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Plant Growth Regulators for Efficient in vitro Regeneration of *Solanum lycopersicon*

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Abstract

Leaf, apical meristem and nodal explants of *Solanum lycopersicon* were cultured in many permutations and concentrations of BAP and IBA to improve *in vitro* environments and to detect the reaction of tomato cultivar. All the explants always formed calli with the diversity of color and textures after three weeks. Callus induction was observed at a low concentration of hormonal arrangement in case of leaf explant; however, high concentration was effective in case of apical meristem and nodal explants. No early morphogenic response was detected, after 6 weeks morphogenic response of callus was perceived at 1.2 mg/L BAP + 2 mg/L IBA and clear shoot primordia were formed by nearly all explants. Calli consuming shoot embryonic were transferred to hormones-free MS media for additional differentiation and growth. Whole redevelopment i.e. Shoot and root development were detected after 8 weeks. A regular of 10 shoots with insufficient roots was detected on BAP+IBA (1.2mg/L+2mg/L) from calli of leaf explants. While 7-8 shoots with numerous roots and 5 shoots with several roots in case of apical meristem and nodal callus individually.

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Introduction

The capability of plants to reproduce asexually has been efficiently exploited in plant biotechnology, which has been done the application of clonal micropropagation (Through somatic embryogenesis, organogenesis, and nodal culture). Specific families have high regeneration abilities, but others such as (specifically woody species) in vitro vegetative propagation may be extra difficult [5]. Plant tissue culture is the repairs of plant cells, organs or tissue in sterile, environmentally and nutritionally helpful situations *in vitro* [21, 23].

Plant tissue culture is a different technique of propagation and is widely used for profitable propagation of an enormous number of plant type, such as including many medicinal plants [12, 20]. *In vitro* culture is permitted from exogenous fungal and bacterial pollutants and might be virus-free, if formed from meristem-tip culture. This suggestion can successfully use for the manufacture and source of healthy plants for overall agriculture. [9, 15]. The significant development controllers frequently used in diverse preparations and concentration in tissue culture media for research determinations are auxins, cytokinins, and gibberellins [3, 17].

Tomato (*Solanum lycopersicum* formerly *Lycopersicon esculentum* Mill.), belong to the *Solanaceae* family; it is the second largest best important vegetable production in all worldwide marketable distribution. Tomato by its nature is a permanent (every year) but commercially grow as a once-a-year crop. The attractiveness of tomato between customers has through it the main source of vitamins A and C in diets. Three types of tomato fruits are most common in worldwide such as smaller-size "cherry" tomatoes, fresh market tomatoes, and processed tomatoes. Sort out tomatoes have a bright red color and high solids content that kinds them appropriate for production of tomato paste, ketchup or sauce. Tomato cultivates best under the temperature assortment of 20-27°C. Fruit situation is poor because when average temperature cross 30°C or decreases below 10°C. Tomato grows benefits from crop revolution. Tomato grows after paddy rice because of decreases the occurrence of diseases and nematodes [14, 17, 19].

Lycopersicum is sustained as the progenitor of cultured tomatoes, and the species happen as a thin outflow from agriculture worldwide. Every tomato is diploid (2n = 24; while rare tetraploid methods happen) [8]. Tomato is observed as a prototypical organism for development and genetic studies [1, 25]. All of the world people accepted tomato as a food which could be used as a source of nutrients in the subcontinent. Micropropagation of select tomato cultivars can preserve their physical quality generation after generation [22, 8].

Nutritionally tomato is significant for observing of health and diets [12]. The caloric assessment is a smaller amount and a normal sized of tomato is (148 g) increases only 35 calories. It also comprises about 20-50 mg of lycopene/100g of the fruit weightiness [10, 18]. Lycopene is the best prevailing antioxidant in the carotenoid family and help is known to stop cancer [8].

Tissue culture is utilized for micropropagation of high esteemed business cultivars. Tomato is a self-pollinated crop by nature and is developed by means of seeds. Different attractive hereditary attributes, for example, high lycopene content are hard to be held because of the occurrence of segregation. In this way, a proficient in vitro plant recovery framework may help the proliferation of the financially vital cultivars without losing their genetically upgraded nutraceutical constituents. In tomato, unusual shoot regeneration can be realized directly or indirectly done in-between callus phase [2, 6]. Certainly, both calli and shoots might be formed together [7, 9].

