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Effect of Auxin and Cytokinin on *in Vitro* Tomato Production by Tissue Culture Technique and Its Impact on the Production of Somatic Embryos and Somaclonal Variations

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Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2767-178X

Online ISSN: 2767-181X

DOI:
10.37229/fsa.fjas.2024.01.15

Received: 30 November 2023
Accepted: 1 January 2024
Published: 15 January 2024

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Growth regulators (auxin and cytokinin) were used to induce explants to develop for callus and regeneration in whole plant of tomato (*Lycopersicon esculentum*). Explants (hypocotyl, cotyledon, stem, crown and root) of Tomatte and Super set cultivars were utilized as a starting material for callus induction. Explants were cultured on MS medium provided with different concentrations of hormones. The maximum percentage of callus induction (callogenesis %) was obtained from hypocotyls and stems on MS medium provided with 2mg/l IAA ($C_{10}H_9NO_2$) and 0.5mg/l BAP ($C_{12}H_{11}N_5$), it was (100%) for both hypocotyls and stems in Tomatte cultivar, but with Super set recorded (99.6%) for hypocotyles and (98.6%) for stems explants. However, the total percentage of callus initiation from all tested explants was (86.67%) for two cultivars at two seasons. This ratio between auxin and cytokinin not only induced callus but also given (10.5%) somatic embryo and somaclonal variation (3%) at first season in Super set cultivar and at second season somatic embryo recorded (12.67%) and somaclonal (3.33%), while Tomatte cultivar could not induced any values for somatic embryo at two season but somaclonal variation recorded (1.33 %) at first season and (0.7%) at second season. So the cultivar also has important role to induced somatic embryo and somaclonal variation beside hormones ratio and type. However, the highest percentage for shoots formation resulted from medium provided with 1.5 mg/l IAA, 1mg/l BAP and 1mg/l kinetin ($C_{10}H_9N_5O$), which recorded (96.55%) from Tomatte cultivar and (94.83%) from Super set at first season, (95.9%) from Tomatte and (93.5%) from Super set at second season. For rooting stage all media were gave (100%) for rooting % but medium (2) was the best, and Tomatte was better than Super set, however Tomatte cultivar was accomplished at first season (12.33) for number of leaves per plantlet, (13.57cm) for shoot length, (9.33) for number of root per plantlet and (17.33 cm) for root length. Also second season recorded (12.67) for number of leaves per plantlet, (13.23 cm) for shoot length (cm),

(9.67) for number of root per plantlet and (16.87 cm) for root length, but Super set was less

Key words: *Lycopersicon esculentum*, growth regulators, callogenesis, somatic embryo, somaclonal variation.

1. Introduction

The tomato (*Lycopersicon esculentum* Mill), is the generality significant vegetable crops in the Solanaceae family. It is also the crop that most people consume every day throughout the world. Since numerous bacteria, fungi, and viruses affect tomatoes, it is important to treat these diseases at the seed, seedling, and plant stage in order to prevent them from spreading to the human-eating (product). The major crucial aspects of the tissue culture technique is the ability to produce a huge number of healthy plants from a single seed. One explant can produce several copies of plant in a short time under controlled conditions, unmindful of the season and weather throughout the year, as in the case of tomatoes or any other tomato-related plant. Numerous biotechnology strategies have been used over the past 20 years to boost tomato crops. In vitro culture has a variety of uses in tomato production, including the creation of virus-free plants, genetic modification, and numerous basic research projects (**Jamous and Abu-Qaoud 2015 a**). In breeding program, the multiplication of intermediate genotype is not determined by seeds because the special features loss when plants produce from the germination maybe genetic segregation occurs **(Mendez-Hernandez *et al*., 2019)**. Additionally, tissue culture is employed in plant improvement, the generation of secondary metabolites, and the expansion of strains resistant to salinity **(Al-Khateeb *et al*., 2020 and Youssef *et al* 2020)**, drought (**Tu *et al*., 2020)**, and heat stressors. To increase the number of callus selections and formation the ideal cultivars in the shortest amount of time, in vitro procedures are crucial **(Sherbeni *et al*. 2019),** also production of homozygous, doubled haploid (DH) pure lines via Androgenesis process **(Corral-Martı ´nez *et al*., 2011**). Due to their flexibility and totipotency, plant cells in culture can be directed in their development using certain media manipulations. The key media elements in determining the plant cells' developmental course are plant growth regulators. So MS medium **(Murashige and Skoog 1962)** were utilized and provide with two main classes of plant growth regulators Auxins and cytokinins in various ratios to produce callus and whole plant by tissue culture **(Haridy, 2011 and 2016).** When callus was planted on MS+V media with a high ratio of auxins (IAA) Indole-3-acetic acid) to cytokinins (BAP) Benzylaminopurine) the maximum fresh weight of callus was obtained. The development of cultured organs is significantly influenced by the ratio of auxins and cytokinins. Auxins (IAA) encourage cell division, cell development, and callus formation. Cytokinins (BAP) encourage cell division, stimulate the activity of proteins and enzymes, and boost RNA production **(Koning, 1994)**. Plant tissues are cultured *in vitro* on synthetic media in a sterile environment. The hypothesis of plant cells' totipotency, which forms the basis of the tissue culture procedure, characterizes the ability of individual cell to express the full genome through cell division. Plant cells possess totipotent potential, but just as important and necessary for the regeneration of the whole plant is the capacity of individual cells to alter their growth, metabolism, and development. Cells in some organisms can dedifferentiate and revert to their totipotent state (**Singh and Kumar, 2020).** Also the practise of cultivating embryos from seeds and ovules in a nutritional medium is known as embryo cultivation in plants. In embryo cultivation, the plant either develops directly from the embryo or indirectly via the callus and subsequent shoot and root production. The method was created to thaw dormant seeds, assess the viability of seeds, and grow haploid plants and rare species. So found a useful technique used to abbreviate the reproduction process of plants by developing excised embryos (**Zou *et al*., 2019**), which also reduces the length of the seeds' long dormant period. With the particular goal of mass reproduction, intra-varietal hybrids of the economically significant energy plant "Jatropha" have been successfully created. Regeneration of plant and Somatic embryogenesis has been done in Jucara Palm embryo cultures for fast cloning and enhancement of chosen individuals. There are certain constraints to the *in vitro* approach, the occurrence of spontaneous genetic or epigenetic modifications that result in cytological defects, phenotypic mutations, sequence changes, and DNA methylation in *in vitro* regenerated plants, however, these differences could have an impact on the quantity and quality of plants (**Cruz-Mendı´vil *et al*., 2011**) through various approaches as well as genetic transformation. But there are factors influencing the occurrence of somatic

