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In vitro Propagation of Ancestral Tomato Plant Using 6-Benzyladenine and α-Naphthaleneacetic Acid

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Abstract

The aim of this study was to ensure rapid reproduction of ancestral tomatoes under sterile conditions for the continuation of their generation. *In vitro*, the clonal propagation of tomato plants was investigated on medium containing different concentrations of the hormones 6-Benzyl Adenine (BA) and α-Naphthalene Acetic acid (NAA) in explants. Hypocotyl, cotyledon, and cotyledon nodes explants were taken from 20-day-old tomato seedlings, and their combinations containing BA and NAA were cultured on Murashige Skoog (MS) medium. Subsequently, all explants were transferred to hormone-free MS medium. As a result of the study, the cotyledon explant showed the greatest increase in callus diameter at a concentration of 0.25 BA + 0.4 NAA ppm. This concentration was followed by a two-fold increase at 0.50 BA + 0.8 NAA ppm concentration. The number of shoots in the callus was observed to be significantly in the

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cotyledon explant and was highest at 0.25 BA + 0.2 NAA ppm concentration. Significant results were found in hypocotyl explants regarding callus formation and development (callus number (5/5)). Explants taken from the cotyledonary node were observed to be planted more in pots. Acclimatization and fruit formation were highest in explants taken from the cotyledonary node of the ancestor tomato.

Keywords: BA; NAA; Micropropagation; Organogenesis; *Solanum lycopersicum* L.

6-Benziladenin ve α-Naftalenasetik Asit Kullanılarak Ata Domates Bitkilerinin *in vitro* Çoğaltılması

Öz

Bu çalışma, ata domatesin neslinin devam edebilmesi için steril koşullar altında hızlı bir şekilde çoğalmasını sağlamayı amaçlamaktadır. *In vitro* ortamda, domates bitkilerinin eksplantlarına farklı konsantrasyonlarda 6-Benzil Adenin (BA) ve α-Naftalen Asetik asit (NAA) hormonlarını içeren besi ortamındaki klonal çoğaltımları araştırılmıştır. Hipokotil, kotiledon ve kotiledon boğumu eksplantları 20 günlük domates fidelerinden alınmış ve bunların BA ve NAA içeren kombinasyonları Murashige Skoog (MS) ortamında kültüre alınmıştır. Daha sonra, tüm explantlar hormonsuz MS ortamına aktarılmıştır. Araştırma sonucunda kotiledon eksplant kallus çapında en büyük artışı 0,25 BA + 0,4 NAA ppm konsantrasyonunda göstermiştir. Bu konsantrasyonu 0,50 BA + 0,8 NAA ppm konsantrasyonlarındaki iki kat artış takip etmiştir. Kallustaki sürgün sayısı önemli ölçüde kotiledon eksplantında gözlenmiş ve en çok 0,25 BA + 0,2 NAA ppm konsantrasyonunda görülmüştür. Kallus oluşumu ve gelişimi (kallus sayısı) açısından hipokotil eksplantlarında anlamlı sonuçlar bulunmuştur. Kotiledon boğumdan alınan explantlarda saksıya daha fazla ekim olduğu gözlenmiştir. Aklimatizasyon ve meyve oluşumu en fazla ata domatesin kotiledon boğumundan alınan eksplantlarda görülmüştür.

Anahtar Kelimeler: BA; NAA; Mikroçoğaltım; Organogenez; *Solanum lycopersicum* L.

1. Introduction

The tomato fruit is recognized as an essential resource for hydroxycinnamic acid derivatives and vital nutrients and vitamins [1]. Lycopene is a carotenoid group compound found in abundance in tomatoes. It has numerous biological activities, including acting as an antioxidant [1]. Interest in micropropagation methods is growing as plant biotechnology advances. Tomato production under *in vitro* conditions, one of the first steps of plant biotechnology and genetic engineering, emerges as an alternative production method. The superior races of tomato plants can be made more meaningful through tissue culture studies. Varieties, combinations, and explant