Keeping in view the importance of tomato plant, the main objectives of the current study was to improve situations for redevelopment of a number of disease-free plants complete tissue culture and the result of different plant growth controllers on micropropagation of diverse tomato explants.

Materials and Methods

Plant Material

An experimental study was carried out on *Solanum lycopersicon*. Explants for experiments were taken from the plants grown in the *green house* at Shaheed Benazir Bhutto University, Sheringal, Dir (U), Pakistan. Leaves, Node, Apical meristem of *Solanum lycopersicon* were used as explants for *in vitro* culture.

Preparation of stock solution for MS media

Murashige and Skoog (MS)Medium is an inorganic salt solution, used as the basal medium during this experimental work. The main mechanisms of the MS medium i.e. inorganic nutrients, trace elements, diverse development hormones, and vitamins were ready independently in stocks and stored below an suitable situation at low temperature.

Preparation of the Medium necessary amounts of agar and sucrose were weighed. Suitable quantities of the

various stock solutions dependent on the concentration of the medium were dignified in a flask with the graduated cylinder. Correspondingly required growth controllers previously or later autoclave, as high temperatures degrade/decompose sucrose and growth controller was more to the medium. The ending volume of the solution was found by adding distilled water. Weighed sucrose was added and comprehensively dissolved in it. After dissolving the sucrose well, the pH of the medium was adjusted with 0.1N NaOH or 0.1N HCl. After the adjustment of pH at 5.8, agar was added to the medium and dissolved by heating and constant stirring, till near the boiling of the solution. The medium was then transferred into flasks that were strongly plugged with cotton wool. The flask comprising the medium, petri dish box, distilled water flask and surgical apparatuses (wrapped in aluminum foil) were sterilized in an autoclave at 151psi at a temperature of 121°C for 15 minutes, the medium was permissible to cool and then used.

Preparation and Sterilization of Explants

Explants were wisely selected from the mother plant of *Solanum lycopersicum*. These were finally washed with tap water and then softly soaked in insignificant profitable detergent solutions for a few seconds and again washed carefully with tap water and then washed in distilled water. Plant material (Pieces of shoots containing 2-3 buds) was then surface sterilized with dissimilar levels of mercuric chloride ($HgCl_2$) for dissimilar times waits. Nodal segment and buds were sterilized for 3 minutes, by 1% mercuric chloride ($HgCl_2$) solution.

Aseptic transfer of plant tissue

Sterilized transfer of plant tissue was approved out in a Laminar Flow: Functioning surface and wall of laminar Flow were spread out with 95% ethyl alcohol. Tools and Petri dishes were sprayed with 95% ethyl alcohol and placed in Laminar Air Flow Chamber for 10-15 minutes earlier working to avoid some chances of contamination. For earlier inoculation hands were spread with 95 % ethyl alcohol, the explant has a large number of fungal and bacterial contaminants on their surfaces; therefore, they were surface sterilized with 1% $HgCl_2$ for 3-4 minutes. The explants were then given 4-5 washing in sterilized water so as to remove all suggestions of the sterilizer. Explants were ready and cut to the obligatory size from the rest of the shoot and moved aseptically to the culture pots. The experimental cultures were incubated in biotrons with a photoperiod of 16/8 light/dark period in 24 hours cycle. The temperature was familiar at 25±1°C.

Results

Callogenesis

For the formation of disease-free cultures, leaf, apical meristem and nodal parts of tomato plants after surface sterilization by growing aseptically on MS medium containing diverse concentrations and a recipe of cytokines and auxins. Respectively explants were cultured on MS-Medium with the combination of BAP + IBA i.e. 0.15 mg/L + 0.02 mg/L; 1.2mg/L + 2mg/L; 2.2mg/L + 1mg/L. Callus was successfully induced and also observed different morphogenic changes within 8 weeks. The results of each explant reply to growth controller are accessible in Table 1.

Callogenesis response of leaf explants of tomato to callus growth was a diverse activity in BAP + IBA unrelatedly of the high or low concentration of phytohormones. Callus development was resolute in 1.2 mg/L BAP + 2mg/L IBA (**Figure 1a**). Callus produced was spineless and light green in color, while sensible callus production was perceived on MS medium containing 2.2mg/L BAP 1mg/L IBA. Soft and green callus production was sawed after 3 weeks. However, soft and green callus with light brown limit was produced on MS medium including 0.15mg/L BAP and 0.0.2 mg/L IBA (**Table 1**).