embryogenesis, as well as the control of somatic embryogenesis. Link the species' genetic pattern to the absence or low response. A species' high or poor ability for somatic embryogenesis is correlated to the presence or absence of particular cells in the explant, which is inherent in their totipotency. The capability to maintain somatic embryogenesis capacity necessitates the utilization of settings that promote the proliferation of specific and particular cells. Other parameters that influence somatic embryogenesis regeneration capacity contain development stages of explants donor plant, physiological factors of explant donor plant, the position of explants relative to the plant, in vitro culture conditions, and most importantly plant growth regulators, also **Kim *et al*., (2007)** stated that through germination of somatic embryo was frequently observed the secondary embryogenesis in the lower part of the hypocotyl or radicle ends of germinating somatic embryos. Another important phenomenon is somaclonal variation, which defined as variance in tissue culture-derived plants **(Larkin and Scowcroft, 1981)**. Later, it was shown that a wide range of changes in nuclear and cytoplasmic genetic components led to the observed phenotypic variance, with many of them being epigenetic in origin. In plant species which produces by Tissue culture a wide extend of genetic alteration can be used in plant breeding operations. Useful Mutants with agronomic features, such as disease resistance, drought or salt tolerance, can be separated in a short time during in vitro selection. The successful using of somaclonal variation is greatly depending on its genetic establishment in latter generations, which can be supported by molecular markers as AFLPs, RAPDs, SSRs, and other.

Despite the development of a few cultivars in plants like *Brassica juncea*, rice, and many more, the promise of somaclonal diversity has still to be completely realized by breeders. **(Jain, 2001)**. And it may arise from the somaclonal variation undesirable characteristics, and in this case the researcher completely excludes them and remains only on the desirable characteristics and works to increase them. Also **Alatar *et al*., (2017)** study the effect of hormones and cultivar on induced somatic embryo and Somaclonal variations. The goal of this investigation was to enhance an effective and repeatable regeneration plants for this important crop by optimizing multiple parameters such as balance or ratio between auxin and cytokinin, genotypes, explant types, media for *in vitro* multiplication and plant regeneration. However in this investigation using different concentrations of auxin and cytokinin to obtain callus, shoot and root without any contamination from pathogens by tissue culture technique, and without any variation in phenotype (it is aim for most of researcher and commercialism companies), and also produced somatic embryo and somaclonal variations, they are important for researcher, this variant (somatic embryo or somaclonal variation) may be occur from the effect of hormones on the behavior of cells during divisions to form callus and during differentiation to form shoots and roots.

2. Material and Methods

This experiment was performed at the Plant Tissue Culture Lab., Central Lab of Organic Agriculture, Agricultural Research Center. Egypt, during two successive seasons of 2022 and 2023.

2.1. Growth regulators, plant materials and seeds sterilization

2.1. a Growth regulator

Two type of growth regulators were used

1. Auxin as (IAA) Indole-3-acetic acid ($C_{10}H_9NO_2$), (IBA) Indole-3- butyric acid ($C_{12}H_{13}NO_2$) and (NAA) Naphthalene-1-acetic acid ($C_{12}H_{10}O_2$) (synthetic hormone).
2. Cytokinin as (BAP) 6-Benzylaminopurine, ($C_{12}H_{11}N_5$) and Kinetin ($C_{10}H_9N_5O$).

2.1. Plant materials and seeds sterilization

The one of the major disincentives for success of plan tissue culture is contamination of *in vitro* cultures from various sources. Sterilization of seeds is an essential pre-requisite to obtaining an aseptic seedling for any *in vitro* regeneration experiment.

In this study, used sterilizing agent viz. sodium hypochlorite (NaOCl) on efficiency of seeds germination in *Lycopersicon esculentum*. Seeds of two tomato cultivars (Tomatte and Super set) were used in this study (100 seeds for each cultivar); seeds of the two cultivars were washed by running tap

water for 1 hour. The surface sterilized by 70% aqueous ethanol for 60 seconds, followed by 23% sodium hypochlorite (NaOCl) (Chlorox) for 10 minutes containing 1% (v/v) tween 20 as a wetting agent, and rinsed by sterile distilled water three times (**Haridy, 2011 and 2016)**.

2. 2. Preparing the germination Media

4.4 g /l MS salts and vitamins (MS+V) **(Murashige and Skoog, 1962)**, which used as a basal medium, and supplemented with 30 g/l of sucrose and 8 g/l of Agar and adjusted The PH at 5.8, and finally all the media in Table 1, 2 and 3 autoclaved at 121C^{o} for 20 minutes.

2. 3. germination stage

Tomato seeds were cultured in jars containing 25 ml of MS+V, 10 seeds were planted in each jar, three replicate were used each one contained 10 jars then transferred to incubator and kept for 7-15 days at 22-24C^{o} under 16 h of daylight and 8 h dark period.

2.4. Callus induction

Callus initiation need to test some sources of explants for callus induction or formation, in this experiment, a plantlet which *in vitro* established was cut into five different explants (hypocotyl, cotyledon, stem, crown and root) according to (**Ajenifujah-Solebo *et al*., 2013) and (Haridy, 2011 and 2016)** from both cultivars. Each explant cultured as it is except cotyledons and stem were cut into two pieces, 10 segments for each jar, with three replicates, each replicate contained 9 jars, all jar contain 25 ml MS+V medium (three different media) basal media supplied with different concentrations of growth regulators (Table 1) auxin ((IAA) Indole-3-acetic acid ($C_{10}H_9NO_2$)) and cytokinin ((BAP) 6-Benzylaminopurine, ($C_{12}H_{11}N_5$)) (**Cai *et al*., 2015**), to obtain the highest percentage of callus formation. The cultures were incubated in a growth chamber at 24±1°C with 16 h of photoperiod.

Table (1). Effect of different media on the percentage of callus formation and production

Media components	Media 1	Media 2	Media 3
MS+V	4.4	4.4	4.4
Sucrose	30.0	30.0	30.0
IAA	1.0	1.5	2.0
BAP	0.25	1.0	0.5
Agar	8.0	8.0	8.0

2.5. Shoots organogenesis and Multiplication

Callus which formed in the previous step were cut to a small pieces with interval about 1.0x 1.0 cm **(Haridy, 2011 and 2016)** and transferred into three treatments of MS+V basal media supplemented with different concentrations from auxin (deferent concentration of IAA 0.5- 1.5 ml/L) and cytokinin (deferent concentration of BAP 1-1.5ml/L) (**Amer *et al*., 2020**) and (Kinetin ($C_{10}H_9N_5O$) 1ml/L with medium (1) only) to induced shooting and also a high number of shoots as showed in (Tables 2), compared to the MS basal medium as a control treatment (without growth regulators addition), 6 small callus cultured in each jar, with three replicates, each replicate contained 9 jars. The cultures were incubated for 2-3 weeks. The number of shoots per explant, shoot length (cm) and number of leaves per shoot were determined. The cultures were incubated in a growth chamber at 24±1°C with 16 h of photoperiod in air conditioned growth room, illuminated by 40W (watts) white fluorescent lights. The intensity of light was regulated between 1000-2000 lux **(Altaf Hussain *et al* 2011).**

