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In Vitro Propagation in Strawberry (*Fragaria x ananassa* Duch.)

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ABSTRACT

Strawberry cultivation is a highly profitable venture producing maximum returns in short period of time. Strawberry is conventionally cultivated by runners which produce plants that are disease prone. Therefore, quality planting material is essential for successful cultivation. A major drawback of cultivation of strawberry is unavailability of runners during the cropping season. *In vitro* propagation of strawberry aims at mass propagation of disease free plants in a short period of time wherein use of several types of media, explants and combinations of growth regulators have been standardized for successful *in vitro* propagation of strawberry. This review focuses on the developments and recent advances made with reference to *in vitro* regeneration in strawberry.

1. Introduction

Strawberry is conventionally propagated by runners which are labour intensive and plants produced are disease prone with low rooting. Micropropagation of strawberry produces disease free plants with improved characters like crown dimension and flowering procedure. Tissue culture permits mass propagation in a short period of time by direct or indirect regeneration with plants produced *in vitro* are better than conventionally propagated plants in terms of yield, pest resistance, vigour, number of runners and leaves per plant (Rahman *et al.,* 2015). *In vitro* propagation of strawberry was first reported by Boxus in 1974 following which different types of medium, plant growth regulators, explants and genotypes has been used (Haddadi *et al.,* 2010). Over time it was found that media used in micropropagation of strawberry is affected by hormones, vitamins, amino acids and physical state of media (Kadhimi *et al.,* 2014). The following review discusses the progress made in the field of *in vitro* regeneration for propagation of strawberry.

2. *In Vitro* Regeneration

Diengngan *et al.* (2014) studied disinfection procedures for explants in strawberry using sodium hypochlorite at concentration of 0.5% for 7, 5 and 3 minutes and mercuric chloride (HgCl2) 0.1% concentration for 5 minutes, 3 minutes and 3 minutes 10 seconds respectively, with lowest contamination and browning of explants observed on treatment of explants with HgCl2 0.1% for 3 min 10 sec. Kichaoui (2014) studied methods for preventing contamination and oxidative browning in *in vitro* explants. Surface sterilization of runner tips with sodium hypochlorite solution was done at concentrations 0.5, 1, 1.5, 2.0 and 2.5% respectively. Pretreatment with Polyvinylpyrrolidone (PVPP) at concentrations 1, 1.5 and 2% was done to study effects on explant browning and survival percentage. Maximum aseptic cultures and reduction of explant browning was obtained on treatment with 1.5% sodium hypochlorite and 0.1% mercuric chloride. Pre-treatment with 2% PVPP recorded the highest percentage of explant survival. Lal *et al.* (2003) developed an efficient protocol for plant regeneration in three cultivars of strawberry *viz.* Ofra, Oso Grande and Chandler using modified versions of Murashige and Skoog (MS) and Knop's media. The regeneration frequency of the cultivars Ofra, Oso Grande and Chandler was observed to be at a maximum when MS media was supplemented with 4 mg/l of 6-Benzylaminopurine (BAP). Knop's media fortified with 4.0 mg/l Indole-3-butyric acid (IBA), 0.4 mg/l BAP and 0.4 mg/l Gibberellic acid (GA3) yielded maximum regeneration. Sakila *et al.* (2007) used nodal segments of strawberry on MS medium supplemented with different concentration of