varieties of plant growth regulators form the basis of existing literature on tomato organogenesis and micropropagation [1]. Regeneration somatic embryogenesis started to be explained also as a necessary method for genetic transformation in tomatoes. These methods are also crucial for generating large numbers of elite transgenic plants. However, this potential can only be realized when diversity-specific protocols for morphogenesis are established [1]. It has been observed that cytokinins, the plant growth hormones used in these protocols, play an essential role in somatic embryogenesis in tomato plants [2, 3]. They indicated that cotyledon explants have organogenesis advantages compared to hypocotyl and leaf explants [4]. Micropropagation is the process of propagating and rooting plants from organized meristems, from immature or mature somatic cells, either directly (organogenesis or somatic embryogenesis) or indirectly (callus, protoplast, etc.) [5]. However, vegetable propagation of tomato plants has recently been used for the commercial production of genetically superior cultivars of heterozygous genotypes. The demand for those new cultivars has been rapidly increasing, and an immediate need is the efficient development of techniques to propagate them [6-8]. Plant varieties, explant types, and the media composition of plant growth regulators are three critical factors affecting micropropagation. The regeneration capacity of the explants taken from the plant depends mainly on the plant variety and the explant type [9].

This study aims to achieve the micropropagation of the tomato plant using the most suitable combination of cytokinin and auxin for the micropropagation of the plant, specifically using hypocotyl, cotyledon node, and cotyledon explants. This article is an essential protocol for the rapid propagation of tomato plants. Tomato plants grown in aseptic conditions were removed from the pots, and the total number of specimens, number of callus, callus diameter, number of shoots in the callus, plant height, shoot branches, and number of plantlets in the pots were analyzed. This research seeks to optimize the tissue culture conditions for tomato plants in order to enhance regeneration efficiency and minimize contamination risks during in vitro propagation.

2. Material and Methods

2.1. Plant Material and Disinfection of Seeds

Ancestor tomato seeds (*Solanum lycopersicum* L.) were provided by a farmer in Türkiye's Elazig region. The tomato variety is heirloom (traditional). Before 1970, ancestral seeds were multiplied every year and sown continuously. Seeds were disinfected in 5% commercial sodium hypochlorite (NaOCl) solution for 25 minutes, followed by rinsing with sterile distilled water 5 times for 5 minutes.

2.2. Seedling Growth

Different parts of the seedlings obtained by germinating the seeds in MS medium for 3 weeks were used as explants.

2.3. Culture Conditions and Culture medium preparation

A total of 6,4 g/L of plant agar (Duchefa), 30 g/L of sucrose, and 4.4 g/L of MS medium [10] were added to the medium, which was then autoclaved at 121 °C for 15 minutes with the pH adjusted to 5.8. The 0.5xMS version of this medium was poured into culture vessel boxes. The prepared explants were transferred to sterile culture medium in a laminar flow cabinet with the help of forceps. An acclimatization chamber with a photoperiod of 22 °C, relative humidity of 70 ± 5%, light intensity of 250 µmol $m^{-2}s^{-1}$,16 hours light and 8 hours dark was used as a growth medium.

2.4. Shoot Organogenesis and Callus Regeneration from Hypocotyl, Cotyledon Node, and Cotyledon Explants

The hypocotyl, cotyledon, and cotyledon nodes of 20-day-old tomato shoots were separated into three parts. The MS medium (Duchefa) was made up of NAA and BA. NAA and BA were dissolved in 99% ethanol and 1 N sodium hydroxide, respectively, in distilled water. The medium, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 ppm NAA with 0.25 ppm BA and 0.1, 0.2, 0.4, 0.6, 0.8, and 1 ppm NAA with 0.50 ppm BA, were combined for 12 concentrations (Table 1). The samples were kept in the air conditioning cabinet at 16/8 photoperiod for two months. The inside of the cabinet was measured at approximately 22-24°C, and the relative humidity was 80 percent. The explants were then transferred to the MS medium devoid of hormones. The samples were placed in MS media without plant growth regulators for rooting.

2.5. *Ex vitro* Acclimatization

Seedlings were transplanted from MS medium into pots. Pots, peat, and perlite were disinfected before transfer. These tomato seedlings were transplanted into pots with a 3:1 mixture of peat and perlite. The stretch film surrounding the pots was removed one week after the seedlings were acclimated to their surroundings. Tomato seedlings were irrigated regularly every 2 days. Pollination occurs under controlled laboratory conditions on plants grown in tissue culture. To do this, pollen was provided, and tomato flowers were shaken by a light blow in the morning. There, aseptic conditions were used to produce tomato fruit and ancestor tomato seeds. Explants were cultured in 5 culture vessels per medium and 5 plant explants per culture vessel. At least 5 repetitions were used in this study.