Table (2). Effect of different media with different concentrations of growth regulators on the Shoots organogenesis and Multiplication

Media components	Medium 1	Medium 2	Medium 3	Control medium
MS+V	4.4	4.4	4.4	4.4
Sucrose	30.0	30.0	30.0	30.0
IAA	1.5	1.0	0.5	0.0
BAP	1.0	1.0	1.5	0.0
Kinetin	1.0	0.0	0.0	0.0
Agar	8.0	8.0	8.0	8.0

2.6. Rooting Stage

Multiplicated shoots from two tomato cultivars were harvested and grown on MS media enriched with various compositions, of rooting growth regulators, (IAA) at 0.5-1.5 mg/l, (NAA) Naphthalene-1-acetic acid ($C_{12}H_{10}O_2$) at 1.0 mg/l, and (IBA) Indole-3- butyric acid ($C_{12}H_{13}NO_2$) at 1.0- 1.5 mg/l, as shown in table (3). Each jar had ten shoots cultured in three repetitions, with each replicate including nine jars. After three to four weeks of culture, the rooting percentage, number of roots per plantlet, root length (cm), shoot length (cm), and number of leaves per plantlet were determined (**Sherbeni *et al.*, 2019**). The cultures were incubated in a growth chamber at 24±1°C with 16 h of photoperiod.

Table (3). Effect of different media with different concentrations of growth regulators to obtain the best number of roots

Media components	Medium 1	Medium 2	Medium 3	Control medium
MS+V	4.4	4.4	4.4	4.4
Sucrose	30.0	30.0	30.0	30.0
IAA	0.5	1.0	1.5	0.0
NAA	1.0	0.0	0.0	0.0
IBA	1.0	1.5	0.0	0.0
Agar	8.0	8.0	8.0	8.0

2.7. Somatic embryo and somaclonal variations

The ratio of [somatic embryos] is calculated from this equation,

$$\text{Somatic embryo } \% = \frac{\text{number of callus containing somatic embryos}}{\text{total number of callus}} \text{X } 100$$

Also the ratio of somaclonal variations is calculated from this equation

$$\text{somaclonal variations } \% = \frac{\text{the number of variations}}{\text{total number (for only the stage observation)}} \text{ X } 100$$

2.8. Statistical analysis

All treatments with different combinations of the growth regulator (callus induction from different type of explants, shoot multiplication experiments from callus and rooting stage) each experiment was conducted in two seasons. Collected variable, were summarized and analyzed in one-way analysis of

variance, using Costat soft, comparative analysis was conducted for the significant results using LSD at 0.05 probability.

3. Results

3.1. Callus induction

Seeds were grown on MS medium, after germination plantlet were cut into five e different explants (hypocotyl, cotyledon, stem, crown and root) from both cultivars. The result showed that, there were significant differences among all different media, but the best media induced the highest percentage of callus formation was medium (3) for all the explants which contained 2g /l IAA, and 0.5g /l BAP, as showed in table (1), followed by medium (2) and then the medium (1). Hypocotyl and stem recorded the high percentage (100%) for callus induction with medium (3) in Tomatte cultivar. Also in Super set cultivar hypocotyls were recorded (99.6%) and stem (98.6%). This result was in agreement with **Chaudhry *et al*. (2004) and Ajenifujah-Solebo *et al*. (2013)**. Also **(Klimek-Chodacka *et al*., 2020)** reported that the callus development was achieved on 83–100% of hypocotyl and cotyledon explants.

Table (4). Effect of different media with different concentrations of growth regulators on percentage of callus induction from explants (hypocotyl, cotyledon, stem, crown and root) of Tomatte and Super set cultivars

Media	Tomatte					Super set				
	Cotyle-don	Hypoc-otyl	Stem	Crown	Root	Cotyle-don	Hypoc-otyl	Stem	Crown	Root
Media1	30.3^{c}	52.3^{c}	53.6^{c}	50.0^{c}	43.0^{c}	29.3^{c}	50.3^{c}	51.6^{c}	50.0^{c}	41.6^{c}
Media2	47.6^{b}	77.0^{b}	75.3^{b}	73.6^{b}	66.0^{b}	45.3^{b}	76.3^{b}	75.6^{b}	74.0^{b}	63.6^{b}
Media 3	55.3^{a}	100.0^{a}	100.0^{a}	92.3^{a}	85.6^{a}	51.3^{a}	99.6^{a}	98.6^{a}	96.3^{a}	81.3^{a}

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$

Table (5). Effect of different media with different concentrations of growth regulators in callus formation (%) (callogenesis %) for Tomatte and Super set cultivars at two seasons

Media	First Season				Second season			
	Tomatte		Super set		Tomatte		Super set	
	No of callus	Callog enesis %	No of callus	Callogene sis %	No of callus	Callogene sis %	No of callus	Callog enesis %
Media 1	229.33	45.87^{c}	223.00	44.60^{c}	236.33	47.33^{c}	225.00	45.00^{c}
Media 2	334.66	67.93^{b}	335.00	67.00^{b}	338.33	67.67^{b}	333.33	66.67^{b}
Media 3	433.33	86.67^{a}	427.33	85.47^{a}	433.33	86.67^{a}	422.00	84.40^{a}

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

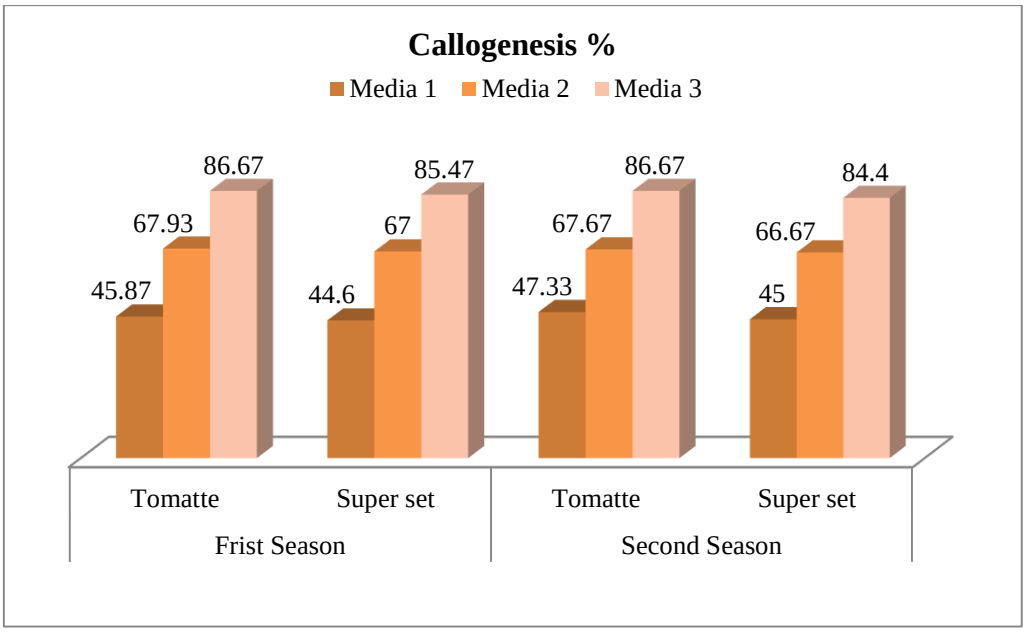

Fig (1). Diagram showing the Effect of different media with different concentrations of growth regulator in Callogenesis (%) for Tomatte and Super set cultivars at two seasons