Actively developing calli in a situation of apical meristem was observed after three weeks on MS medium with 0.15 mg/L BAP and 0.02 mg/L IBA. Callus shaped was nodular, soft and light brown with black acnes (**Fig. 1b**). However, mixture and concentration of BAP + IBA (1.2 mg/L + 2 mg/L) formed nodular, soft and light brown callus after 3 weeks. As the concentration of these phytohormones growths, the low quantity of soft and brown callus production was observed on 2.2 mg/L BAP and 1 mg/L IBA (**Table 1**).

Green morphogenic calli were brought within 3 weeks from nodal explant on MS medium with 0.15 mg/L BAP and 0.02 mg/L IBA. Tough and green callus with black acnes was observed at 1.2 mg/L BAP and 2 mg/L IBA produced rigid close and green callus. Comparable opinions were made on MS medium covering 2.2 mg/L BAP and 1 mg/L IBA.

Effect of Phytohormones on Plant Regeneration

Later 3 weeks, soft and green calli of leaf explants recover meristematic movement and shoot embryonic development was observed on the similar medium

Table 1: Effect of plant growth regulators on callus production & plant regeneration of different tomato explants.

Sr. #	Explant	Hormonal concentration	Callus texture & Color (after 3 weeks)	Callogenic Response	Morphogenetic potential on hormone free MS media
1	Leaf	BAP (0.15 mg/L) +IBA (0.02 mg/L)	Soft, Green with light brown margins	+++	-
2	Leaf	BAP (1.2 mg/L) +IBA (2 mg/L)	Soft and light green	+++	10 shoots with few roots
3	Leaf	BAP (2.2 mg/L) +IBA (1 mg/L)	Soft and green	++	3 shoots with 5-6 roots
4	Apical Meristem	BAP (0.15 mg/L) +IBA (0.02 mg/L)	Soft, nodal & light brown with black spot	++	7-8 shoots with many roots
5	Apical Meristem	BAP (1.2 mg/L) +IBA (2 mg/L)	Soft, nodal and light brown	+++	5-6 shoots with 2 roots
6	Apical Meristem	BAP (2.2 mg/L) +IBA (1 mg/L)	Soft, brown	++	-
7	Node	BAP (0.15 mg/L) +IBA (0.02 mg/L)	Hard and green with black spot	+++	5 shoots with numerous roots
8	Node	BAP (1.2 mg/L) +IBA (2 mg/L)	Hard, compact and green	++	2 shoots with many roots
9	Node	BAP (2.2 mg/L) +IBA (1 mg/L)	Hard, compact and green	++	-

Note: Multiple comparison proportions*, Significant ♦ (P<0.002).

(1.2 mg/L BAP + 2 mg/L IBA; 2,2 mg/L BAP + 1 mg/L IBA). However, calli create on medium having 0.15 mg/L BAP + 0.02 mg/L IBA did not show several morphogenetic perspectives. However, after several days these embryonic shoots were finally produced shoots and transferred to the MS medium without phytohormones. While observed 10 number of shoots with few roots and 3 number of shoots with 5-6 roots were observed after 8 weeks on MS medium without any hormones previously grown on 1.2mg/L BAP + 2mg/L IBA and 2.2 mg/L BAP + 1mg/L IBA respectively (**Table 1**).

Callus of apical meristem formed on low concentration of BAP + IBA (0.15 mg/L BAP + 0.02 mg/L) showing high morphogenetic likely than callus produced on 1.2 mg/L BAP and 2 mg/L IBA. So, the result of shoot primordial was observed from callus on both above-mentioned concentrations. However, callus was produced on higher concentration (2.2 mg/L BAP + 1 mg/L IBA) unsuccessful to yield shoot primordial. This primordial shoot increased and extended after shifted on normal MS medium without free hormone (**Fig. 1c**). The results show, seven to eight shoots with several roots development were observed from shoot primordia on concentration of 0.15 mg/L BAP + 0.02 mg/L IBA (**Fig. 1e**) However 5- shoots with no roots were formed from shoot primordia in MS-medium having 1.2 mg/L BAP + 2 mg/L IBA (**Table 1**).

