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Scientific Research

Hormonal Requirements Trigger Different Organogenic Pathways on Tomato Nodal Explants

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ABSTRACT

In this work, nodal-disk segments (4 - 6 mm in diameter × 5 - 6 mm in length) were obtained from established shoot culture, resulted from disinfected tomato seedlings, and they were suitable to induce different organogenic pathway under the influence of specific hormonal treatment. Application of BAP (1 - 2.5 mg/l) alone or in combination of 0.5 mg/l NAA resulted in induction of shoot formation. Somatic embryogenesis was rarely appeared (6%) when relatively low concentration of BAP (1.5 mg/l) with low concentration of IAA (0.5 mg/l IAA) was applied. Root induction was triggered when nodal explants or shoot cuttings were cultured on MS medium with (1 mg/l IAA, IBA or NAA) or without auxins, but the best result was obtained when 1 mg/l IAA was used. Application of 0.5 mg/l NAA stimulated callus formation but the best result was obtained when the three different phytohormoes were used (0.5 mg/l 2,4-D + 1 mg/l NAA + 0.5 mg/l BAP). These results indicated that nodal segments, as described in this protocol, can be used as alternative to other types of explants such as cotyledon, hypocotyl and leaf explants.

Keywords: Growth Regulators; Nodal Segment; Organogenesis; Tissue Culture; Tomato

1. Introduction

Tomato is one of the major vegetable crops for human being; it belongs to the family *Solanaceae*. The botanical name of tomato is *Lycopersicon esculentum* Mill. It is a diploid plant with 2n = 24 chromosomes. Tomato is a natural perennial plant, but it is commercially cultivated as an annual crop. It is grown in almost every country of the world—in the field, greenhouses and net houses [1].

Tomato is an important crop worldwide for fresh market or processing. It is necessary for the human being because it contains high amounts of antioxidant vitamins such as vitamins A and C, fiber, and considerable quantities of antioxidants such as flavonoids, zeaxanthin, b-carotenes, lutein, and lycopene [2]. Lycopenes protect cells and other structures in the human body from harmful oxygen-free radicals, therefore they decrease the risk of cancers [3,4]. In addition, tomato has also great potential for transgenic applications, it was used for production of oral vaccines [5] and creation of a new anthocyanin-enriched food for cancer prevention [6], and as model for functional genomics, proteomics and metabolomics to improve abiotic and biotic stress tolerance [7-9].

Genes were conventionally transferred by plant breeders from specific varieties to the related species by sexual hybridization to develop new cultivars with the desirable traits including high yield, stress and disease resistances. These methods have been faced a great challenge to develop new cultivars and sustain food production for the ever-growing human population. The adoption of new technologies such as plant tissue culture and recombinant DNA may achieve the goals. Since 1930s, plant tissue culture has been progressing. Consequently, tissue culture techniques could be used for mass propagation [10], and induction of somaclonal variation [11].

In vitro regeneration from cultivated tomato explants on synthetic medium has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation. Consequently, numerous studies on plant regeneration from a wide range of tissues and organs of wild and cultivated tomato germplasm were conducted for selection of cell lines for biotic and abiotic stresses [7,12], transformation [13], development of haploids [14], production of somatic hybrids [15], and mass propagation [16].

In tomato, adventious shoot formation was obtained

directly [17], or indirectly through the callus formation [18], and both the shoot and root could be obtained together [19]. Response of shoot formation on tomato explants higher in the order of leaves, cotyledons and hypocotyls for the cell cultivars were proved [20]. Any part of plant species (preferably young explants) was used to induce specific type of organogenesis under the influence of phytohormones [21]. In general, nodal explants were rarely used as plant material for induction of different organogenic pathway in tomato. Therefore, this study was used to describe a simple protocol for induction of different type of organogenesis on nodal tomato explants which can be used to improve tomato via gene biotechnology.

