnation. Adam *et al.* (2008) reported that sodium hypochlorite and teepol were found to be highly effective to eliminate fungal contamination in strawberry. Use of bavistin and $HgCl_2$ solution along with swirling at 110 rpm on shaker results 100 per cent contamination free explants of *Pongamia pinnata* explants (Singh *et al.,* 2016).

Shoot Induction from Explants

In the present investigations, most appropriate explant(s) and culture media for *in vitro* propagation of strawberry genotypes were identified by using shoot tip and leaf as explants and MS medium (Murashige and Skoog, 1962) as basal medium supplemented with different combinations of TDZ, IBA and PVP was kept constant at 0.1 mg l^{-1}.

Earlier various researchers *i.e.* Husaini and Srivastava (2011), Liu and Sanford (1988), Jones *et al.*, (1988), Nehra *et al.* (1989), Greene *et al.* (1991) and Isac *et al.* (1994) on leaf explants and meristematic callus (Jones *et al.*, 1988), meristem tips and axillary buds (Lee and Park, 1980) stolon tips and axillary buds (Waithaka *et al*.,1980), vegetative buds (Mahajan,1997), anthers (Laneri and Damiano, 1980), petiole segments and flower buds (Foucault and Letouze, 1987) and immature embryos (Wang *et al.*, 1984). Regeneration of strawberry plants by organogenesis from leaf blade and petiole explants obtained from *in vitro* micropropagated plantlets was investigated by Husaini and Srivastava (2011).

Shoot tip explants have been widely used for shoot induction and multiplication in many strawberry cultivars using modified MS and Knops media supplemented with different PGR's and their combinations (Haddadi *et al.,* 2010; Negi *et al.,* 2008; Kaur and Chopra, 2004; Sood *et al.* 2004; Lal *et al.*, 2003; Bhatt and Dhar, 2000; Greene *et al.*, 1991; Liu and Sanford, 1988; Lee and Park, 1980).

Optimization of plant Growth Regulators for shoot Induction in leaf Explants of Strawberry

Leaf explants of strawberry cvs. Chandler and Ofra were collected from greenhouse and open field grown plants. These were cultured on MS basal media supplemented with TDZ, IBA and PVP alone as well as in combinations. The response varied with the nature (cultivar) of explants used and their type and concentration of growth regulators. MS media supplemented with 2.5 mg l^{-1} TDZ found more suitable in both the combinations of IBA (0.5 and 1.0 mg l^{-1}) for shoot induction in leaf explants of Chandler (81.34 and 81.33 per cent) and Ofra (79.34 and 79.33 per cent). Lowest shoot regeneration was recorded for IBA alone and combine with lowest concentration of TDZ i.e. 0.5 mg l^{-1}. The Figure 4.1.1 and 4.1.2 shows positive response in terms of per cent shoot induction number of shoots explant^{-1} and mean shoot length (cm) with increasing concentration of TDZ alone and in combination with both the concentration of IBA till 3.5 mg l^{-1} at the peak, their after showed negative response. PGR's play major role in bud breaking because of no response shown under control (without PGR) and 0.1 mg l^{-1} PVP. These results might be due to which exhibited both auxin and cytokinin like

effect and it modify endogenous plant growth regulators, either direct or indirectly and produce reactions in cell/tissue, necessary for its division/regeneration and other possibilities include modification in cell membrane, energy levels, nutrient absorption, transport and assimilation, *etc.* (Guo *et. al.* 2011)

The days taken for emergence of micro shoots from leaf explants varied from 11-32 days in cv. Chandler and 14-37 days for cv. Ofra on various shoot induction media. It might be due to suitable amount of auxins and cytokinins and genetic makeup or physiological condition of the explant, which played an important role for early shoot initiation. Some workers also reported rapid shoot regeneration in leaf explant of strawberry (Hammoudeh *et al.*, 1998 and Thakur *et al.,* 2010.)