Table 1: Combinations of NAA and BA in the medium

Medium No	BA (ppm)	NAA (ppm)
MS 1	0.25	0.1
MS 2	0.25	0.2
MS 3	0.25	0.4
MS 4	0.25	0.6
MS 5	0.25	0.8
MS 6	0.25	1
MS 7	0.50	0.1
MS 8	0.50	0.2
MS 9	0.50	0.4
MS10	0.50	0.6
MS 11	0.50	0.8
MS 12	0.50	1

2.6. Statistical Analysis

Data analysis was carried out using Statistical Analysis Software's analysis of variance (ANOVA). A crucial F test for the analysis of variance served as the foundation for the Least Significant Difference (LSD) test. Results were analyzed using the Mean Standard Error. One-way analysis of variance (ANOVA) and the Tukey test were used for multiple comparisons to identify significant differences (p: *<0.05; **<0.01; ***<0.001) with SPSS 15.

3. Results

This study indicates that acclimatization and fruit formation were highest in explants taken from the cotyledonary node of the ancestor tomato. The test's significance level (p) was used to assess the accuracy of the values acquired for this investigation. The tests for somatic embryonic development and organogenesis, which were carried out independently using different concentrations and combinations of BA and NAA, produced significant results (p). In the event that the values we discovered after the test result exceeded 0.05, the results were deemed insignificant. When grown in MS medium without hormones, explants from tomato seeds with

proven germination showed no improvement. Therefore, the lowest concentration of 0.25 ppm BA+0.1 ppm NAA was used as the control group. The studies resulted in measurements of callus number, callus diameter, callus shoot number, shoot length, shoot branch number, and potted plant count at various concentrations. It has not been documented because the callus and shoot length are less than 1 cm in some concentrations. No statistical evaluation was performed between the groups.

3.1. Callus Regeneration and Shoot Organogenesis from Hypocotyl Explants

As a result of the measurements made, at concentrations of 0.50 ppm BA and 0.6 ppm NAA, the callus count was found to be significant ($p<0.05$). At concentrations of 0.25 ppm BA+1 ppm NAA (48%) and 0.50 ppm BA + 0.1% NAA (60%) the diameter of the calli increased significantly ($p<0.05$). At concentrations of 0.25 ppm BA+0.6 ppm NAA (40%) and 0.25 ppm BA+1 ppm NAA (57%), the majority of the concentrations, which generally vary in the number of shoots in the callus, showed a significant decrease in data ($p<0.001$). It was discovered that there was little increase in shoot length at different concentrations. At 0.50 ppm BA+0.2 ppm NAA concentrations, however, shoot length was found to be significant ($p<0.001$). It was found that the number of shoot branches increased at concentrations of 0.25 ppm BA+0.6 ppm NAA (66%), 0.50 ppm BA+0.2 ppm NAA (66%) ($p<0.001$), and 0.50 ppm BA+0.6 ppm NAA (67%). The number of plantlets in pots was not observed to increase for hypocotyl (Table 3).

3.2. Acclimatization, Shoot Organogenesis, and Callus Regeneration from Cotyledon Node Explants

Table 4's measurements indicate that the number of calli dropped at concentrations of 0.25 ppm BA + 0.2 ppm NAA (13%), 0.25 ppm BA + 0.4 ppm NAA (13%), 0.25 ppm BA + 0.6 ppm NAA (7%), 0.25 ppm BA + 0.8 ppm NAA (7%), 0.25 ppm BA + 1 ppm NAA (13%), 0.50 ppm BA + 0.6 ppm NAA, 0.50 ppm BA + 0.8 ppm NAA (13%), and 0.50 ppm BA + 1 ppm NAA (20%). The callus's diameter decreased considerably at concentrations of 0.25 ppm BA + 0.4 ppm NAA, 0.25 ppm BA + 0.6 ppm NAA (44%), 0.25 ppm BA + 0.8 ppm NAA (44%), 0.25 ppm BA + 1 ppm NAA (32%), and 0.50 ppm BA + 0.6 ppm NAA. The following information was discovered to be declining in relation to the number of shoots in the callus: 0.25 ppm BA + 0.2 ppm NAA (19%), 0.25 ppm BA + 0.4 ppm NAA (25%) ($p<0.001$), 0.25 ppm BA + 0.6 ppm NAA (12%), 0.25 ppm BA + 0.8 ppm NAA (12%), and 0.50 ppm BA + 1 ppm NAA (25%) ($p<0.001$). Concentrations of 0.25 ppm BA + 0.2 ppm NAA (22%), 0.25 ppm BA + 0.6 ppm NAA (26%), 0.25 ppm BA + 0.8 ppm NAA (42%), 0.25 ppm BA + 1 ppm NAA (26%), 0.50 ppm BA + 0.2 ppm NAA (48%) and 0.50 ppm BA + 1 ppm NAA (79%) resulted in a significant decrease in