2. Materials and Methods

2.1. Preparation of Plant Materials

Tomato seeds (Cassel rock) were surface-sterilized in 5% commercial bleach solution for 10 min followed by 5 min treatment in 75% (v/v) ethanol. After three successive rinses in sterile distilled water for 5 min each, seeds were placed on MS medium [22] supplemented with 3% sucrose, without growth regulators, for seed germination. The medium was solidified with 8 g/l agar at pH 5.8. Vitamins (mgl-1) were: myo-inositol (100), vitamin B1-hydrochloride (4), nicotinic acid (4), pyridoxal hydrochloride (0.7), biotin (0.04) and folic acid (0.5). Seeds were germinated at 25˚C ± 2˚C with 16-h photoperoid. A two-three cm section of seedling containing shoot apical meristem was cut and transferred for further growth to establish shoot culture. Subculture of these shoot cultures was fulfilled on basal MS medium in 11 cm Petri dishes. These shoot cultures were used as a source of tomato nodal segments. The nodal-disk segments (4 - 5 mm radius × 4 - 6 mm long) were cut and cultured vertically or horizontally on MS medium where the half of the explant was immersed in the medium containing different hormonal treatments. In each treatment, fifteen explants were placed horizontally on prepared medium in 11 cm width Petri dishes. Three replicates of each treatment were incubated at tissue culture rooms at 25˚C ± 2˚C. After 6 weeks, the type of organogenesis and other parameters were determined.

2.2. Induction of Root Formation

To obtain roots, shoot cuttings or nodal explants (4 - 5 mm radius × 5 - 10 mm long) obtained from established shoot culture were vertically cultured on MS medium containing 1.5% sucrose and 1 mg/l of NAA, IAA or IBA. Three replicates of each treatment were incubated, each one containing 15 segments, at tissue culture rooms at 25˚C ± 2˚C. After 6 weeks, root frequency, root number and the length of root system/plantlet were determined.

2.3. Induction of Compact Callus Formation

To obtain callus, nodal explants (4 - 5 mm radius × 5 - 10 mm long) obtained from established shoot culture were placed vertically or horizontally on MS medium containing 3% sucrose and different concentration of hormones as indicated in **Table 1**. Three replicates of each treatment were incubated at tissue culture rooms at 25˚C ± 2˚C. After 6 weeks, frequency and the weight of callus were determined.

3. Results and Discussion

Tomato is a subject for research in many research institutes in Egypt aiming to improve its productivity to fulfill the market needs and export. There are several species of the genus *Lycopersicon*, and they represent an important source of genes, conferring resistance to various diseases and pests of cultivated *L. esculentum* [23]. They require the delivery of appropriate genes into the plant cells and are followed by regeneration of the transformed cells [15,24]. Consequently, establishment of tissue culture protocol, as has been described in this work, is essential prerequisite for improvement of tomato plants.

The suitable size of the explants was easily obtained as a nodal-disk segment from *in vitro* grown shoots. The cultured shoots were grown on basal MS medium for 6 weeks; therefore they reached about 10 cm long with 4 - 6 mm in diameter. The obtained nodal segments were like nodal disk and they were suitable to induce different organogenic pathway under the influence of specific hormonal treatment. The obtained explant size of the nodal segment was suitable for shoot regeneration where the very small structures such as individual cell, cell clumps, and shoot tip meristems were in general considered much more difficult for the induction of organogenesis [25], but more shoots were formed from small explants than from large ones [26,27].

Nodal explants were obtained easily and in large quantity higher than other types of explants such as shoot tip, cotyledons and hypocotyls. The influence of explant on organogenesis depends on several factors, including the

Table 1. Effect of explant position on shoot regeneration. Nodal segments were cultured on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA. Means ± standard deviation of three independent experiments, 30 explants were used for each one.

Treatment	Shoot formation frequency (%)	Number os shoots/explant
Vertical position	76	7 ± 1.5
Horizontal position	86	3.33 ± 0.57

genotype, the age of explant, the size of explants, the method of inoculation [28], the time of the year and the environment where the source plant was grown [29]. Tomato shoot culture was used as donor plant materials (nodal segment) where they existed under elite environment and could be obtained at any time through the year. In general, the response of tomato explants from seedlings grown *in vitro* culture differed from those raised in a greenhouse [30]. The established shoot culture, as was used in this work, was preferable as plant materials for induction of different forms of organogenesis as was reported in other works [7,11,21,29].

The *in vitro* organogenic responses of cultured explants of tomato were affected by the type and the concentration of growth hormones (**Table 2**) as was described in this work and others [1,28]. Response of shoot regeneration from the explants higher in the order of leaves, cotyledons and hypocotyls for the cell cultivars were proved [31]. In this work, shoot organogenesis was observed when nodal explant segments were cultured on MS medium supplemented with various concentrations of BAP (1.0 - 2.5 mg/l), but maximum shoot organogenesis was obtained when BAP was used in combination with NAA (2 mg/l BAP and 0.5 mg/l NAA). These results indicated that nodal explant segments, as described in this protocol, can be used as alternative to other types of explants (cotyledon, hypocotyl and leaf explants) in tomato without negative effect on the number of shoots when appropriate concentration of phytohormones was used (**Table 3**). Combination between BAP (2 mg/l) and NAA (1.5 mg/l) was previously reported to induct shoot formation [32,33], and it was affected by the type of explants [34].

