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**EFFECT OF BENZYL ADENINE ON CALLUS FORMATION IN APPLE**

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**Abstract**

Effect of benzyl adenine on callus formation in apple cotyledon explants *in vitro* was observed. The cotyledons were separated from embryo and cultured on MS media supplemented with different concentrations of BA. Maximum callus formation was observed in cultures supplemented with 1.0 mg l<sup>-1</sup> BA followed by BA 2.0 mg l<sup>-1</sup>, BA 3 mg l<sup>-1</sup> and control the least. Maximum 100% callusing was observed after 15 days of culturing the cotyledons on MS media provided with BA 1 mg l<sup>-1</sup>. As per time period to callus formation, the observations depicted that callus formation initiated after 15 days of cotyledon culture on MS media, 7 days, and 8.8 days on control, 1 mg l<sup>-1</sup>, 2 mg l<sup>-1</sup> and 3 mg l<sup>-1</sup>, respectively provided with different concentrations of BA. Regarding days to completion of callus formation 45, 30, 38 and 40 days time period was observed under control, BA 1 mg l<sup>-1</sup>, BA 2 mg l<sup>-1</sup> and BA 3 mg l<sup>-1</sup>, respectively. The aim of this research venture was to develop a well defined media protocol for regeneration of apple from cotyledon and to enhance the existing status of apple germplasm in the Pakistan.

**INTRODUCTION**

Apple (*Pyrus malus*) is most important and widely grown temperate fruit of the world that requires a distinct cold period. It is considered as one of the most ancient fruits. The apple fruit is very nutritious, aromatic and rich in minerals and vitamins. Apples have been widely used as a good fruit from the earliest times and its curative powers are clearly illustrated by the proverb "an apple a day keeps the doctor away".

In Pakistan apples are grown in Baluchistan (Quetta, Kalat, Ziarat), NWFP (Swat, Chitral, Hazara, Para Chanar), Punjab (Murree Hills) and Northern regions like Gilgat, Skardu, Hunza and Kashmir (Dhirkot, Rawlakot, Bagh, Neelum valley). The largest production is from Quetta region of the Baluchistan province. Apple ranks fifth in acreage of fruit in Pakistan. In Pakistan, it's first in the temperate hilly zone where the grower's economy is dependent on fruit.

Traditionally, apple plants are propagated by budding or grafting the scion cultivars on rootstock in nursery where they are allowed to grow for a period of one to three years before transfer to orchard site. This method is used because scion cultivars are not true to type from seed and are difficult to propagate by conventional layering or cutting techniques. Propagation and establishment of rootstock, budding or grafting with the scion and growing to form 1-2 year old tree in the nursery is, however, a lengthy and increasingly expensive procedure. As a result the

methods of reducing tree costs have become of considerable importance. Interest has been developed towards producing trees with shoots and roots of the same genotype, both to meet increased demand and to propagate trees at lower cost. Tissue culture propagation stands out as a logical method to produce own rooted plants because it has the potential for large scale increase of plants in minimum possible time.

The application of tissue culture technology to the micro propagation and regeneration of apple plants has also increased in importance, because it offers mass production of disease free plants. It has now become accepted alternative to conventional propagation procedures. Self rooted apple plants propagated through tissue culture techniques may have potential for commercial orchard use and have been proposed as a potential alternative to trees budded or grafted on vigorous or moderately vigorously moderately rootstock. Sexual and asexual means of propagation through conventional methods have one drawback or the other. This is particularly true in relation to scion stock incompatibility and disease incidence.

The regeneration of self rooted plants through somatic embryogenesis could become a very useful technique for large scale propagation. If methods are developed to generate apple from callus, cells or protoplasts, either by somatic embryogenesis or by organogenesis, then it is conceivable to do screening *in vitro* to cells, plant parts, or young plants to induce mutations which might prove useful in breeding or in production of tree and fruit. The ability to combine mutagenic treatments with *in vitro* screening provides apple breeders with a very powerful tool for crop improvement.

Maari et al., (1994) indicated that *Pyrus communis* cultivars established best on medium containing 2.0 mg l<sup>-1</sup> BAP, 0.1 mg l<sup>-1</sup> BA and 0.2 mg l<sup>-1</sup> GA<sub>3</sub>. Hutchinson (1985) cultured shoot tips of apple cultivar Northern spy in mid-spring and mid-summer and obtained few globular heart shaped embryos. Somatic embryos were produced from leaf explants of Golden Delicious cultured on MS media with B<sub>5</sub> 10.0 mg l<sup>-1</sup> BA and 3.0 mg l<sup>-1</sup> NAA in the dark (Liu et al., 1983). Similarly, embryo free cotyledons produced callus when cultured on medium with several vitamins, malt extract, inositol and coconut milk (Kouider et al., 1985).