As shown in Table (5), (Fig, 1 and Fig 4- A and B) the highest total percentage of callus formation or callogenesis (%) (that was produced from different plant parts for each medium separately) was produced from medium (3), and recorded in the Tomatte cultivar (86.67%) at the first and second seasons. Super set cultivar was recorded at the first season (85.47%) and at second season (84.40%). Then the medium (2)

3.2. Shoot organogenesis and Multiplication

The effect of using growth regulators in different media on the callus differentiation to form shoots had a positive effect (Table 6), the highest rate of differentiation reached to (96.55%) (Fig., 2 and Fig., 4- C and E) as appeared in medium (1). Where number of shoot per callus was (5.8) for Tomatte cultivar at the first season and at the second season was (95.9%) for shoot formation (%) and (5.4) for number of shoot per callus. And in super set recorded at the first season for shoot formation (%) (94.83%) and number of shoot per callus (5.33) and at the second season was (93.5%) for shoot formation (%) and (5.1) for number of shoot per calli. Following for medium (1), the medium (2), then medium (3), while the control without any detection of cells differentiation or any growth and it deteriorated and eventually died. Growth regulator exhibited a significant effect on the shoot number with significant between hormone which include in different media.

Table (6). Effect of different media with different concentrations of growth regulator in shoots organogenesis and Multiplication

Media	First Season				Second season			
	Tomatte		Super set		Tomatte		Super set	
	Shoots formation %	No of shoots / callus	Shoots formation %	No of shoots / callus	Shoots formation %	No of shoots/ callus	Shoots formation %	No of shoots/ Calli
Media 1	96.55^{a}	5.8^{a}	94.83^{a}	5.33^{a}	95.9^{a}	5.4^{a}	93.5^{a}	5.1^{a}
Media 2	67.0^{b}	2.9^{b}	61.5^{b}	2.6^{b}	63.7^{b}	2.4^{b}	60.5^{b}	2.5^{b}
Media 3	25.4^{c}	1.5^{d}	19.7^{c}	1.2^{c}	23.5^{c}	1.7^{c}	20.0^{c}	1.2^{c}
Control	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

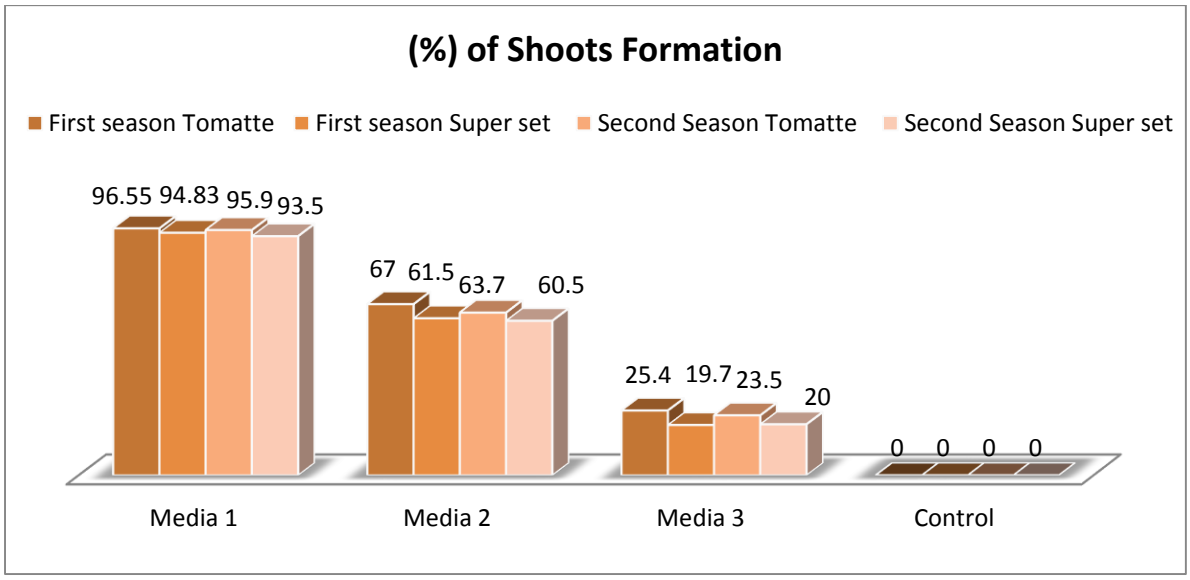

Fig. (2). Diagram for the Effect of different media with different concentrations of growth regulator in (%) of Shoots Formation for Tomatte and Super set cultivars in two seasons

Table (7). Effect of different media with different concentrations of growth regulator in number of leaves per shoot and shoot length (cm) for Tomatte and Super set cultivars at two seasons

Media	First Season				Second season			
	Tomatte		Super set		tomatte		Super set	
	No. of leaves/shoot	Shoot length (cm)	No. of leaves/shoot	Shoot length (cm)	No. of leaves/shoot	Shoot length (cm)	No. of leaves/shoot	Shoot length (cm)
Media 1	4.67^{a}	4.77^{a}	4.67^{a}	4.23^{a}	4.33^{a}	3.43^{a}	3.67^{a}	3.40^{a}
Media 2	3.33^{b}	3.17^{b}	3.0^{b}	3.33^{a}	3.13^{ab}	3.23^{a}	2.33^{b}	3.13^{a}
Media 3	2.33^{c}	2.9^{b}	2.67^{b}	3.17^{a}	2.33^{b}	2.33^{a}	2.67^{b}	2.37^{a}
Control	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

The highest number of leaves per shoot (4.67) for two cultivars which culture in medium (1) at first season, and at second season recorded (4.33) for Tomatte cultivar and (3.67) for super set cultivar as shown in Table (7). Also the shoots length was (4.77 cm) for Tomatte cultivar and (4.23 cm) for Super set at first season and (3.43 cm) for Tomatte, and (3.40 cm) for Super set cultivar at second season.

3.3. Rooting

Also the rustle in table (8) appeared the medium (2) was the best medium for all measurements (number of leaves/plantlet, shoot length, number of root/plantlet, rooting %) at the two seasons for the two cultivars, except root length (cm) there were no significant different among the three media and control medium for the cultivar Super set at the first season. And no significant different between media (1) and (3) for all measurements for the two cultivar at the second season, except number of roots per plantlet for Super set which recorded no significant with control, and there was a significant between these two media and control, but no significant for media (3) and control medium for all measurements of the two cultivar at the second season, except number of leaves/plantlet and number of root/ plantlet whereby both recorded significant with control for Tomatte cultivar. While root length reached to 17.33

cm at the first season in Tomatte cultivar and 16.87 cm at the second season, and with Super set the root length reached to 12.33 cm at the first season and 12.63 cm at the second season as shown in Fig (3). By the end of this stage, we obtained a complete plant (Fig., 4- F) without any contamination.