shoot length ($p<0.001$). The count of shoot branches markedly decreased ($p<0.001$) at concentrations of 0.50 ppm BA + 0.02 ppm NAA (42%), and 0.50 ppm BA + 0.06 ppm NAA (9%). In an MS medium devoid of hormones, it was observed that the explants taken from the cotyledon node of the tomato plant were rooted. The number of potted plantlets for the cotyledon node showed no statistical significance.

Table 2: Morphological parameters of hypocotyl explants on MS medium supplemented with BA and NAA from plant growth regulators

PGR concentration (ppm) Hypocotyl	Total Number of Samples	Count of Calli	Callus Diameter (cm)	Number of shoots in callus/Explant	Shoot Length (cm)	Count of Shoot Branches /Explant
0.25BA+0.1NAA	5	5.00±0.00	1.80±0.20	5.00±0.58	-	1.50±0.29
0.25BA+0.2NAA	5	3.67±0.33*	2.27±0.30	5.00±0.58	-	-
0.25BA+0.4NAA	5	4.33±0.33	1.79±0.33	-	-	-
0.25BA+0.6NAA	5	4.33±0.67	2.15±0.24	3.00±0.58***	-	4.00±0.58***
0.25BA+0.8NAA	5	4.33±0.33	1.38±0.19	-	-	-
0.25BA+1NAA	5	4.00±0.58	2.66±0.10*	2.17±0.44***	-	1.00±0.00
0.50BA+0.1NAA	5	3.33±0.88*	3.83±0.17*	1.00±0.00	1.00±0.00	3.50±0.29
0.50BA+0.2NAA	5	4.33±0.33	2.05±0.18	1.67±0.33	2.00±0.29***	4.00±0.00***
0.50BA+0.4NAA	5	4.00±0.00	1.63±0.19	-	-	-
0.50BA+0.6NAA	5	5.00±0.00*	1.77±0.18	1.33±0.33	-	2.50±0.29**
0.50BA+0.8NAA	5	4.67±0.33*	1.73±0.15	-	-	-
0.50BA+1NAA	5	4.67±0.33*	1.69±0.11	-	-	-

*: Compared to control; *<0.05; **<0.01; ***<0.001 is important at probability levels. Average of the data ± Standard error, some of them show statistical differences (n:5)

Table 3: Morphological parameters of cotyledon node explants in cultures on MS medium supplemented with BA and NAA from plant growth regulators

PGR concentration (ppm) Cotyledon node	Total Number of Samples	Count of Calli	Callus Diameter (cm)	Number of shoots in callus/Explant	Shoot Length (cm)	Number of shoot Branches /Explant	Number of Plants Taken in Pot
0.25BA+0.1NAA	5	5.00±0.00	2.70±0.15	5.33±0.33	12.11±0.65	3.83±0.60	2.00±0.00
0.25BA+0.2NAA	5	4.33±0.33**	2.17±0.17	4.33±0.33**	9.40±0.31***	3.22±0.40	3.67±0.33
0.25BA+0.4NAA	5	4.33±0.67**	1.50±0.29*	4.00±0.58**	12.00±0.58	3.33±0.33	4.00±0.58
0.25BA+0.6NAA	5	4.67±0.33***	1.77±0.15**	4.67±0.33***	9.00±0.58***	4.50±0.29	4.67±0.33
0.25BA+0.8NAA	5	4.67±0.33***	1.77±0.22*	4.67±0.33***	7.00±0.29***	3.00±0.00	4.00±0.58
0.25BA+1NAA	5	4.33±0.33**	1.83±0.17*	3.00±0.58	9.00±0.58***	3.00±0.00	2.00±0.00
0.50BA+0.1NAA	5	5.00±0.00	2.80±0.35	5.00±0.00	14.00±0.58	6.33±0.88	1.67±0.33
0.50BA+0.2NAA	5	4.00±0.58	3.50±0.29	5.00±0.00	6.33±0.33***	2.23±0.14***	2.00±0.00
0.50BA+0.4NAA	5	4.67±0.33	3.27±0.41	5.67±0.33	13.33±0.17	6.00±0.58	2.33±0.33
0.50BA+0.6NAA	5	5.00±0.58*	2.70±0.29*	3.00±0.00	-	3.50±0.29***	-
0.50BA+0.8NAA	5	4.33±0.33*	2.17±0.10	5.20±0.15	-	-	-
0.50BA+1NAA	5	4.00±0.58*	2.00±0.33	4.00±0.58***	2.59±0.21***	2.67±0.33***	5.00±0.58

