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RESEARCH ARTICLE

Researches on the Reproduction of "Ottoman Strawberry" with Tissue Culture

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ABSTRACT

Plant tissue culture is widely used as auxiliary methods for vegetative propagation due to give results in a shorter time and to obtain mass plant production than other vegetative propagation methods. The combined effect of BAP and IBA hormones (0.1 mg/l IBA; 0.2 mg/l IBA; 0.3 mg/l IBA; 0.5 mg/l BAP; 0.5 mg/l BAP + 0.1 mg/l IBA; 0.5 mg/l BAP + 0.2 mg/l IBA; 0.5 mg/l BAP + 0.3 mg/l IBA) was studied relative to average number of shoots per explant and root formation (%) of 42-day-old in strawberry cultivar "Ottoman Strawberry" shoot tip cuttings. Our results have shown that concentration of BAP + IBA (0.5+ 0.1 mg/l) in MS medium successfully resulted in the induction of average number of shoots and root formation per explant when compared to other treatment doses and control. Alone concentration of BAP had not significant effect on development of average number of shoots per explant and root formation. Development of average number of shoots per explant and root formation was inhibited as the BAP + IBA concentration increased or decreased except for MS culture medium 5 to 0.5 mg/l BAP and 0.1 mg/l IBA. Consequently, in strawberry cultivar "Ottoman Strawberry" tissue culture, application of alone BAP in the concentrations used is discouraged, while application of BAP + IBA (0.5 + 0.1 mg/l) concentrations is recommended.

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Introduction

Strawberry (*Fragaria x ananassa* Duch.) cultivars are an important source of nutrients with regard to excellent dietary sources, flavor of potassium, ascorbic acid, fiber and simple sugar sources of energy and other secondary metabolites (Perez et al., 1997; Wang and Galletta, 2002). In general, strawberries are grown in temperate, Mediterranean, taiga, grassland and subtropical climates (Hancock and Luby, 2014). In Turkey, *Fragaria ananassa* is cultivated in the Mediterranean coastal regions, and it is followed by a later product in the Aegean coastal regions of Anatolia in March (Karacam et al., 2015). Additionally, strawberry, which has a special local name, is known as "Ottoman Strawberry" in general. It is a unique taste with its pale pink color, rich aroma, excellent flavor and oval seem.

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It is very rich in taste and aroma, but its mean fruit size is very small (roughly 5-8 g), and its flesh firmness is highly undesirable owing to its softness that tenders it inappropriate for transportation.

The reproduction of strawberries is carried out vegetatively, by rooting stolon plants that are formed under long day and high temperature conditions. A well-developed strawberry plant can form 10-15 stolons, and more than 100 seedlings can be obtained from these stolons. However, intense manual labor and labor required to obtain seedlings make it difficult and limited the reproduction of strawberry farming. In addition, the need for strawberry seedlings can take quite a long time to be achieved by traditional methods. Significant improvements have been recorded in the plant breeding sector with renewed biotechnological methods, and these revolutionary innovations are continuing rapidly (Doğan and Çağlar, 2018; Kim et al., 2020). In context, biotechnological methods are used in order to realize more economical, higher quality plant production by

bringing solutions to problems that are difficult to solve with traditional methods known in plant production. Because the use of tissue culture methods causes the formation of genetically homogeneous plant populations, the production of strong and healthy plants can be provided (Murashige and Skoog, 1974; Blazina et al., 1991). Additionally, plant tissue culture is the process of obtaining new tissue, a complete plant or herbal products by using plant parts (explants) such as a whole plant, organ, tissue or cell in aseptic conditions and in an artificial nutrient medium. From this point of view, it is of great importance to fulfill the need for strawberry seedlings using micro propagation methods. However, there are a limited number of studies on *in-vitro* culture and shoot tip reproduction of strawberry cultivars grown in Turkey.

It has been reported in previous studies that somaclonal variation has been used in plants such as apples, bananas, strawberries, peach, blackberries as a useful tool in micropropagation (Hwang and Ko, 1987; Mc Pheeters and Skirvin, 1989; Hammerschlag and Ognianov, 1990; Chevreau et al., 1998; Sahijram et al., 2003).In those studies, very different chemical doses in the starting culture for shoot regeneration and rooting stages have been tried. In present study, we aimed to determine the reproduction possibilities by using one of the tissue culture techniques, shoot tip culture method and to specify the most appropriate hormone concentrations "Ottoman strawberry", which stands out with its unique aromatic taste and smell in Turkey.