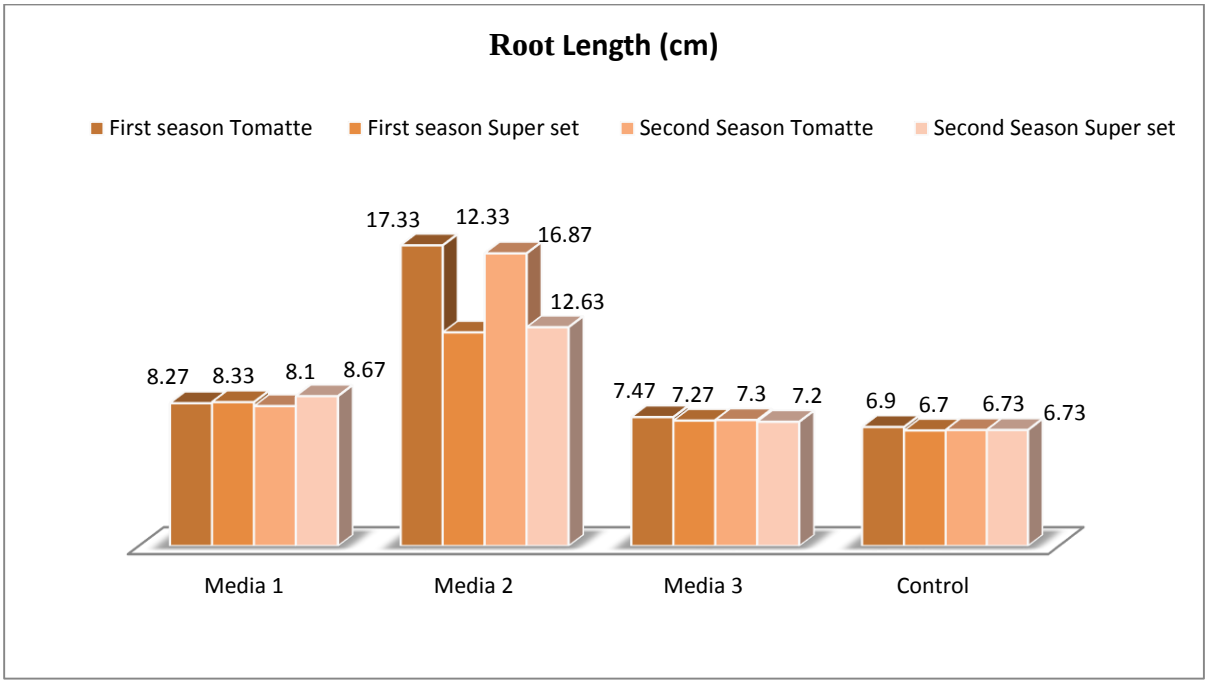

Fig. (3). Diagram for the Effect of different media with different concentrations of growth regulator in Root length (cm) for Tomatte and Super set Cultivars at two seasons

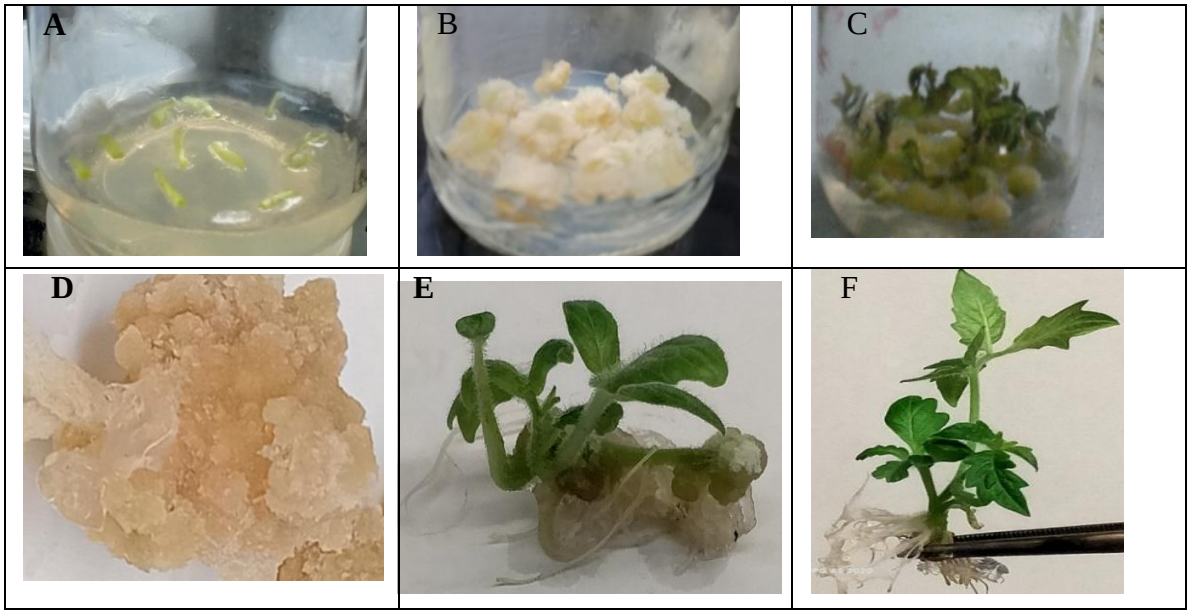

Fig. (4): Establishment of plant regeneration system from two tomato cultivars (Tomatte and Super set); (A) stem explants, (B) callus formation, (C) shoots initiation, (D) huge calli, (E) shoots multiplication and (F) whole plant.