*: Compared to control; *<0.05; **<0.01; ***<0.001 is important at probability levels. Average of the data ± Standard error, some of them show statistical differences (n:5)

3.3. Callus Regeneration and Shoot Organogenesis from Cotyledon Explants

Although the callus number was excessive, the measurements suggested no concentration significance (Table 5). A significant decrease in callus diameter was only observed at a concentration of 0.25 ppm BA + 0.4 ppm NAA (58%). The concentrations of 0.25 ppm BA + 0.8 ppm NAA (52% decrease) ($p<0.01$), 0.50 ppm BA + 0.2 ppm NAA (70% decrease) ($p<0.001$), 0.50 ppm BA + 0.6 ppm NAA (40% decrease), 0.50 ppm BA + 0.8 ppm NAA (40% increase),

and 0.50 ppm BA + 1 ppm NAA (40% decrease) ($p<0.05$) were found to be significant for the number of shoots in the callus. Despite the callus showing numerous shoots, the plant height showed slight elongation. However, at concentrations of 0.50 ppm BA + 0.2 ppm NAA (25% increase) and 0.50 ppm BA + 0.6 ppm NAA (70% decrease) ($p<0.001$), shoot length was found to be statistically significant. Only at a concentration of 0.25 ppm BA + 0.4 ppm NAA (319%) did the count of shoot branches increase. There was no increase in the quantity of plantlets cultivated for cotyledons in pots. However, the number of plants could only be detected at a concentration of 0.50 ppm BA + 0.1 ppm NAA ($p<0.001$).

Table 4: Morphological parameters of cotyledon explants in cultures on MS medium supplemented with BA and NAA from plant growth regulators

PGR concentration (ppm) Cotyledon	Total Number of Samples	Number of Callus	Callus Diameter (cm)	Number of shoots in callus/Explant	Shoot Length (cm)	Number of Shoot Branches /Explant
0.25BA+0.1NAA	5	4.67±0.33	1.90±0.21	4.17±0.60	3.17±0.17	1.67±0.33
0.25BA+0.2NAA	5	4.67±0.33	2.40±0.31	5.00±0.58	-	2.00±0.00
0.25BA+0.4NAA	5	5.00±0.58	3.00±0.58 *	3.83±0.44	-	7.00±0.58 ***
0.25BA+0.6NAA	5	4.67±0.33	2.71±0.29	4.67±0.33	-	-
0.25BA+0.8NAA	5	4.67±0.33	2.29±0.10	2.00±0.00**	2.67±0.33	1.33±0.33
0.25BA+1NAA	5	4.67±0.33	2.63±0.20	3.83±0.73	-	-
0.50BA+0.1NAA	5	4.33±0.67	2.50±0.29	3.33±0.33	3.33±3.33	2.00±0.58
0.50BA+0.2NAA	5	4.67±0.33	2.35±0.30	1.00±0.00***	4.17±0.17**	1.67±0.33
0.50BA+0.4NAA	5	4.67±0.33	2.23±0.19	3.00±0.00	-	-
0.50BA+0.6NAA	5	4.00±0.58*	2.53±0.46	2.00±0.00*	1.00±0.29***	-
0.50BA+0.8NAA	5	5.00±0.00	2.92±0.52	4.67±0.33*	-	-
0.50BA+1NAA	5	4.33±0.33	2.32±0.41	2.00±0.00*	-	-

*: Compared to control; *<0.05; **<0.01; ***<0.001 is important at probability levels. Average of the data ± Standard error, some of them show statistical differences (n:5)

Callus formation was visible in the hypocotyl parts of germination-proven tomato seeds that were removed after one week. Hair rooting and growth were later observed after the hypocotyl parts were exposed to different concentrations of BA and NAA. When the cotyledon nodes from the explant depicted in Fig. 1 were added to concentrations with BA and NAA, they

began to shoot after 4-5 days. The cotyledon node region is where the callus is formed. After three weeks, callus and then shoots started to develop on the cotyledon parts. Explants whose development continued after three months were moved to the MS medium free of hormones. There were not many potted hypocotyl and cotyledon explants. They were used in all concentrations of the cotyledon node and applied to the pot. The ancestors' tomato seedlings bloomed in pots to produce tomato fruit (Fig. 1).