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Benzyl adenine (BA) (0.5-2.0 mg/l) with Kinetin (KIN) (0.1 and 0.5 mg/l) or GA3 (0.1 and 0.5 mg/l). Medium with BA + KIN yielded higher numbers of shoots than with BA + GA3 while maximum response of shoot proliferation and development was observed in medium containing 1.5 mg/l BA+0.5 mg/l Kinetin. However, an increased dose of BA decreased the rate of shoot multiplication, while BA in combination with Gibberellic acid (GA3) instead of Kinetin had a positive impact on shoot length, with no improvement on the rate of shoot proliferation and shoot number. Maximum multiple shoot induction was obtained in media supplemented with 1.5 mg/l BA + 0.1-0.5 mg/l KIN. Biswas *et al.* (2008) used runner tips in MS media supplemented with different concentrations of 1-Naphthalene acetic acid (NAA), BA and Kinetin used singly or in combinations. MS medium supplemented with 0.5 mg/l lBA produced the best response towards multiple shoot regeneration shoot proliferation, highest number of shoots and harvestable shoot per culture while media supplemented with low BA produced best percentage of culture response. It was observed that proliferation of shoots from the runner tips depended on the types and concentration of the cytokine used. The percentage of explants showing proliferation and number of shoot per culture increased gradually with decrease in BA concentrations from 4.0 –0.1 mg/l. Haddadi *et al.* (2010) used runner tips for 'Camarosa' strawberry on MS medium supplemented with 0, 2, 4, and 8 µM Thidiazuron (TDZ) and 0,4, 9, 18, and 27 µM BAP for shoot induction. Maximum number of shoots per explant was observed on MS medium supplemented with 2µM TDZ and µM BAP. The mean number of shoots produced per explant was directly proportional to the TDZ concentration whereas it was indirectly proportional to the concentration of BAP. Hasan *et al.* (2010) studied the effect of different concentrations of cytokinins, benzyladenine and adenine and auxins on the rate of multiplication of strawberry using nodal segments. Explants were inoculated on MS basal medium supplemented with different concentration of cytokinin, auxin, additives and GA3. Seven different combination of BA as cytokinin and NAA as auxin were used where cytokinin/auxin used ranged from 1.0-3.0 mg/l and 0.1-0.3 respectively while 1.0 mg/l GA3, 120.0 mg/l adenine and additives like 150 ml/l coconut water were used. The concentration of growth regulators was found to be inversely proportional with shoot formation. Shoots were produced in all the treatments studied with maximum number of shoots obtained in media supplemented with 1.0 mg/l BA and 0.1 mg/l NAA while highest shoot length was observed at the concentration of 2.0 mg/l BA + 0.2 mg/l NAA +120 mg/l adenine. Mir *et al.* (2010) used nodal segments of strawberry to study the effect of media supplemented with various combinations and concentrations of BA, NAA and IBA.MS medium supplemented with 2 mg/l BA + 0.5 mg/l NAA produced highest shoot production. BA alone or with NAA in low concentrations was required for shoot multiplication and elongation while MS medium containing 2 mg/l IBA produced maximum number of roots. Kichaoui (2014) studied the response to different strengths of MS (1/8, 1/4, 1/2) and full strength of MS medium, effect of different concentration of IBA *i.e.* 0.5, 1.0, and 1.5 mg/l as well as different concentration of activated charcoal (0.2, 0.5 and 1.0 mg/l) were also studied. Data on root parameters *viz.* root number/plant, root length and shoot length were examined. Maximum auxiliary buds were observed when MS medium was supplemented with 1 mg/l IBA. Bhandari and Roy (2015) studied *in vitro* production of strawberry using MS, B5 and NN (Nitsch and Nitsch) basal media supplemented with different combination and concentration of plant growth regulators. The best response for shoot apex development was observed in MS basal medium whereas B5 and NN basal medium showed poor and no response respectively. Variable response in *in vitro* culture of strawberry was observed at different concentrations of NAA, BAP and GA3. Maximum number of shoot bud proliferation and shoot length was observed in MS basal media supplemented with equal concentration of NAA (5 mg/l) and BAP (5 mg/l) and low concentration of GA3 (0.5 mg/l) compared to B5 basal media which showed poor response with shoot bud proliferation explants and shoot length. The response percentage and shoot bud proliferation decreased with increase in concentration of NAA and BAP. The best response was observed with 5 mg/l BAP and 5 mg/l NAA with 0.5 mg/l of GA3. Munir *et al.* (2015) studied response of four strawberry cultivars *viz.* Chandler, Osogrande, Toro and Islamabad Local liquid and solid media, MS and Knop's media and response to different sources of sugar. It was observed that solid media supplemented with 0.5 mg/l GA3 exhibited maximum survival while using meristems while highest percentage of bud initiation was recorded in Osogrande in media supplemented with 0.5 mg/l BAP. It was observed that maximum bud formation per culture (25, 20 and 15) was obtained in MS media supplemented with 1.5 mg/l BAP and 0.1 mg/l IBA in cultivars Osogrande, Chandler and Islamabad Local respectively in *in vitro* shoots derived from meristem while the response varied when Knop's media was used. Maximum number of buds in cultivar Osogrande was observed in sucrose based MS media supplemented with 0.8 mg/l Kinetin and 0.2 mg/l NAA while Chandler and Islamabad Local showed maximum number of buds in sucrose based MS media supplemented with 0.6 mg/l Kinetin and 0.2 mg/l NAA. Moradi *et al.* (2011) studied the effects of different combinations of plant growth regulators in *in vitro* micropropagation of strawberry and developed an effective

