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INVITROPROPAGATION OF *FRAGARIA ANANASSA* SEED UNDER 2,4-DICHLOROPHENOXYACETIC ACID AND 6-BENZYLAMINOPURINE GROWTH REGULATORS

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ABSTRACT

An efficient micropropagation system for strawberry (*Fragaria ananassa* L.*)* has been developed to improve strawberry production. An experiment to evaluate the effect of growth regulators under tissue culture conditions and the growth of cultured plants under hydroponic conditions was carried out in the plant tissue culture lab and hydroponic greenhouse of Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore Pakistan. For shoot induction, the sterilized strawberry seeds were cultured on Murashige and Skoog media which have different concentrations of 6-benylaminopurine viz., 0µM, 1µM, 5µM and 10µM and a constant 10µM 2,4-D (2,4-Dichlorophenoxyacetic acid). The highest average number of shoots were observed at same concentrations of 10µM BAP and 10µM 2,4-D during 4-5 days of treatment while the shoot induction was also occurred at 0µM BAP and 10µM 2,4-D concentrations. The other two concentrations 1µM and 5µM showed no effect which also caused no shoot induction. The plant grown from tissue culturing of seeds give better results under hydroponic conditions. It was concluded from our study that the growth of strawberry under controlled conditions (on MS media) and then under hydroponics may be helpful to improve plant growth and production.

Keywords: micropropagation, *Fragaria ananassa*, hydroponic, 2,4-Dichlorophenoxyacetic acid, 6-Benzylaminopurine, growth regulators

INTRODUCTION

Plant tissue culture mainly involves the process of growing new plants in a controlled environment. These plants may be those which we have genetically altered in some way or may be those for which we need multiple copies all exactly alike [1, 2]. By the help of appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots with the help of cytokinin and with the help of auxin new roots can be induced. These plantlets when divided, mostly at the shoot stage, to produce large numbers of new plantlets. The new plantlets which are produced invitro can then be placed in soil which afterward grown in the normal manner [3, 4]. The main tool of plant biotechnology is invitro culture which exploits the totipotency nature of plant cells [5-8]. Tissue culture is called as cell, tissue and organ culture of any organism or plant by applying the invitro conditions [9, 10]. With the help of tissue culturing, large scale propagation of disease free clones and gene pool conservation can be occurred. A large scale plant propagation of elite superior varieties has been produced by ornamental industry which has been applied in invitro propagation. Due to in vitro propagation of plants, hundreds and thousands of plant tissue culture laboratories have been build up in developing countries the main cause is cheap labour costs. As compared to conventional propagation methods, micropropagation is costly [11-13].

In the strawberry production system, mulching is an important component. Mulches are of synthetic and organic types which are usually based on climatic conditions and availability of raw materials in different parts of the country. The plants which are grown under black polythene produce more fruits than with bare soil [14, 15]. Many other beneficial effects of organic mulches on strawberry production have been reported [16, 17]. There is a need of skilled and manageable expertise for high demand of soilless culture of strawberry. It is important to maintain the quality of substrate and water for cultures of strawberries. The recent substrates are either peat moss or mixture of peat moss and coir. The nutrient solution should be calculated according to the soil factors and the requirements of various cultivars being produced. The amounts of macro nutrients (Si) and micro elements (Cl, Fe, Zn) and pH should be accurate for substrate grown strawberries and salinity is also a major factor of sensitivity [18, 19]. In modern days, computer controlled fertigation systems have been developed to manage the

nutrient solutions for substrate grown strawberries. In Belgium and the Netherlands, it is very important to re-use the drainage water to make it pollutant free mandated by the EU. So for this purpose new installations have been established that collect, filter and recycle any excess nutrient solution. Ultraviolet-radiations and sand filtration are the most common sterilization processes for eliminating and reducing harmful pathogens, such as Pythium, Phytophthora and other water molds [20-22]. Present study was conducted to access the effects of using growth regulators for growing strawberry under control conditions.

MATERIALS AND METHODS

Research work for seed germination was done in tissue culture lab of Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore Pakistan.

Selection of media and its composition

A nutrient medium for plant regeneration mostly consists of organic and inorganic salts, irons, a carbon source, some vitamins and growth regulators. In the shoot proliferation of strawberry seed MS [23] medium was used as a basal medium for plant regeneration [24].