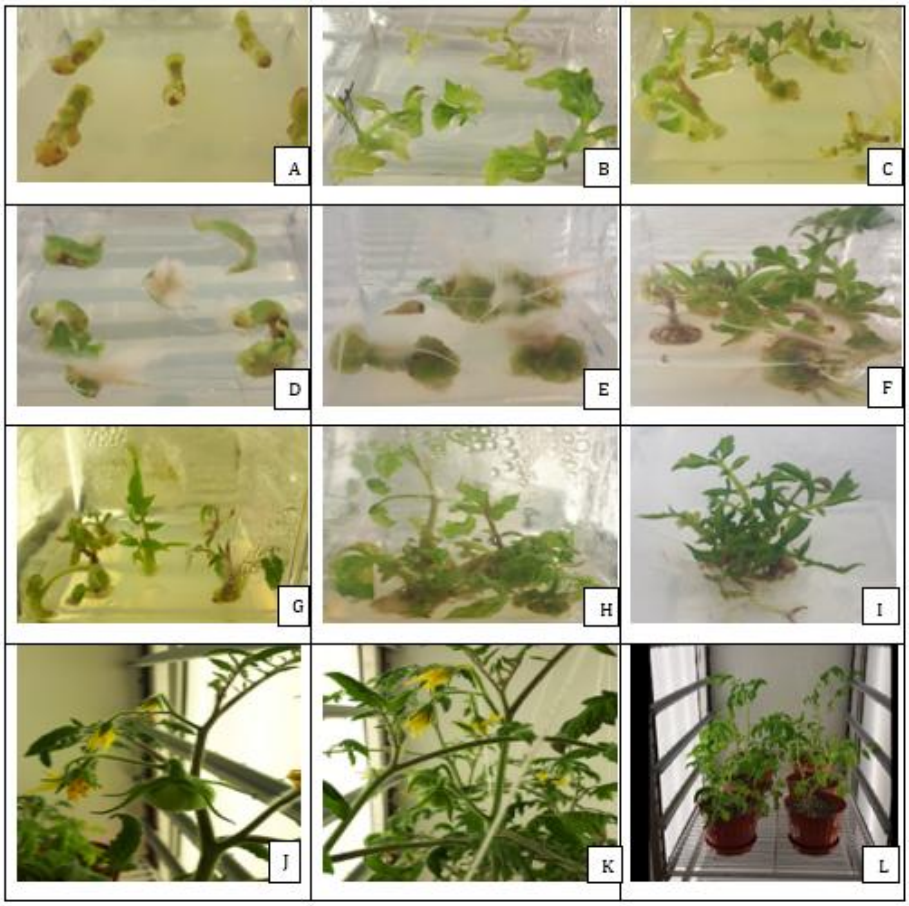

Figure 1: The flowering and fruit formation of the ancestor tomato ((A) Initiation of hypocotyl callus; (B), (C) Beginning of cotyledon node development; (D) Beginning of cotyledon rooting after callus; (E) Rooting the hypocotyl; (F) Callus and rooting of the cotyledon node; (G) (H), (I) Development of cotyledonary node; Start of acclimatization of tomato plant; (J), (K), (L))

Figure 2 shows explants of the hypocotyl, cotyledon node, and cotyledon from tomato seedlings that were treated with various NAA combinations at concentrations of 0.25 ppm BA and 0.50 ppm BA. Using callus taken from the tomato plant hypocotyl, cotyledon node, and cotyledon explants, seedlings were produced. The parameters of this study, conducted in climate-controlled chambers—callus, callus diameter, shoots in callus, shoot length, shoot branches, and plants in pot—were presented graphically.