Callus formation from stem internodes of several apple rootstocks was initiated on four NAA based media (James et al., 1984). Similarly Evaldsson (1985) obtained callus when stem segments of cultivar Akero were cultured on MS medium with IBA and BA. Michael and Gray (1993) observed that addition of IAA to the medium with BA increased callus formation. Lane (1982) reported that BA at concentration of 0.87 mg l<sup>-1</sup> was required for establishment of shoot meristems of Macspur and Harrold Red apple. Balerioual and Mullins (1982) reported that nodal explants of cultivars Jonathan, Richard and Golden Delicious established best on medium containing 1.75 mg l<sup>-1</sup> BA. During studies Belaizei et al. (1989) depicted that 0.085 mg l<sup>-1</sup> IBA and 0.035 mg l<sup>-1</sup> BA gave better results for establishment during preliminary growth phase for apical buds. Regeneration of adventitious shoots has been obtained from *in vitro* leaved of apple cultivars with vitamins, BA and IBA (Fasolo et al., 1990). However, BAP 0.77 mg l<sup>-1</sup> in combination with the TIBA 0.17 mg l<sup>-1</sup> gave the highest percentage of regeneration (Belaizei et al., 1990). Present studies were under taken to develop protocols for efficient micro propagation and regeneration of apple cultivars.

## **MATERIALS AND METHOD**

The present studies were conducted at Institute for Horticultural Sciences, University of Agriculture, Faisalabad. In the present study MS medium (Murashige and Skoog, 1962) was used. The MS media was prepared by taking the specific amount of stock solutions. Sugar was added and pH was adjusted with the help of pH meter. It was adjusted at 5.7. Agar was added and the whole composition was boiled to dissolve the agar uniformly. The media was poured in to the glass test tubes and were wrapped with the help of polythene paper and rubber band and were sterilized in autoclave at 121°C and 1.5 lbs psi for 15-20 minutes. After autoclaving the test tubes

were taken out and kept in growth room. All the material except ex-plant was kept in inoculation room and UV was kept on for 20-25 minutes for sterilization.

The seed of apple were surface washed with the help of Tween 20 for two minutes and sterilized with the help of 70% Ethyl alcohol for 3-5 minutes. The seed were taken in the inoculation room provided with laminar air flow. The seed were washed with double distilled water and surface sterilized with Mercuric Chloride 0.1% for 3-5 minutes. The seed were washed three to four times with double distilled water to remove the effect of chemicals. After peeling embryo was cut and cotyledon were get. Then each cotyledon was cultured in media provided with different concentration of Benzyl Adenine along with control. The cultured test tubes were kept in the growth room facilitated by fluorescent tube lights of total light intensity 2500 lux maintaining 16 hours day light and  $25\pm 2^{\circ}\text{C}$  temperature. The cultured tubes were wrapped with black paper for one week. The cultures were observed for callus formation.

## RESULTS

The apple cotyledon cultured as explant on MS media supplemented with different concentrations of BA depicted that 37% callusing was observed in the control which was minimum, while maximum 100% was depicted by the media having BA  $1\text{ mg l}^{-1}$  and 80% in media with  $2\text{ mg l}^{-1}$  BA and 90% in BA  $3\text{ mg l}^{-1}$ . After 22 days Maximum 100% callusing was found in medium provided with BA  $1\text{ mg l}^{-1}$ ,  $2\text{ mg l}^{-1}$ ,  $3\text{ mg l}^{-1}$ , respectively and the least 71% in control (without BA). After 29 days all the media combinations showed 100% callusing in the explants. The earlier callusing was found in the medium having BA  $1\text{ mg l}^{-1}$  (Table 1).

As per days to initiate callus it was found that control exhibited the callusing after 15 days of culturing while BA  $1\text{ mg l}^{-1}$  induced callus after 7 days. Benzyl adenine with concentration of  $2\text{ mg l}^{-1}$  and  $3\text{ mg l}^{-1}$  showed the callus initiation days 8 each. Subject to the completion of the callusing in the cultures it was found that medium supplemented with benzyl adenine  $1\text{ mg l}^{-1}$  the duration was 30 days. The control exhibited 45 days which was maximum. While in media supplemented with BA  $2\text{ mg l}^{-1}$  38 days and 40 days in BA  $3\text{ mg l}^{-1}$  were observed. The medium provided with BA  $1\text{ mg l}^{-1}$  showed the callus initiation after 7 days of culture, and completion time was 30 days which proved the best combination (Table 2). The study depicted that low dose ( $1\text{ mg l}^{-1}$ ) of hormones enhanced callus formation in apple. The aim of this research venture was to develop a well defined protocol for most suitable media combination for regeneration of apple from cotyledon and to enhance the existing status of apple germplasm.

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**Table 1:** Effect of BA on callus formation percentage in apple cotyledon explants *in vitro*

BA (mg l <sup>-1</sup> )	15 days	22 days	29 days
0	37	71	100
1	100	100	100
2	80	100	100
3	90	100	100

**Table 2:** Time period to callus formation

BA (mg l <sup>-1</sup> )	Initiation	Completion
0	15 days	45 days
1	7 days	30 days
2	8 days	38 days
3	8 days	40 days