Table (8). Effect different media with different concentrations of growth regulator on growth characteristic and rooting (%) of plantlet during rooting stage for Tomatte and Super set cultivars at two seasons

Media	First Season										Second season									
	Tomatte					Super set					Tomatte					Super set				
	No. of leaves/ plantlet	Shoot length (cm)	No. of roots/ plantlet	Root length (cm	Rooting (%)	No. of leaves/ plantlet	Shoot length (cm)	No. of roots/ plantlet	Root length (cm	Rooting (%)	No. of leaves/ plantlet	Shoot length (cm)	No. of roots/ plantlet	Root length (cm	Rooting (%)	No. of leaves/ plantlet	Shoot length (cm)	No. of roots/ plantlet	Root length (cm	Rooting (%)
Media 1	5.3^{b}	7.53^{b}	7.33^{b}	8.27^{b}	100	5.0^{b}	7.4^{b}	7.67^{b}	8.33^{a}	100	6.0^{b}	7.4^{b}	7.0^{b}	8.1^{b}	100	5.67^{b}	7.27^{b}	7.33^{b}	8.67^{b}	100
Media 2	12.33^{a}	13.57^{a}	9.33^{a}	17.33^{a}	100	7.33^{a}	9.73^{a}	9.0^{a}	12.3^{a}	100	12.8^{a}	13.2^{a}	9.67^{a}	16..9^{a}	100	7.67^{a}	9.6^{a}	9.67^{a}	12.63^{a}	100
Media 3	5.33^{b}	6.53^{b}	6.0^{c}	7.47^{b}	100	4.67^{b}	6.53^{bc}	6.33^{c}	7.27^{a}	100	5.7^{b}	7.1^{b}	6.33^{b}	7.3^{b}	100	5.33^{b}	6.27^{bc}	5.67^{c}	7.2^{bc}	100
Control	4.67^{b}	6.03^{b}	4.67^{d}	6.9^{b}	100	4.33^{b}	5.67^{c}	5.33^{d}	6.7^{a}	100	4.8^{c}	6.8^{b}	4.67^{c}	6.7^{b}	100	4.67^{b}	5.67^{c}	5.33^{c}	6.73^{c}	100

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

3.4. Somatic embryo and somaclonal variations

In the stage of callus formation which derived from medium (3), some bodies appear above the mass of callus, which have different shapes; globular (Fig., 5- A and B arrow head), torpedo stage (Fig., 5- C, early torpedo and D, Late torpedo stage), heart shape (Fig., 5- E) these bodies are called somatic embryos. The somatic embryo % in Super set was (10.5%) (Table 9) at the first season and (12.67%) at the second season, while Tomatte cultivar did not recorded any value for somatic embryo, these embryos develop and form whole plants pass by young shoots as shown in (Fig., 5- B arrow, F, G, H, I, and J). While the somaclonal variations % recorded in Super set cultivar (3 %) at the first season and (3.33 %) at the second season, and Tomatte cultivar recorded less value at the two seasons (Fig 5-K and L).

Table (9). Percentage of somatic embryo and somaclonal variations derived from medium provided with 2g /l IAA, and 0.5g /l BAP

Phenomena (Abnormal shape)	First season		Second season	
	Tomatte	Super set	Tomatte	Super set
Somatic embryo (%)	0.0	10.5	0.0	12.67
Somaclonal variations (%)	1.33	3.0	0.7	3.33

4. Discussion

Callus tissues were produced under sterilized conditions from the two tomato cultivars; Tissue culture technique was used to test the effect of different concentrations of growth regulator on callus growth. Plant tissue cultures are powerful tools for metabolism studies. Recent advances with plant tissue cultures involve studies of the mode of action or selectivity of growth **(Mumma and Hamilton**, **1982).** Despite the various regeneration outcomes, it is still imperative to develop a trustworthy in vitro system that can accommodate the majority of tomato types. According to studies by **Ichimura and Oda (1995) and Jamous and Abu-Qaoud (2015 b)**, direct regeneration can differ depending on the hormone concentrations and combinations utilised, the culture medium used, the temperature, the amount of light, the genotype, and the explant employed. Therefore, the goal of this study is to develop an in vitro regenerative system for tomato employing seedling explants of the cultivars Tomatte and Super set. In this work, we examined how cultivar, hormone, and explant type affected adventitious shoot and root regeneration. **Chaudhry and Rashid (2007)** found that cultivar and explants type have a substantial impact on the regeneration ability. Also maybe Plant morphogenic effects a result of different ratios of plant hormones formed by roots as by rhizosphere bacteria (**Muller *et al*., 1989) and (Challaraj and Maruthamuthu, 2015**) for plants grown in soil and also in tissue culture the ratios of plant hormones play important role to produced whole plant by tissue culture form explants which divides by metosis to form callus and in second stab differentiation to shoot and finally root.

4.1. Callus formation

Plant regeneration from callus was achieved for Tomatte and Super set cultivars. The effects of IAA and BA on callus induction were examined, and BA had a significant favorable effect, which agrees with **(Liang *et al*., 2020)** they studied the effects of additional hormones such as KIN, 2,4-D, and BA on callus and shoot induction, and they were discovered that KIN (cytokinin) was not required for callus induction, 2,4-D caused poor quality callus, while BA had a significant favorable influence on callus induction. However, **(Chaudhry *et al*., 2010)** found the used of MS medium rich with BAP (5 mg/l), NAA (2 mg/l), Kin (4 mg/l) and IAA (2 mg/l) induced maximum callogenesis, while in this study using IAA and BA only were successful to obtain the maximum callogenesis.