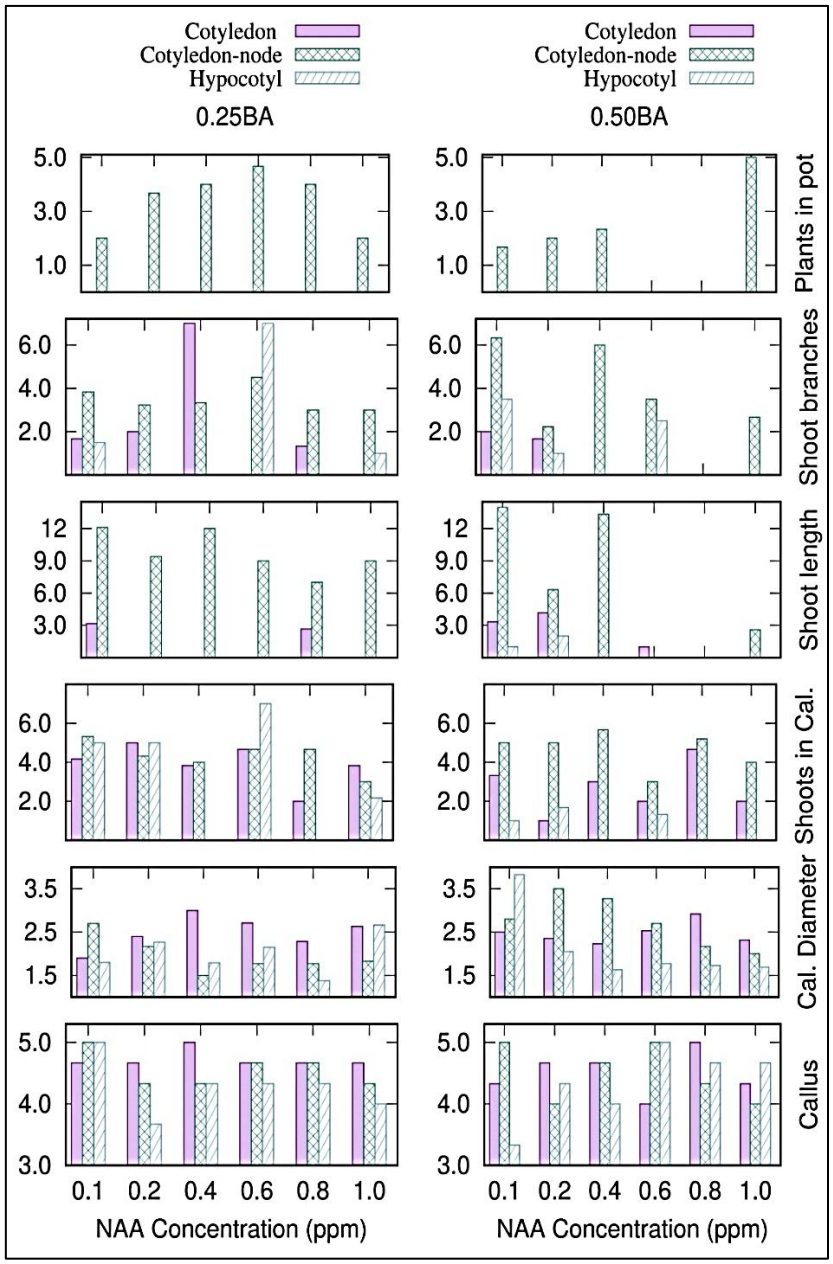

Figure 2: Comparison of different NAA combinations with 0.25 ppm BA and 0.50 ppm BA concentrations of hypocotyl, cotyledon node, and cotyledon explants compared to the control, $p<0.05$ is critical at probability levels. Average of the data ± Standard error, some of them show statistical differences

4. Discussion

In vitro conditions, cultured explants are based on regeneration induction [11]. The concentration of NAA, a derivative of auxin, is higher in the number of calli in the explants taken from the hypocotyl part, demonstrating that it is more effective than BA, a derivative of the cytokinin [12]. As a result, efficiency rose along with concentration. Unusually, the diameter of the callus shrank as the concentration level rose. The number of shoots in the callus was best seen at a concentration of 0.25 BA + 0.6 NAA ppm. The best shoot length was detected at a