Material and Method

The study was carried out in the Aegean Agricultural Research Institute, Plant Tissue Culture Center. As plant material, explants (3-3.5 cm) in *in-vitro* stock cultures derived from the first sub-culture of the shoot tip explants of the "Ottoman strawberry" cultivar were used. Explants were cultured on MS (Murashige and Skoog, 1962) medium containing macro, micronutrients and vitamins was used and 30 g/l sucrose and 7 g/l agar were added to the medium. Seven different nutrient media containing different combinations of BAP and IBA hormones (0.1 mg/l IBA; 0.2 mg/l IBA; 0.3 mg/l IBA; 0.5 mg/l BAP; 0.5 mg/l BAP + 0.1 mg/l IBA; 0.5 mg/l BAP + 0.2 mg/l IBA; 0.5 mg/l BAP + 0.3 mg/l IBA) were used. The control medium was not supplemented with any hormone. The prepared growth medium was adjusted to pH 5.7 before adding agar and was poured as 40 ml into 175 ml jars. The jars were then autoclaved for 20 minutes under a pressure of 121°C and 1 atm. Autoclaved media were placed inside the sterile cabinet, allowing the media to cool until they reached room temperature. The explants transferred to culture in the jars were kept in a conditioning chamber at a temperature of 24 ± 2°C (with cool white fluorescent light, 3000 lüx), 6h light and 8h dark. The experiment was set up as a completely randomized design. Treatment of experiments with 3 replicates and 5 explants per replicate were established according to the test pattern, and the number of shoots and root formation per explant was determined at the end of the 6^{th} week. Variance analysis of the data was performed with JMP 7 statistical package program on the number of shoots per explant. The differences among the significant averages were compared with the *LSD* test. Rooting occurring in the media were evaluated as absent/present and so were only analyzed the percentage of root formation.

Results and Discussion

The success of sterilization methods *in-vitro* studies affects the success of the entire study (Şahin and Doğan, 2019). With the use of explants in *in-vitro* stock culture, all potential contaminations were avoided and the experiment's homogeneity was achieved using explants (3-3.5 cm) with the same growth strength. The average number of shoots per explant varied between 1.00 and 10.80, and the media influence ($p \leq 0.01$) was found to be statistically significant (Table 1). The lowest number of shoots was observed in the MS medium of 1, 2, and 3 which contained different concentrations of IBA and also in the MS medium 8 where hormone was not used. It is clear that BAP is essential for shoot development. Studies in different plant species support this finding (Sajjad et al., 1994; Gürel and Gülsen, 1998; Asmono et al., 2020).

Table 1. Effects of BAP and IBA doses on the average number of shoots per explant.

MS medium number	Plant growth regulators(mg/l)		Average number of shoots per explant (number)
	BAP	IBA	
1	0	0.1	1.07 ± 0.76 **c***
2	0	0.2	1.00 ± 0.72 **c**
3	0	0.3	1.00 ± 0.75 **c**
4	0.5	0	10.13 ± 0.69 **a**
5	0.5	0.1	10.80 ± 0.62 **a**
6	0.5	0.2	5.40 ± 0.43 **b**
7	0.5	0.3	5.60 ± 0.28 **b**
8	0	0	1.40 ± 0.46 **c**

*Means followed by different letters are significantly different at 0.01 level of significance.

The highest number of shoots was determined from MS medium 5 with 0.5 mg/l BAP and 0.1 mg/l IBA (Figure 2), and it was followed by MS medium 4 with 0.5 mg/l BAP only (Figure 1). No statistically significant difference was observed between these two culture mediums. In the case when both 0.5 mg/l BAP and0.1mg/lIBA dose were increased to higher than 0.1 mg/l, the number of shoots decreased significantly (Table 1). In previous in vitro propagation studies it was tested that using BAP is more effective than other types of cytokines (Arinaitwe et al., 2000; Arab et al., 2014; Erawati et al., 2020) and determination of optimal BAP concentration and combination is important (Şahin et al., 2016; Yahya et al., 2019). For example, higher levels of BAP induce callus formation, apical shoots necrosis, decrease in shoot number, and also rosette plant formation in embryo culture (Ahmad et al., 2003; Şahin et al., 2016; Yahya et al., 2019).