Shoot Induction

Shoot tip explants of strawberry cvs. Chandler and Ofra were collected from greenhouse and open field grown plants. These were cultured on MS basal media supplemented with TDZ, IBA and PVP alone as well as in combinations. The response varied with the nature (cultivar) of explants used and their type and concentration of growth regulators. MS media supplemented with 2.5 mg l^{-1} TDZ found more suitable in both the combinations of IBA (0.5 and 1.0 mg l^{-1}) for shoot induction in shoot tip explants of Chandler (76.80 and 76.67 per cent) and ofra (73.34 and 73.33 per cent). Lowest shoot regeneration was recorded for IBA alone and combine with lowest concentration of TDZ i.e. 0.5 mg l^{-1}. The Figure 4.1.3 and 4.1.4 shows positive response in terms of per cent shoot induction number of shoots explant^{-1} and mean shoot length (cm) with increasing concentration of TDZ alone and in combination with both the concentration of IBA till 3.5 mg l^{-1} at the peak, their after showed negative response. PGR's plays major roll in bud breaking because of no response shown under treatment control (without PGR) and 0.1 mg l^{-1} PVP. These results might be due to TDZ which exhibited both auxin and cytokinin like effect and it modify endogenous plant growth regulators, either direct or indirectly and produce reactions in cell/tissue, necessary for its division/regeneration and other possibilities include modification in cell membrane, energy levels, nutrient absorption, transport and assimilation, *etc*. (Guo *et. al.* 2011)

The days taken to emergence of micro shoots of leaf explants varied from 8-31 days in cv. Chandler and 9-37 days for cv. Ofra on various shoot induction media. It might be due to suitable amount of auxins and cytokinins and genetic makeup or physiological condition of the explant, which played an important role for early shoot initiation. Some workers also reported rapid shoot regeneration in leaf explant of strawberry (Hammoudeh *et al.*, 1998 and Thakur *et al.,* 2010.). Strawberry cultivar Chandler is more responsive to shoot induction as compared to Ofra. These result are in accordance with Kaushal *at al*. (2004) and Bhanakher, (2008).

Shoot Multiplication

In present investigation, differences in shoot multiplication frequency were observed between two different explants of strawberry cultivars, with maximum frequency noticed in leaf explants followed by shoot tip. The maximum number of shoots per leaf explant was obtained on MS medium fortified with 1.5 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) for Chandler (19.60) and in Ofra (19.60). Whereas maximum shoots regenerated from shoot tip explant for Chandler (16.27) and Ofra (15.53) on MS medium fortified with 1.0 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}). Similar results were quoted by Shashi (2011) who described that the synergetic effect between cytokinin and auxin up to certain optimum level. Cytokinins are known to play a major role in shoot multiplication. 6- benzylamino-purin (BAP) is the the cytokinin that is mostly used for *in vitro* shoot proliferation of strawberry (Haddadi *at al.,* 2010). Negi *et al.* (2008) reported that different combinations of growth regulators BAP, IBA, kinetin and GA_3 were tried for further multiplication. Cytokinin in presence of auxin increased the cell division efficiency and cell division enhanced the shoot multiplication.

MS medium containing 1.5 BA + 0.5 IBA + 0.1 PVP gave maximum mean length of shoots on both type of explants Chandler (5.20 cm) and Ofra (3.93 cm) 4.83 cm on Chandler and 4.37 cm on Ofra leaf and shoot tip explants, respectivey. The increase in shoot length was probably due to increase in cell division by BA and elongation of these cells by IBA.

The numbers of leaves shoot^{-1} were recorded highest on HiMedia on Chandler (4.07) and Ofra (4.57) for shoots regenerated from leaf explant. Whereas shoots regenerated from shoot tip for Chandler (4.53) and Ofra (4.33) were noticed on 0.5 BA + 0.5 Kn + 2.0 GA_3 + 0.1 PVP (mg l^{-1}) and HiMedia, respectively. A synergistic effect of optimum concentrations of BA and Kn were responsible for increase in number of leaves. Bhat a*t al.* (2012) reported that addition of kinetin with BAP resulted maximum number of leaves.