concentration of 0.50 BA + 0.2 NAA ppm [12]. The best concentration of 0.25 BA + 0.6 NAA ppm was detected in the number of branches in the shoot. Additionally, it is well known that many plant species can produce more shoots when small amounts of auxin are combined with cytokinin [13]. In a study, the meristematic end of the hypocotyl of the Pontaroza cultivar showed more significant regeneration than the other explants. Shoot induction difference was the highest in the UC-97 variety, with 57.2%. Cultivar-specific genetic variations are the cause of these variations [14]. Shoot tip and cotyledon explants of 8-10-day-old tomato (*Lycopersicon esculentum* cv Omdurman) seedlings were taken and cultured separately with different concentrations of BA, 2-isopentenyl adenine, and kinetin or NAA hormones. It was found that the best rooting of developing shoots was in 1/2 MS medium supplemented with 0.5 mg L^{-1} NAA [15]. Various explants of plants, such as cotyledons, hypocotyls, leaves, stem parts, internodes, apical meristem, shoot tips, and inflorescences, were used for organogenesis [11]. Numerous shoots were visible. The potted plant was found to have a maximum concentration of 0.50 BA + 0.1 NAA ppm [11, 15-17]. In one study, maximum shoot regeneration was obtained in 1.0 BAP + 0.5 NAA ppm, and the highest root induction was obtained in combinations 0.5 NAA + 1.0 IAA ppm [22]. Regeneration occurred in hypocotyls through somatic embryogenesis and shoot organogenesis when seedlings were harvested without growth regulators [18-21]. Shoots are either formed directly or indirectly [14]. Shoot elongation was determined by adding different concentrations of BA to 2-week-old tomato (*Lycopersicon esculentum* Mill.) seedlings. The somatic embryos were only loosely attached to the hypocotyl tissue and had a suspension-like shape with a distinct protoderm covering [21]. Cotyledons, hypocotyl, and leaf segments from four tomato cultivars were used for callus induction using BA and NAA hormones for efficient regeneration [22].

The number of calli in explants taken from the cotyledon node has been shown to increase with a concentration of 0.50 BA + 0.6 NAA ppm. The callus count decreased with increasing concentration of NAA. Even though earlier research found that 8- to 10-day-old cotyledons of tomatoes were better than other sources of samples, including hypocotyls, stems, and leaves for promoting shoot organogenesis of tomatoes [23, 24], in these investigations 10–13-day-old cotyledons were successfully used as a source of explants. Tomato cotyledons that were 10 to 12 days-old were successfully used as explants for tomato regeneration [25]. Furthermore, the cotyledon is the best explant for tomato in-vitro regeneration [26]. As the concentration increased, the callus's diameter decreased, and it was detected at the concentration of 0.50 BA + 0.2 NAA ppm [12]. The concentration of 0.50 BA + 0.4 NAA ppm produced the greatest number of shoots on the callus. This resulted in the formation of multiple shoots. Comparing the concentration to the control group, shoot length decreased [12]. When explants were taken from tomato seedlings cultivated in BA medium, the greatest regeneration was seen. The inhibitory effect occurred in

seedlings grown in a medium without BA [21]. At 0.50 BA + 0.4 NAA ppm concentration, the maximum shoot length was achieved. Compared to other explants, the shoot tip was more successful [15]. As the concentration rose, fewer shoot branches were seen. A concentration of 0.50 BA + 0.1 NAA ppm was found to have the maximum number of shoot branches. In potted plants, 0.50 BA + 1 NAA ppm was the most effective concentration. It has been discovered that as the concentration of potted plants increases, the yield also increases.

The number of branches in the shoot was found to be highly significant at a concentration of 0.25 BA + 0.4 NAA ppm. A concentration of 0.50 BA + 0.1 NAA ppm was found to significantly increase the number of potted tomato plants [11, 15-17]. Organogenesis was seen in the tomato plant's cotyledon section, where a callus formed over the entire surface beginning at the cotyledon's edge [18-20, 27].

5. Conclusions

Many combinations of cytokinins and auxins can supply shoot multiplication in tomatoes from hypocotyl, cotyledon nodes, and cotyledon explants. However, the type and concentration of PGRs can be successfully optimized using tomato organogenesis to achieve high multiplication rates. Therefore, it is necessary to create protocols for cultivars that are crucial for commerce. Plants can only be produced from transformed cells using methods such as regeneration and somatic embryogenesis. Many superior transgenic plants must also be created using these methods. Somatic embryogenesis in tomato plants is still not at a stage where producing high-viability, high-quality embryos is financially viable. So, it necessitates the analysis of tissue culture data as well as the field effectiveness of tomato plants that have been grown.

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