procedure for propagation using nodal segments. MS medium supplemented with different concentrations and combination of growth regulators such as BAP (6-benzylaminopurine) 0, 0.5, 1 and 1.5 mg/l alone or with Kinetin 0, 0.2 and 0.5 mg/l or IBA (Indole-3-Butyric Acid) 0, 0.2 and 0.5 mg/l was used. MS medium supplemented with BAP and KIN produced the highest number of microshoots per explant. Highest bud induction was observed in MS media supplemented with BAP 0.5 mg/l and KIN 0.2 mg/l. Ara *et al.* (2012) developed a protocol for direct regeneration of strawberry from runner tips and nodal segment using different growth regulators. MS medium supplemented with different concentration and combinations of 6-benzylaminopurine, 6-furfuryl amino purine, Indole-3-butyric acid and Gibberellic acid was used. The maximum percentage of shoot formation was observed in media supplemented with 1.5 mg/l BAP+ 0.5 mg/l Kinetin while highest number of shoots per explant and the length of longest shoot were observed in medium supplemented with 2.0 mg/l BAP+ 0.5 mg/l GA_3. It was observed that nodal segments produced more number of shoots/culture than shoot tips while shoot tip explant was better for shoot initiation. Mozafari and Gerdakaneh (2012) studied the effect of media, hormone combinations and cultivar on the regeneration and morphological characteristics of two strawberry cultivars. Meristems were cultured on three different growth media *viz.* Murashige and Skoog (MS), B5 and Nitsch and Nitsch (NN) media supplemented with various growth regulator combinations. A strong interaction was observed between plant growth regulators, culture conditions and cultivar used for micropropagation. MS medium was superior to NN and B5 medium for most of traits studied. However, maximum number of shoots was obtained on NN medium and the lowest number was obtained on B5 medium. The best response of number of shoot regeneration was observed on growth media supplemented with BA 1.0 mg/l + IBA 0.05 mg/l + GA_3 0.05 mg/l whereas the lowest response was observed in media supplemented with Kinetin 5 mg/l + 2, 4-D 0.5 mg/l + GA_3 0.05 mg/l. Murti *et al.* (2012) studied methods to improve the regeneration rate of *in vitro* regeneration in strawberry using IBA in combination with high concentration of TDZ (Thidiazuron). MS medium supplemented with different concentrations of TDZ in combination with IBA was used. Lowest regeneration rate was observed on MS medium supplemented with 34.1 µM TDZ when used alone as opposed to high regeneration rates when TDZ was combined with IBA. The optimum concentration of TDZ for regeneration rate was 39.6 µM when combined with 0.5 µM IBA and 42.5 µM when used singly. In all TDZ concentrations, 2.5 µM IBA produced the highest number of plantlets per explant.

Ashrafuzzaman *et al.* (2013) studied *in vitro* propagation of strawberry using runner segments and runner tips. BAP concentrations *viz.* 0, 0.5, 1.0, 1.5 and 2.0 mg/l for shoot induction and four IBA concentrations *viz.* 0, 0.5, 1.0, and 1.5 mg/l for root induction were used. Data on various parameters such as number of explants cultured, average number of shoots/culture, average length of shoots/culture, average number of shoots per culture, days to root induction, average number of roots per culture and average length of roots/culture were recorded and mean values were calculated. Medium supplemented with 0.5 mg/l BAP produced the highest average number of shoots, length of shoots and leaves whereas no shoot was produced at 2.0 mg/l concentration of BAP. BAP free media produced the lowest average length of shoot and average number of leaves. Diengngan *et al.* (2014) studied the effect of medium supplemented with BAP (0.5, 1, 1.5 and 2mg/l) alone and in combination with GA_3 (0.5 and 1mg/l). MS medium supplemented with 1.5 mg/l BAP produced maximum shoot proliferation percentage, shoots per explant and minimum number of days to shoot initiation while maximum length of shoots was obtained in medium supplemented with 1.5 mg/l BAP in combination with 0.5 mg/l GA_3. Harugade *et al.* (2014) used nodal segments of strawberry on MS medium supplemented with different concentration of growth regulators *viz.* Benzyl adenine (BA), Kinetin and Gibberellic acid for *in vitro* regeneration. The numbers of shoots in medium supplemented with BA + Kinetin were higher than medium supplemented with BA + GA. The highest rate of response was obtained in media supplemented with 1.5 mg/l BA in combination with 0.5 mg/l KIN. The rate of shoot multiplication reduced on increasing BA concentration beyond 1.5 mg/l, while the maximum number of shoots per explant and highest average length were obtained at 1.5 mg/l BA + 0.1 mg/l KIN. The shoot length increased when BA was supplemented with GA_3. Danial *et al.* (2016) studied *in vitro* propagation of strawberry in presence of BAP singly or in combination with GA_3, Kinetin, IBA and TDZ. TDZ and BAP in combination with 0.25 mg/l of GA_3, Kinetin or IBA at concentrations 0, 0.5, 1.0, 1.5 and 2.0 mg/l respectively were used for shoot multiplication while IAA and IBA at concentrations 0, 0.25, 0.5, 0.75 and 1.0 mg/l were used to study their effect on rooting. It was observed that the maximum number of shoots /explants, maximum shoot length and number of leaves /explants was obtained in 2.0 mg/l BAP in combination with 0.25 mg/l IBA able while growth medium supplemented with 1.5 mg/l of TDZ produced more shoot number/explants. Media supplemented with 2.0 mg/l BAP and 0.25 mg/l GA_3 produced significantly higher number of branches per explant with greater mean length of branches than control and other treatments. Supplementation of BAP was significantly effective in increasing the number of shoots per explant as compared to the control where 1.5 mg/l of BAP + 0.25 mg/l Kinetin