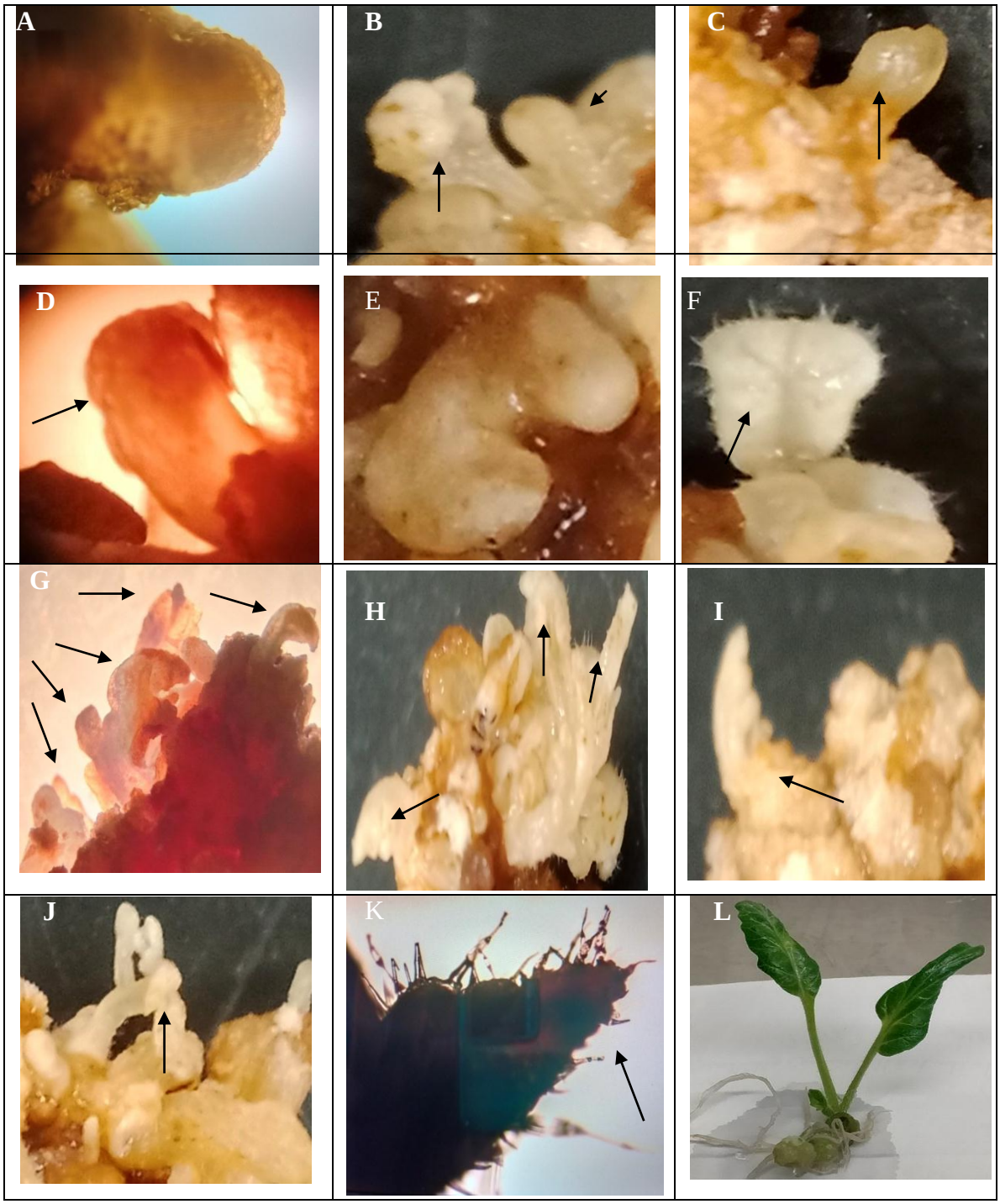

Fig (5). Somatic embryo and somaclonal variations from treatment with 2 mg/l IAA and 0.5 mg/l BAP in Super set cultivar; (A and B arrow head) globular stage, B (arrow) young shoot, (C) early torpedo, (D) Late torpedo stage, (E) heart stage, (F, G, H, I, and J) all arrows refer to a young shoot and (K and L) somaclonal variation (abnormal shape).

4.2. Shoot organogenesis and shoot Multiplication

At two seasons, the optimum medium for shoot organogenesis for Tomatte and Super set cultivars was medium (1), which contained diverse hormones such as IAA, BAP, and Kinetin. Table (6) shows that there are substantial variations between the control media and the shoot % per callus and number of shoots generated of both tomato varieties. Following that, shoot length (cm) and number of leaves per shoot Table (7) (Fig 4) .Hormone compositions at these levels caused a greater number of shoots

per callus and a higher percentage of shoot development, therefore, this medium was used to form the induced shoot organogenesis and shoot multiplication at the same time.

IAA, a phytohormone, is a colourless heterocyclic solid molecule that is thought to be the most significant plant auxin. IAA, like all auxins, has a variety of functions, including stimulating cell elongation and cell division, with all of the consequences for plant growth and development **(Chaudhary *et al*., 2004)** and **(Chaudhry and Rashid, 2007)**. in this study investigated the effects of IAA, BAP, and Kinetin on shoot development, and found that the using IAA at a level (1.5 ml/L) in conjunction with 1.0 ml/L of BAP and 1.0 ml/L of Kinetin resulted in a greater shoot percentage. auxins and cytokinins aid in cell division and elongation, whereas cytokinins aid in differentiation. As a result, cell division and differentiation or organogenesis requires the proper concentration of these growth hormones. As a result of the presence of cytokinin, the amount of cytokinin used is also crucial in the induction of many shoots **(Abdellatef and Khalafallah 2007 and Al-Kaaby 2016)**. As a result, the type of explant as well as the type and concentration of growth regulators influence the frequency with which adventitious shoot regeneration occurs **(Gubis *et al*., 2003).** Increase the shoot number in the shoot Multiplication stage, where the shoots number are multiplied by repeated subcultures until the necessary number of plants is obtained, however more subcultures increase the occurrence of mutations. As a result, the type and concentration of hormones applied influenced the frequency of shoot number. Micro-propagation is currently a trusted and popular practise for large-scale, rapid plant multiplication **(Akbas *et al*., 2009)**. Also, **(Baye *et al*., 2021)** investigated the influence of BAP on tomato shoot start. **Also** the number of shoots produced/explant was dependent on BAP concentration, and that the number of shoots produced rose as BAP concentration increased.

4.3. Rooting

During the rooting stage, all shoots grown on different media developed roots, but medium (2), which included 1mg/l IAA and 1.5 mg/l IBA, formed the best rooting. However, as compared to the control (MS media without hormones), these results are consistent with **(Liang *et al*., 2020)**, as they stated that the half-strength MS medium provided with 2.5mg/L IBA or NAA induced the maximum rooting percentage (98-99 %), but in this study the two hormones were used together. Also there was increase in the number of leaves per plantlet, shoot length, number of roots per plantlet. The action of auxins on rooting initials could explain these findings **(Sherbeni *et al*., 2019).** However, another media (medium (1) and medium (3)) had a less significant effect on shoot length, number of shoots per callus Table (7), number of leaves per plantlet, shoot length (cm), number of root, and root length (cm) for two cultivars across two seasons, and establishment of robust root growth, also the roots were robust and encircled by a dense layer of root hairs this observation corroborates with **(Singh and Kumar, 2020)**. Furthermore, in the first season, the root number each shoot ranged from 4.67 to 9.33 for Tomatte and 5.33 to 9 for Super set, while at the second season, it ranged from 4.67 to 9.67 for Tomatte and 5.33 to 9.67 for Super set. (Table 8).

4.4. Somatic embryos and Somaclonal Variations

However, the ratio of hormones that are used in the composition of the medium (3) which used for the formation of callus had another effect, "the production of somatic embryos" by 10.5 % at the first season and 12.67 % at the second season of the Super set cultivar, while the Tomatte cultivar was not record any value for the formation of somatic embryos. Also, this medium (3) had the ability to cause somaclonal variation for the Super set cultivar by 3% at the first season and 3.33 % at the second season, but the cultivar Tomata was less likely to induce somaclonal variation, as it was recorded at the first season 1.33 % and the second season 0.7 %, and therefore the cultivar has an effect on the formation of somatic embryos **(Woo *et al* 2021)** and somaclonal variation also the ratio of growth regulators had affect (**Konieczny *et al*., 2009 and Eeuwens *et al*., 2002).**

Somatic embryogenesis is the process by which somatic cells or tissues, including haploid cells, grow into differentiated embryos and allow plants to regenerate. Somatic embryogenesis considered a biotechnological procedure used to multiply significant economic cultivars. If no fertilization occurs, this mechanism is a sort of plant cell totipotency in which embryos form from somatic or vegetative cells. Several factors influence the success or failing of the somatic embryogenesis formation, involving

the source of the explant, culture media, and in vitro environmental circumstances. Somatic cells pass through embryonic stages by producing constructs similar to zygotic embryos but without gametes combining. Somatic embryogenesis may be ideally ideal for the mass propagation and commercial production of endangered species of crops **(Bidabadi and Jain, 2020)**. On MS media supplied with 0.2 mg/L IAA and 0.5 mg/L BA, and in suitable environmental conditions (the cultures were incubated in a growth chamber at 24±1°C with 16 h of photoperiod Super set cultivar explants generated somatic embryos. Somatic embryos of globular, torpedo, and heart were obtained. While the Tomatte cultivar was unable to accomplish this effect. The effect of medium (3) (which was used to initiate callus) on the subsequent development of callus into somatic embryos was also examined (Fig 5). Although somatic embryogenesis did not emerge in the medium (1 and 2) treatments, somatic embryogenesis was induced in tomato when growth factors were used, according to **(Gill *et al.*, 1995)**. The induced sometic embryos are affected by the type and quantity of hormones employed, as well as the cultivar used, hence **(Azadi *et al*., 2018)** reported that small protocol optimizations for each cultivar are required. Future studies are also required to increase the frequency of somatic embryo start and maturation.