Using tomato nodal segments, organogenesis pathway was controlled using specific hormonal treatments. Application of BAP (1.0 - 2.5 mg/l) alone or in combination of 0.5 mg/l NAA resulted in induction of shoot formation (**Table 2**, **Figures 1**-**3**). Somatic embryogenesis (**Figures 3** and **4**) rarely appeared (6%) when relatively low concentration of BAP (1.5 mg/l) and IAA (0.5 mg/l IAA) was applied. Root induction was triggered when 1 mg/l NAA was used but application of 0.5 mg/l NAA stimulated callus formation (**Table 2**). Shoot formation can be initiated with or without the appearance of callus growth.

Table 2. Effect of different growth regulators on organogenic pathways of tomato explants under the influence of growth regulators. Means ± standard deviation of three independent experiments, 30 explants were used for each one.

BAP (mg/l)	NAA (mg/l)	Morphogenetic frequency (%)	Organogenesis type	No. of shoots/explant	No. of roots/explant	Fresh weight/explant (gm)
2.5	-	55	Buds	3.33 ± 0.57	-	2.07 ± 0.37
2.0	-	63	Buds	3.67 ± 0.57	-	2.30 ± 0.17
1.5	-	43	Buds	2.33 ± 0.57	-	1.57 ± 0.15
1.0	-	40	buds	1.67 ± 0.57	-	1.33 ± 0.15
-	1.0	82	Roots	-	3.67 ± 0.57	1.53 ± 0.30
-	0.5	43	Callus	-	-	0.93 ± 0.15
2.5	0.5	80	Buds	5.33 ± 0.57	-	2.03 ± 0.40
2.0	0.5	90	Buds	8.00 ± 1.00	-	2.40 ± 0.10
1.5	0.5	67	-Buds -Embryos	4.00 ± 1.00	-	1.47 ± 0.05
1.0	0.5	55	Buds	2.33 ± 0.57	-	1.27 ± 0.20

Table 3. Effect of explant type on shoot organogenesis on tomato nodal segments. Different segment types were cultured on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA. Means ± standard deviation of three independent experiments, 30 explants were used for each one.

Explant type	Shoot formation frequency (%)	Number of shoots/explant
Nodal segment	82	7 ± 1.0
Cotyledons	85	6 ± 1.5
Hypocotyl	56	5 ± 1.5
Leaf	64	3 ± 1.5

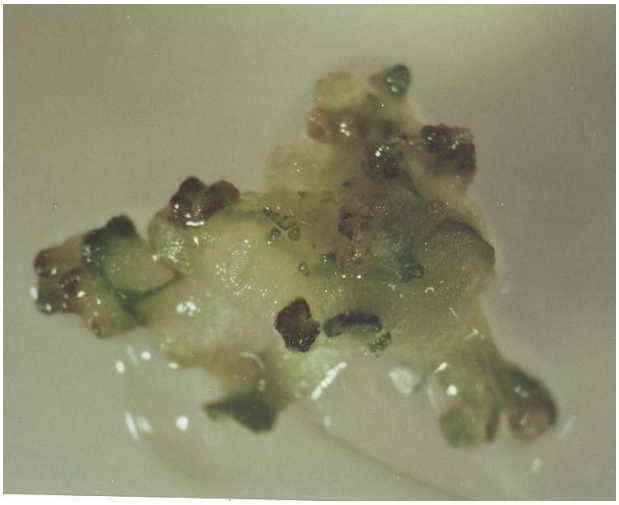

Figure 1. Shoot organogenesis on nodal explant segment cultured for 6 weeks on MS medium supplanted with 2 mg/l BAP.

Figure 2. Shoot formed on MS medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA after 8 weeks culture.