Figure 1. General view from plants in medium 5which is determined as the best medium for shoot development

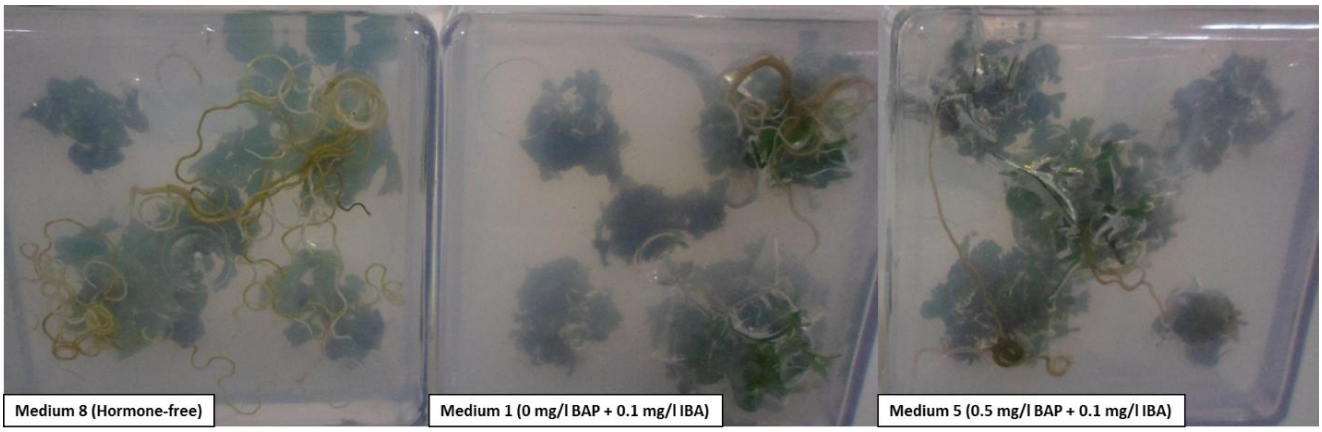

Figure 2. Root formation of different hormone concentration

Previous researches on strawberries have shown that plantlets could be regenerated from leaf derived callus culture (Popescu et al., 1997), somatic embryogenesis (Donnoli et al., 2001), petiole irradiated with gamma rays, and callus culture of leaves (Kaushal et al., 2004). In the present study, we were obtained new plantlets using shoot tip culture technique. For the induction of the second sub-culture of the shoot tip explants, different concentration of BAP and IBA was applied in order to regenerate adventitious shoot tips from explants. Indeed, the BAP requirement for efficient shoot regeneration of plants has also been reported by other studies. For instance, Kaushal et al. (2004) reported that culture medium contains 2 mg/l BAP with 0.5mg/lNAA and 0.5 Kinetin was best for direct shoot regeneration from strawberry leaf explants while Popescu et al. (1997) observed 4.4 mg/l BAP.

There have been a number of reports for shoot regeneration from leaves and adventitious bud (Yonghua et al., 2005; Debnath 2006), petioles (Passey et al., 2003; Debnath, 2006), stems (Graham et al., 1995), peduncular/peduncle base of the flower bud (Foucault and Letouze 1987; Lis, 1993), stipules (Passey et al., 2003), roots (Passey et al., 2003), stolons (Lis, 1993), runners (Liu and Sanford, 1988), anther cultures (Owen and Miller, 1996) mesophyll protoplasts (Nyman and Wallin, 1988), and from immature embryos (Wang et al., 1984) of strawberries. Shoot regeneration from greenhouse-grown strawberry plants or directly field (Nehra et al., 1989) or (Debnath, 2006) or from in vitro-grown shoots (Yonghua et al., 2005) have been reported. However, difficulties occur in forming *in-vitro* cultures due to the possibility of explants from field-grown plants to be highly contaminated. It is generally recommended to take plants from buds which flush from dormant shoots stored indoors or from plants under controlled conditions such as greenhouse or grow room. In our study, therefore, plants were grown under greenhouse conditions, and for *in-vitro* stock culture different doses of IBA and 0.5 doses of BAP medium were used. Culturing explants in a 0.5 mg/l BAP+ 0.1 mg/l IBA medium resulted in successful induction of average number of shoots and root formation. Our findings also showed that regenerated plants in 0.5 mg/l BAP+ 0.1 mg/l IBA medium were more vigorous than other doses and control plants. BAP concentrations at high doses have been widely used for *in-vitro* stock culture in different plants (Manjula et al., 2000; Chatterjee and Gupta, 1998; Ahmed et al., 2002; Kaushal et al., 2004; Amzad et al., 2003).