Root Induction of Micro Shoots

After thriving micro shoots of strawberry by in vitro technique the next important step of the current investigation was to stimulate roots in the developed micro shoots from different explants of the cultivars Chandler and Ofra. Completion of propagation depends on healthy and strong root system. The proliferated micro shoots were transferred to HiMedia alone and combined with 200 mg l^{-1} Charcoal and ½MS basal medium fortified with different concentration of IBA alone and in combination with charcoal to find out there most appropriate concentrations for root induction. It was observed that IBA 1.0 mg l^{-1} along with 200 mg l^{-1} charcoal took minimum number of days (9.63) for root initiation with higher rooting percentage (99.02 %), maximum number of roots (7.20) and longest root length (6.80 cm) were recorded on micro shoots regenerated from leaf and shoot tip explants of Chandler followed by cultivar Ofra. This response was followed by HiMedia (PT 118) in combination with 200 mg l^{-1} charcoal.

The present findings are in agreement with the work of Arun (2013) and Damiano (1978) who reported addition of 1-2 g of activated charcoal L^{-1} of medium promotes elongation of both shoots and roots. Navatel (1979) proposed that activated charcoal can replace the GA_3 in the culture medium. Kaur and Chopra (2004) and Mir *et al.* (2010) also reported similar results. Signifying that this concentration is optimum for effective rooting of *in vitro* derived micro shoots of strawberry cultivars.

Hardening of in vitro Propagated Plantlets

Hardening *i.e.* acclimatization of *in vitro* regenerated plantlets to enable them to adopt in natural environment is a critical

process due to their delicate anatomical and physiological peculiarities. Micropropagated plantlets are difficult to transplant mainly due to their fragile nature and poor control on water loss. The culture media are supplemented with sucrose as carbon and energy sources. This addition decreases considerably the water potential of the medium and increases the risk of bacterial and fungal contamination. Furthermore, the plantlets are usually supplied with large doses of growth regulators. These conditions result in the formation of plantlets of abnormal morphology, anatomy and physiology (Kozai, 1991, Pospisilova *et al*., 1992 and 1997, Buddendorf-Joosten and Woltering, 1994, Desjardins, 1995, Kozai and Smith, 1995). *In vitro* plantlets are grown on sucrose with limited light and gas exchange which reduces capacity for photosynthesis. The special conditions during in vitro culture result in the formation of plantlets of abnormal morphology, anatomy and physiology. After *ex vitro* transfer, these plantlets might easily be impaired by sudden changes in environmental conditions (Pospisilova *et al.* 1999).

In present investigation maximum survival percentage (98.33 %) was found in plantlets regenerated from leaf and shoot tip explant of strawberry cultivar Chandler on cocopeat + vermiculite + vermicompost + sand (1:1:1:1, v/v) hardening media followed by cocopeat + vermiculite + vermicompost (1:1:1, v/v) Schie, (1999) reported that cocopeat had medium ion absorption capacity, aerialporosity and good capacity of water and nutrient preservation. Nourizadeh (2003) obtained that cocopeat and perlite substrate is effective in root due to better interchange of the elements especially cations inside the substrate and proper moisture distribution that finally effect root system and survival of plant. Addition of sand with vermicompost decreased the total porosity, easily available water and total water holding capacity (Zaller, 2007)

Minimum survival percentage (40.90 %) was recorded in Vermiculite + Perlite (1:1, v/v) media because of highly porous nature of perlite and compaction of vermiculite. No significant difference was found in respect of cultivars and explant.

CONCLUSION

On the basis of present investigations, it is concluded that leaf explant is superior over shoot tip explant for both the cultivars of strawberry. MS medium fortified with TDZ (2.5 mg l^{-1}) along with IBA (0.5 or 1.0 mg l^{-1}) and PVP (0.1 mg l^{-1}) was best for shoot induction and 1.0 BA + 0.5 IBA + 0.1 PVP (mg l^{-1}) with MS medium is best over commercially available HiMedia (PT 117) for shoot multiplication. Whereas, ½MS medium supplemented with 1.0 IBA + 200 charcoal (mg l^{-1}) found best for root induction and hardening media combinations of Cocopeat + Vermiculite + Vermicompost + Sand (1:1:1:1, v/v) resulted higher survival in field. These findings are reproducible for *in vitro* propagation of strawberry cultivar Chandler and Ofra.

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