Shoot primordia from callus of nodal explant produced on a medium having 0.15 mg/L BAP + 0.02 mg/L IBA and further produced 5 shoots with numerous roots upon transferring to MS medium without hormone as shown in (**Fig. 1d**). If the concentration of hormones increased, stimulation of shoot primordial decreased on MS medium with 1.2 mg/L BAP + 2 mg/L IBA. Only two numbers of shoots were extended after transferred to hormone-free MS media then the highest number of root development was observed shown in (**Fig. 1f**). However, no morphogenetic possible callus on a high concentration of phytohormones was identified.

Discussion

Tomato (*Solanum lycopersicum* formerly *Lycopersicon esculentum* Mill.), belong to the *Solanaceae* family; it is the second largest best important vegetable production in all worldwide marketable distribution. Tomato by its nature is a permanent (every year) but commercially grow as a once-a-year crop. The attractiveness of tomato between customers has through it the main source of vitamins A and C in diets. Three types of tomato fruits are most common in worldwide such as smaller-size "cherry" tomatoes, fresh market tomatoes, and processed tomatoes. Sort out tomatoes have a bright red color and high solids content that kinds them appropriate for production of tomato paste, ketchup or sauce. Tomato cultivates best under the temperature assortment of 20-27°C. Fruit situation is poor because when average temperature cross 30°C or decreases below 10°C. Tomato grows benefits from crop revolution. Tomato grows after paddy rice because of decreases the occurrence of diseases and nematodes.

Callus development was determined in 1.2 mg/L BAP + 2 mg/L IBA (**Fig. 1.a**). After 3 weeks callus formation was observed like the soft and light green in colour. We were observed 10 numbers of shoots with few roots from the shoot primordial of leaf callus. While enough callus production was detected on MS medium having 2.2 mg/L BAP and 1 mg/L IBA. The same result was observed after 6 weeks like soft and green callus produced shoot primordial.

Proceed to transfer of the shoot primordial without hormone-free MS medium, the results show that 3 numbers of shoots with 5-6 roots were observed after 8 weeks. However, soft and green callus with light brown side was produced on MS medium inclosing 0.15 mg/L BAP and 0.02 mg/L IBA did not indicate every morphogenic reaction (Table 1). Through the similar thought after cultured the tomato leaf explants on MS medium added with 2 mg/L BAP, 1 mg/L ABA and 0.5 mg/L IAA [24, 25]. Generous callus