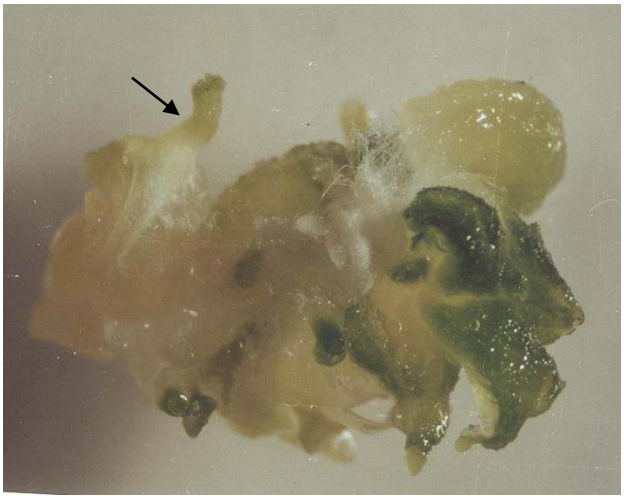

Figure 3. Somatic embryo (arrow) and shoots initiated on nodal explant cultured for 8 weeks on MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA.

Figure 4. Pro-embryo structure on nodal explant cultured for 6 weeks on MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA.

Figure 5. Root organogenesis on nodal explant segment cultured for 8 weeks on MS medium supplemented with 1 mg/l IAA.

Application of BAP alone stimulated direct shoot organogenesis (**Figure 1**) but application of BAP (2 mg/l) with NAA (0.5 mg/l) resulted in induction of shoot formation mediated with the appearance of callus (**Figures 2** and **3**). In tomato, adventitious shoot regeneration can be achieved either directly [17] or indirectly through an intermediate callus phase [18]. The application of phytohormones to trigger specific molecular processes on the explant tissues resulting specific organogenesis pathway was previously reported [21]. As was observed in this work (**Table 2**), variations in quantity and type of PGRs influence both the percentage of explants responding, and the number of shoots/explant. These differences may be governed by both cytoplasmic and nuclear genes, as illustrated in the reciprocal hybrids developed by [35]. Nodal explants can be used for application of tissue culture in the fields of tomato improvements and molecular studies [12,36-39].

Nodal segments of the used tomato cultivar were inoculated on the culture media in polar (straight up, with the physiological base in the medium). The polar orientation resulted in shoot formation higher than horizontall orientation (**Table 1**), where the chemical and physical conditions of the polar culture were more easily than non-polar orientation. In this work, progressive enlargement of the explants was proceeded with the shoot formation (**Figure 1**), but root formation was obtained without enlargement (**Figure 5**). In another work, more shoots

Table 4. Effect of hormone type on callus induction from node segments of tomato shoots. Means ± standard deviation of three independent experiments, 30 explants were used for each one.

2,4-D (0.5 mg/l)	NAA (1 mg/l)	BAP (0.5 mg/l)	Frequency (%)	Weight/explants (gm)
+	−	−	55	2.49 ± 0.14
+	+	−	63	2.11 ± 0.17
+	+	+	100	2.69 ± 0.11

Table 5. Effect of MS medium supplemented with 1 mg/l auxin (IAA, IBA, or NAA) on root induction from 2.5 cm length shoot segments of tomato plantlets after four weeks culture. Means ± standard deviation of three independent experiments, 30 explants were used for each one.

Type of auxin	Frequency (%)	No. of roots/plantlets	Root length/plantlets
Without auxin	76	4.33 ± 1.15	5.07 ± 0.90
IAA	90	11.00 ± 1.00	6.97 ± 0.87
IBA	86	6.00 ± 1.00	3.17 ± 0.28
NAA	80	4.00 ± 1.00	2.17 ± 0.76

were produced from leaf and cotyledon explants placed horizontally than from the ones placed vertically, and hypocotyls explants placed horizontally produce more shoots than those placed vertically straight or upside down [20].

Nodal segments cultured on MS medium supplemented with 1 mg/l of 2,4-D alone or in combination of NAA with or without BAP resulted in callus formation on the nodal explants irrespective the position of the explants on the medium surface, the best results were obtained when the three different phytohormoes were used (**Table 4**).

Root formation on nodal explants or shoot cuttings were obtained on MS medium with or without auxins, but the number and the length of root systems were influenced by the type of auxin, the best result was obtained when 1 mg/l IAA was used (**Table 5** and **Figure 5**). These rooting plantlets were transferred to soil after adaptation under plastic bags for three weeks under green house condition. Root formation was detected when nodal explants were vertically placed on MS medium without phytohormones, which gave an indication about the importance of indigenous auxins in the induction of root formation. Many researchers have speculated that tomato has a high level of endogenous auxin, based on the observations of shoot cultures producing roots without the addition of auxins in the medium [1].

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