On the greenish callus, somatic embryogenesis was detected rather than shoot regeneration. Embryo development phases include globular (Figs. 5- A and B arrow head), early torpedo (Figs. 5- C and D), late torpedo (Figs. 5- E and F), and heart. (Figs. 5- E and F) Furthermore, two types of somatic embryogenesis were detected. The first type happened solely on the callus's surface, and this is the type referred to last. According to **(Chen and Adachi, 1998)**, the second form of somatic embryogenesis is characterized by the generation of numerous or single somatic embryos in various stages towards the medium, but only the first type was found in our work. However, somatic embryogenesis use in tomato plants as synthetic seeds for micro propagation hybrids F1 (**Bhatia *et al*., 2004).**

While Somaclonal variations, it was discovered that a wide range of shifts in nuclear and cytoplasmic genetic elements led to observed phenotypic variance, and that many of them were epigenetic (non-heritable). Somaclonal variation are connected with point mutation, chromosomal rearrangements and recombination, DNA methylation, changed the sequence copy number, and transposable elements, and appear to be regulated by genotype, explant type, culture medium, and donor plant age. The number of subcultures is another crucial factor that can lead to additional diversity depending on the plant type. The tissue culture system itself is a mutagenic system **(Jain, 2001)**. According to **Krishna *et al*., (2016)** and **Nivas and D'Souza (2014)**; oxidative stress damage to plant tissues during in vitro propagation may be the cause of or connected to a large portion of the diversity shown in micropropagated plants. Reactive oxygen species (ROS) including superoxide, hydroxyl, hydrogen peroxide, peroxyl, and alkoxyl radicals are examples of pro-oxidants that are enhanced in response to oxidative stress. These ROS may alter DNA's hyper- and hypo-methylation **(Wacksman, 1997)**, change the number of chromosomes from polyploid to aneuploid, cause chromosome strand breaks and rearrangements, and cause DNA base substitutions and deletions which may result in mutations in plant cells.

According to (**Singh and Kumar, 2020)**, plant tissue culture is the most efficient approach for agricultural improving through the formation of Somaclonal and the Gametoclonal variants. Micro propagation technique has enormous potential for producing high-quality plants, isolating beneficial variants in well-adapted high-yielding genotypes, and improving resistance to diseases and tolerance to stressful conditions. Due to the possibility of Somaclonal variability, certain types of callus cultures give rise to clones with inheritable features different from those of parent plants, resulting in the generation of commercially relevant enhanced varieties. Somaclonal variation is associated with point mutations, chromosomal rearrangements and recombination, DNA methylation, altered sequence copy number, transposable elements, and appears to be influenced by genotype, explant type, culture medium, donor age of the plant depending on plant kind, and the presence of transposable elements. Another key factor that can contribute to more variance is the amount of subcultures. The tissue culture system itself operates as a mutagenesis system because cells have traumatic experiences during isolation and may reprogramme during plant regeneration under conditions that differ from those seen in nature. In newly regenerated plants, reprogramming or reorganisation of events can result in a wide variety of epigenetic diversity **(Jain, 2001)**. At the chromosomal level, somaclonal variation exists. Several tissue culture-

derived plant species and their progenies have shown chromosomal variation. (**Creissen and Karp, 1985)** found that high ploidy and high-chromosome explants are more variable than low ploidy and low-chromosome number species.

Endopolyploidization or nuclear fusion is the most common cause of polyploidy in tissue culture-derived plants. Somaclones have altered karyotypes that include chromosomal rearrangements as well as aneuploidy and euploidy. Non-disjunction, abnormal spindles, lagging chromosomes, and chromosome breakage that results in dicentric and acentric chromosomes can all cause aneuploidy. Tissue culture is thought to disturb normal cell cycle regulators, which limit cell division before the conclusion of DNA replication, resulting in chromosomal breakage. Aberrations are caused by chromosome breakage and its repercussions (deletions, duplications, inversions, and translocations). The breakage events are not random, but they do include late replicating chromosomal regions with heterochromatin. Chromosome breaking can cause mutations directly via a position effect or change in gene expression caused by chromosomal earrangement and placement in close proximity of specified heterochromatic area'. Furthermore, changes in DNA methylation might cause chromosomal breakage (**Jain, 2001**). Furthermore, the degree of chromosomal instability in tissue culture differs between species. Rye, for example, has more chromosomal instability than barley or pearlmillet, which is attributable to repeating sequences in the rye genome's heterochromatin. The prevalence of chromosomal abnormalities is also affected by the age of the callus. In general, the probability of chromosomal instability increases with age of the callus. However, maize callus had no effect on chromosomal alterations as it aged. Also **(Roth *et al.*, 1997)** discovered that embryogenic cell cultures of Abies alba displayed suspensor cell malformation and lack of maturation potential, and that cell chromosome counts revealed trisomy. Application of somaclonal variations, it is well accepted that somaclonal variations arising out of unique tissue culture environment are very often noticed phenomenon in clonally propagated plants, which can advantageously be utilized as a source of new variation in horticultural crops **(Karp, 1987)**. However, suitable tools for detection, evaluation, identification and improvement of resistant clones should be designed in order to realize the benefits of such variations. Crop improvement through somaclonal variation enables breeders to obtain plants tolerant to the biotic or abiotic stress, such as drought, high salinity **(Alhasnawi *et al*., 2014)**, high or low soil pH and disease tolerance. A number of cultivars have been developed through somaclonal variation in different horticultural crops for a range of useful traits (**Krishna *et al*., 2016)**

Conclusions

In conclusion, the current study describes an effective and reproducible method for successful (*Lycopersicon esculentum*) in vitro regeneration by optimizing multiple parameters, which indicated a major benefit over previous approaches of in vitro propagation of this essential crop plants, using the combination of PGRs (auxin and cytokinin) and type of explants that promote Callus, shoot organogenesis and shoot multiplication, rooting. In addition to that, the production of somatic embryos and somaclonal variation is due to three reasons: 1- the type and ratio of hormones that are involved in the media, 2- the plant cultivar and explants types, 3- and in vitro environmental conditions.

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