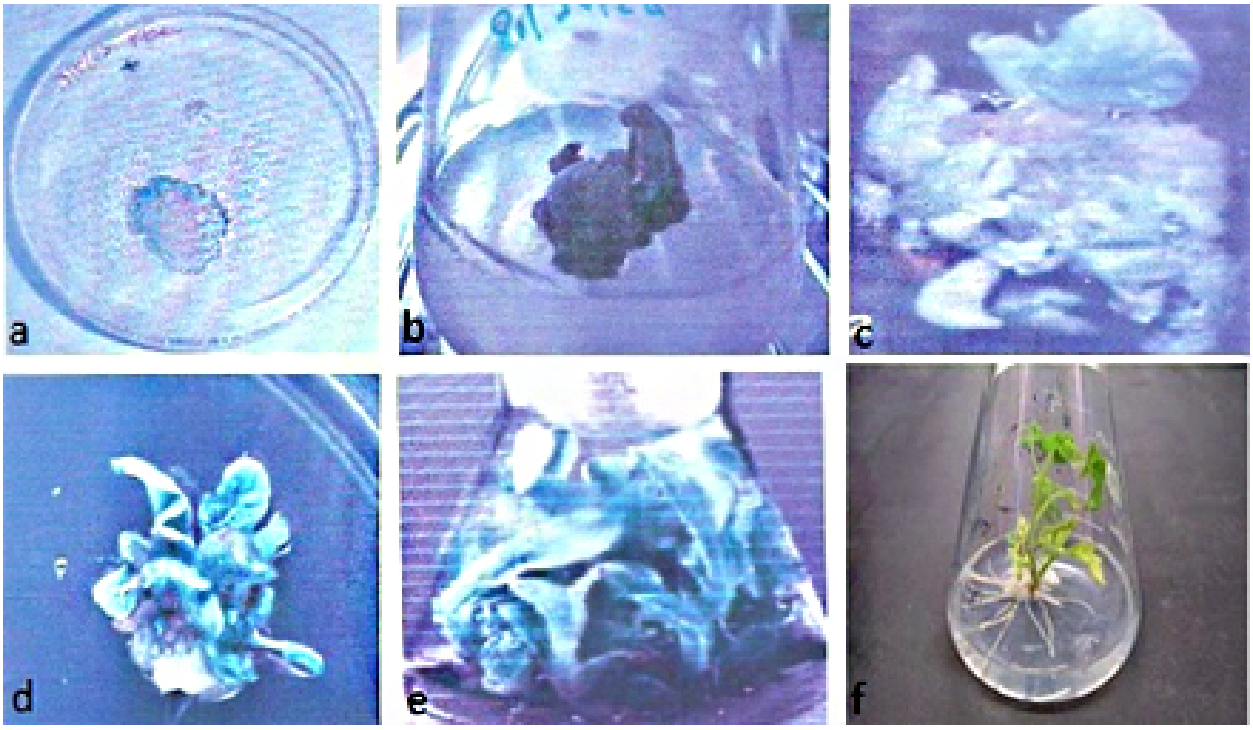

Fig. 1: (a) *In vitro* callogenesis of leaf explant. (b) *In vitro* callogenesis of the apical meristem. (c) Initiation of shoot primordial from callus of the apical meristem. (d) Initiation of shoot primordial from callus of node explant. (e) Shoot multiplication and elongation of the epical meristem. (f) *In vitro* Rooting of regenerated shoots.

stimulation and concurrent shoot initiation were detected. These results are also in accordance with [13]. They also perceived shoot primordia realization on BAP + IBA from the callus of leaf explant and an extreme number of shoot and root amount on hormone-free MS medium. Soft, nodular and light brown callus was formed on MS medium added with BAP + IBA (0.15 mg/L + 0.02 mg/L; 1.2 mg/L + 2 mg/L; 2.2 mg/L + 1 mg/L) after apical meristem. First two hormonal focuses and arrangement formed shoot primordia on the similar medium. Seven to eight shoots with numerous roots and 5-6 shoots with 2 roots were perceived after moved these shoot primordia on MS medium without hormone. Callus produced on high concentration and arrangement of different phytohormone did not show several morphogenic replies. Current study showed conflict with the opinion [4, 16] they found maximum shoot propagation from shoot tip explants in the media having higher concentration of cytokinins i.e. BA (6.66 – 8.88µM) or Kin (9.29 – 13.94µM) and the highest ratio of rooting was reached with medium containing IBA (2.46µM).

Callus produced from nodal explant presented the lowest organogenic response. Result show hard, close and green calli were observed at all concentrations and combinations of BAP + IBA. Yet again no morphogenic response was observed on medium stimulated with a higher concentration of hormones (2.2 mg/L BAP + 1 mg/L IBA) as that apical meristem. The maximum number of shoots and roots (5 shoots with many roots) on MS medium without hormone in a situation of shoot primordia of nodal callus formed on final concentration of BAP + IBA (0.15 mg/L + 0.02 mg/L) was observed. While callus formed on 1.2 mg/L BAP + 2 mg/L IBA produced shoot primordia on the similar medium and moved after 6 weeks to MS medium without hormone. Only 2 shoots with numerous roots extended and increased. A same observation was complete by [4, 11, 13]). They perceived no morphogenic reply of callus in MS medium in a high attention of BAP + IBA (10µM + 5µM) and also observed that lesser attention BAP + IBA (0.5µM + 0.1µM) was appropriate for shoot primordia construction from callus of the nodal explant. They also moved these shoot primordia to the MS medium without hormone and observed growth and elongation of shoots and roots.

Conclusions

The result of different plant growth regulators on the micropropagation of *Solanum lycopersicum* was observed in the current study. For this determination

plantlet redevelopment was verified both through direct or indirect organogeneses via callogenesis explants such as (apical meristem, nodes, and leaves) were used for tissue and callus culture. For callus generation, BAP was used in the MS medium 1.2 mg/L BAP was originated for stimulation soft and light green callus was formed on this concentration. The combination of BAP and IBA was used for shoot proliferation. Concentrated shoot proliferation was found on medium holding 1.2 mg/L BAP and 2 mg/L IBA. The shoots formed were green and healthy. Additionally, this research can also be helpful to evaluate callus initiation size of *Solanum lycopersicum*, which might be studied in more research platforms.

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