Preparation of MS Media

Murashige and Skoog medium was prepared by using MS media named MS media with salts and vitamins 4.9 g/l and sucrose 40 g/l, phytagel 4 g/l for solidifying. The volume was considered to be 1 litre. The pH of the medium was maintained to be 5.7 to 5.8 by using 1N KOH or 1N acetic acid before adding phytagel in the medium flask. After adding phytagel, the media was autoclaved at 121^0C for 20 minutes at 15 KPa for sterilization purpose.

Hormones supplementation

After autoclaving, the MS medium was allowed to cool down. Then the medium was supplemented with two different hormones BAP and 2-4-D for shoot induction. Three different concentrations of BAP hormone (1µM,5µM,10µM) and constant concentration of 2-4-D(10µM) for shoot induction was added in four different flasks containing 250ml of MS media each (Table 1). Ashrafuzzaman and his colleagues studied five BAP concentrations 0.0 (Control), 0.5, 1.0, 1.5 and 2.0 mg/l for shoot induction [24].

Table 1: Different concentrations of BAP and 2,4-D hormones supplemented in MS medium for shoot proliferation

No. of concentrations	Concentration of BAP (µM)	Concentration of BAP (µl)	Concentration of 2,4-D (µM)	Concentration of 2,4-D (µl)
Control	0µM	0µl	10µM	24µl
Regeneration media 1	1µM	2.5µl	10µM	24µl
Regeneration media 2	5µM	12.4µl	10µM	24µl
Regeneration media 3	10µM	24µl	10µM	24µl

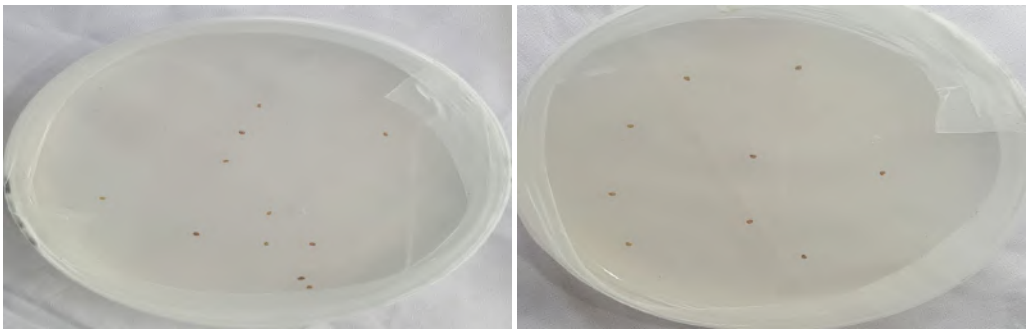
After the addition of hormones, each medium with different concentrations were poured in five petri plates each. The medium was allowed to solidify in the petri plates and then the petri plates were covered with their lids and rapped with parafilm and labelled. All the work was done in laminar flow cabinet to avoid any type of contamination.

Seed Sterilization

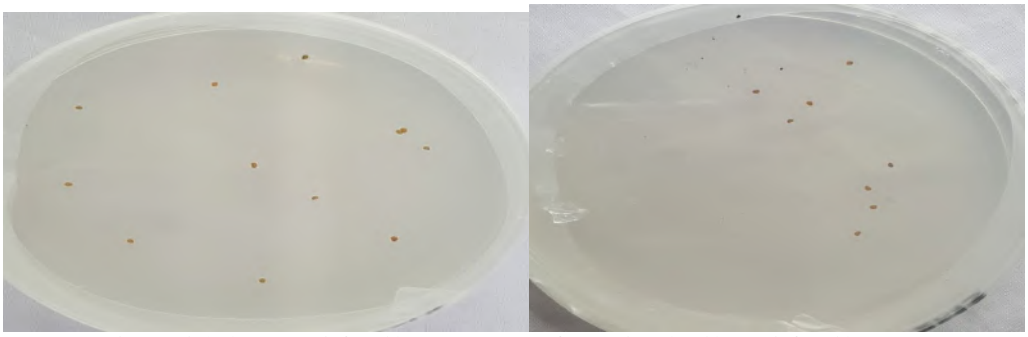
To avoid all types of contamination, the seeds should also be sterilized before inoculation into the medium. The seeds were treated with 10% bleach for about 5 minutes. Then they were washed with autoclaved water for about 3-4 times for 3-4 seconds so that all the bl^{ooo} °°each was washed off from the seeds. Then the seeds were given hormone treatment with 5% hydrogen peroxide and 0.5g GA3 in a reagent bottle. The seeds were soaked overnight in it at 65-70 °C temperature inside the incubator.