In our study, at different combinations of both IBA and BAP rooting at different rates was obtained. The root formation (%) of control and 0.5 mg/l BAP+0.1 mg/l IBA medium was higher than that of other plantlets obtained growth regulator mediums (Figure 2).The percentage of the root formation in only IBA contains medium, plantlets was

13.33%, 26.67% and 20.00% at 0.1, 0.2 and 0,3mg/l, respectively, whereas that was 20.00%, 26.67% and 13.33% in plantlets of 0.5 mg/l BAP, 0.5 mg/l BAP + 0.1 mg/l IBA and 0.5 mg/l BAP + 0.3 mg/l IBA treatments, although no significant difference was found between this treatment and some other treatments regarding this index (Table 2). In the root formation of IBA and BAP medium plantlets the lower ones (13.33%) were obtained with the only 0.1 mg/l IBA and 0.5 mg/l BAP + 0.3 mg/l IBA treatments. Both *ex-vitro* and *in-vitro* methods have successfully been used to acclimatize and root micro propagated shoots of strawberry plants. Proliferated shoots may be rooted *in-vitro* conditions on Boxus plants, on half-strength MS with activated charcoal (0.6 g/l) and IAA (5.7 µM) or modified cranberry medium or half-strength MS without growth regulators (Moore et al., 1991). The roots of the plant grown in vitro-medium are thick, grow horizontally, are fragile, possess no hairy roots, and their easily damaged. Additionally, plants grown in *in-vitro* conditions have poor water balance, low photosynthetic activity, and their morphology and anatomy is far from being optimal (Borkowska, 2001). In the current study, it is seen that the optimum dose rate for the root development of plants grown in the BAP and IBA culture medium is revealed. Given this situation, it was determined that the most suitable hormone dose for root development is 0.5 mg/l BAP+0.1 mg/l IBA with MS medium. On the other hand, as other doses remain below or above the optimum dose limits, this may have caused the growth retardations listed above for plant root growth. In context, when we look at the highest root formation, MS culture medium 5 with 0.5 mg/l BAP and 0.1 mg/l IBA and MS culture medium 8 (hormone-free) come to the forefront with 66.67% and are statistically separated from other mediums (Table 2). As a general principle, suitable concentration of IBA positively affects root formation (Yahya et al., 2019), roots length and mean root number like other auxins, but in some cases it can be differ (De Vries et al., 1988). It is also reported that the most suitable medium for inducing the highest percentage of explant with roots, the highest mean root length and the highest number of roots per explant were 1 µM IBA, 1 µM NAA, and hormone-free MS medium, respectively (Fatemeh et al., 2010).

Table 2. Effects of BAP and IBA doses on root formation (%).

MS medium number	Plant growth regulators(mg/l)		Root formation(%)
	BAP	IBA	
1	0	0.1	13.33 ± 0.11 **b***
2	0	0.2	26.67 ± 0.13 **b**
3	0	0.3	20.00 ± 0.15 **b**
4	0.5	0	20.00 ± 0.12 **b**
5	0.5	0.1	66.67 ± 0.26 **a**
6	0.5	0.2	26.67 ± 0.15 **b**
7	0.5	0.3	13.33 ± 0.21 **b**
8	0	0	66.67 ± 0.32 **a**

*Means followed by different letters are significantly different at 0.01 level of significance.

Conclusions

The effect of IBA and BAP in different doses added to the MS nutrient medium on the rooting and shoot development was evaluated in order to rooting the plantlets and increase the average number of shoots per explant. In this study, the best results in terms of root formation and the average number of shoots per explant were provided by adding MS culture medium 5 with 0.5 mg/l BAP and 0.1 mg/l IBA. Additionally, this study is of particular importance in that it includes the "Ottoman Strawberry" cultivar among *Fragaria x ananassa* Duch. species. In addition, it is thought that the results obtained from this study will play a key role in pioneering in the use of tissue culture in micro propagation, breeding and especially a biotic stress studies, which will be carried out in the varieties and types of this species in the future.

Author Contributions

SD and MS designed the study and were responsible for the performance of the research, collection and interpretation. OK interpreted the results, data analysis, and wrote the manuscript.

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