produced maximum number of shoots and shoot length. Lal *et al.* (2003) observed that MS media supplemented with 4 mg/l of BAP resulted in the highest mean number of roots per explant. It was observed that maximum rooting was obtained in Knop's based media supplemented with 4.0 IBA mg/l + 0.4 mg/l BAP + 0.4 mg/l GA_3. Sakila *et al.* (2007) reported that media supplemented with 1.0 mg/l IBA was suitable for root induction. Haddadi *et al.* (2010) observed that media supplemented with 1 µM NAA or 2 µM IBA produced the largest number of roots. Hasan *et al.* (2010) observed that MS medium supplemented with 0.1 mg/l IAA produced maximum numbers of roots. Mir *et al.* (2010) observed that MS medium supplemented with 2.3 mg/l IBA produced maximum length of roots. Moradi *et al.* (2011) observed maximum number of roots per explant in MS media supplemented with BAP 0.1 mg/l with IBA 0.2 mg/l. The parent plants and the *in vitro* propagated plants showed no observable variations. Ara *et al.* (2012) observed that root initiation was observed in both full and half strength MS medium containing various concentrations of NAA and IBA individually.

Ashrafuzzaman *et al.* (2013) observed that media supplemented with 0.5 mg/l IBA showed the best performance in all the rooting parameters studied. The highest number of roots/culture, longest roots and shortest time for root induction were observed in media containing 0.5 and 1.0 mg/l IBA while no roots were observed in control treatment. Murti *et al.* (2012) observed highest root fresh weight and number of root per plant on medium supplemented with 40.87 µM TDZ. Murti and Yeoung (2013) observed that a low concentration of 0.2 ppm was best for producing single plantlet with longer and higher root number as compared to higher doses of IBA. The effect of IBA at different concentrations did not differ significantly for plant height and higher concentration of IBA inhibited root length development. Diengngan *et al.* (2014) reported that medium supplemented with 0.5 mg/l IBA produced maximum percentage of rooting, number of roots and root length of micro cuttings. Harugade *et al.* (2014) used different concentrations of IBA (0.1-1.5 mg/l) for root induction out of which 1.0 mg/l IBA produced best response for root induction. Kichaoui (2014) observed lowest root response when IBA was used at concentrations 0.5, 1.0 and 1.5 mg/l respectively. Bhandari and Roy (2015) used MS media containing IAA and IBA both or alone at various concentrations for rooting. Best root formation was observed in MS media supplemented with 0.5 mg/l IBA and 0.5 mg/l IAA while rooting was absent in auxin free media. Root induction was optimum in medium supplemented with 1 mg/l IBA and 1 mg/l IAA. Danial *et al.* (2016) observed an increase in number of roots by IBA when compared to the control treatment with best rooting response observed in media supplemented with 1 mg/l IBA. Root length increased on decreasing the concentration of IBA with highest root length observed in media containing 0.50 mg/l IBA.

Constraints

In vitro propagation of strawberry requires runners as explants which are often limited in certain season with runners being produced only during the vegetative development phase. However, offshoots can also be utilized with problems arising during disinfection due to its large size compared to runners. Moreover, browning of *in vitro* culture at initial establishing stage also acts as a major drawback leading to explant death. An effective method of sterilization however will enhanced the survival of explants (Jan *et al.,* 2013).

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