Seed inoculation

The overnight seeds were inoculated inside the petri plates containing MS medium having various concentrations of BAP for invitro multiple shoot regeneration with help of fine forceps. At least 5-6 seeds were sown in each plate for that enough space should be provided to the plant to regenerate itself (Figure 1). All the inoculations and the aseptic manipulations were carried out in laminar flow cabinet to avoid contamination. During the procedure, hands and the cabinet base and all the equipment was treated with 70% ethanol for maintaining aseptic conditions. The physical conditions for growth and development of cultures were maintained at the temperature 25 °C and a light intensity of about 3000-3500 lux was provided by fluorescent tube. The photoperiod was maintained at 12 hours light and 12 hours dark (12L/12D) and the relative humidity was about 60-70% [24]. The successful shoot formation can be seen when small green fresh leaves began to emerge from the seeds. It is the first sign of regeneration.



1) Control (BAP 0µM, 2,4-D 10µM) **2) RM1 (BAP 1µM, 2,4-D 10µM)**



3) RM2 (BAP 5µM, 2,4-D 10µM) 4) RM3 (BAP 10µM, 2,4-D 10µM)

Fig. 1: Inoculation of seeds on MS media containing different concentrations of BAP and constant 2,4-D.

RESULTS

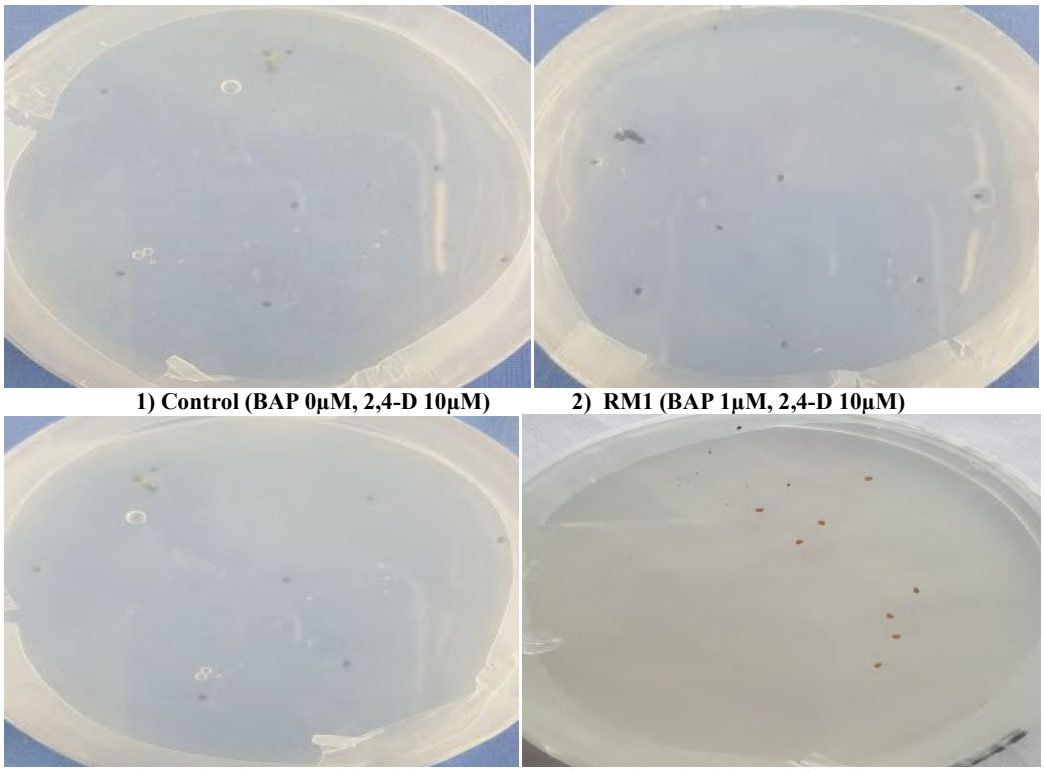
Shoot induction

The shoot started inducing after 4-5 days.

The average number of shoots regenerated was observed to be (1) on 0.0µl and (2) on 24µl of BAP concentrations (Table 2 and Figure 2). No shoots were regenerated on 2.5µl and 12.4µl BAP concentrations.

Table 2: Response of strawberry seed to different BAP concentrations supplemented in MS medium on shoot proliferation

BAP (µl)	Number of seeds inoculated	Average number of shoots regeneration
0.0µl	10	1
2.5µl	9	5
12.4µl	11	7
24µl	9	2 (1 on each plate)



1) Control (BAP 0µM, 2,4-D 10µM) 2) RM1 (BAP 1µM, 2,4-D 10µM)

3) RM2 (BAP 5µM, 2,4-D 10µM) 4) RM3 (BAP 10µM, 2,4-D 10µM)

Fig. 2: Shoot proliferation on different BAP and 2,4-D concentrations

Fig. 3: Strawberry grown under different conditions (A) RM1 (BAP 1µM, 2,4-D 10µM) (B) RM2 (BAP 5µM, 2,4-D 10µM), (C) RM3 (BAP 10µM, 2,4-D 10µM), (D and E) Strawberry plants grown in hydroponic at early stage

DISCUSSION

A large quantity of plant material is being produced by the advantage of tissue culture technology in a very short time as compared to the conventional methods which is through vegetative propagation. Many commercial applications of this technology have been seen in horticulture. So, the in vitro production of strawberry would lead to the development of commercial micropropagation. The strawberry plants derived on commercial scale from in vitro propagation cost four to five times more than the plants which have been produced by conventional propagation [25-27]. The micro propagated strawberry has many benefits. It has the ability to produce virus free plants. It also improves capacity of these plants to produce runners for planting in the field [28, 29]. There are many studies which are related to tissue culture of strawberry. About 30 years ago, the micropropagation of

strawberry was reported [30]. It was found that the best shoots were induced on the media supplemented with having 0.5mg/l BAP, which is a little bit similar to this study in which the best shoot induction was on the media containing 24µl of BAP.

It has been observed that to induce shoot the lower level of BAP (0.5mg/l) was more effective as compared to higher concentrations of BAP which was about 1.0 to 3.0 mg/l [31]. In the present study these results were also observed at high levels of BAP concentration (2.0 mg/l). As in my study, both on the higher (24µl) and lower levels (0µl) of BAP the shoot was being proliferated. Different types of variations at different concentrations of BAP during the growth stage of the plantlets. The results from this study confirmed the importance of 2,4-Dichlorophenoxyacetic acid as a plant growth regulator to promote shoot formation which can be either with or without BAP hormone. Hence it is evident the presence of 2,4-Dichlorophenoxyacetic acid and BAP broke the apical dominance and therefore shoot proliferation occurred. 2,4-Dichlorophenoxyacetic acid was introduced to be the most common plant regulator in 1942. It is the most widely used herbicide in the United States and more than 100 countries. In United States, it is registered as herbicide for the control of broad leaf plants and as a plant growth regulator [32, 33].

This study shows that in the absence of BAP but in the presence of 10µM 2,4-Dichlorophenoxyacetic acid shoots were regenerated from the strawberry seeds. On the other hand, it also reveals that the combination of the high concentration of 10µM 2,4-D and 10µM of BAP promoted a high percentage of shoot proliferation. So, all the overall study shows that the plant growth regulators (PGRs) often act alone but in many of cases these regulators interact with each other for the production of plantlets [34, 35]. So, it was revealed from my study that the presence or absence of BAP did not affect the shoot regeneration from strawberry seeds as much but the presence of 2,4-Dichlorophenoxyacetic acid is necessary for the shoot development. Ashrafuzzaman and his colleagues studied that the highest number of shoots were regenerated at the lowest BAP concentration [24].

CONCLUSION

Overall study showed that, MS medium containing 10µM BAP and 10µM 2,4-D were the most suitable media for strawberry (*Fragaria ananassa)*. The optimized concentration of both of the hormones showed that both are essential plant growth regulators for the shoot production from

strawberry seed. But on the other hand, in the absence of BAP the shoots were also regenerated which means that the hormone 2,4-Dichlorophenoxyacetic acid is one of the most important plant growth regulator and effective for the dicots or broadleaf plants but not for monocots. As strawberry plants are dicots so 2,4-Dichlorophenoxyacetic acid induces their growth and hence in the presence of 2,4-Dichlorophenoxyacetic acid the shoot proliferation of strawberry seed was induced. The presence or absence of BAP not really effected the shoot induction but the presence of 2,4-Dichlorophenoxyacetic acid effects the shoot induction. The plant grown from tissue culture was cultured under hydroponic conditions to get fruits.

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