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In Vitro Propagation of Two Tomato Hybrids (*Lycopersicon Esculentum* Mill.) Via Tissue Culture Technique

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ABSTRACT

This investigation was carried out at the Tissue Culture Lab., Biotechnology Research Center (BTRC) in Tripoli, Libya in 2009 to establish a micro propagation protocol for two hybrids of Tomato in which several factors were studied including the effect of concentration of growth regulator on shoot multiplication, and rooting of explant on MS media. Shoot tips were cultured into (MS medium) with different concentrations of benzyl adenine (BA) + 0.5 mg/l (IAA) compared with MS basal medium as a control treatment. Generally, the results showed that there were no significant differences between several concentrations of (BA+IAA) on the number of leaves and shoot length. The obtained results illustrated was M1 gave high number of shoots/explant, shoot length, and leaves number for two hybrids of Tomato which were 1.90, 3.89, 4.48 respectively compared with the control. The results showed that there were no significant differences between Tomato hybrids on shoot length and the number of shoots. The best result for leaves number was hybrid HH-56 which gave 4.54 leaves. From the foregoing results, MS media supplementing IAA at 0.2 mg/l was the best treatment for rooting stage of hybrid HH-56 and Hybrid AZHAR F1. The plantlets were transferred to plastic cups containing peatmoss + sand at 1:1 (V/V). Acclimatization of plantlets were successfully.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important Solanaceae crop grown throughout the world (Rick, 1980). It is regarded as the second most important vegetable crop in the world after potato (Bhatia *et al*., 2004; Foolad, 2004). It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometimes rightly referred to as poor man's orange (Devi *et al*., 2008). These crop species exhibited extraordinary nutritional value. That is why it is considered preventive food (Raiola *et al*., 2014). Tomato is one of the most popular fruit vegetables in Libya, and is planted in almost 10538 hectares, the production was 218.000 tons in 2019 (FAO STST). The nutritional value of tomato is very high, and is a source of vitamin C, B and a good source of β- carotene (Raziuddin *et al*., 2004). Tomato plays an important role in maintaining human health and strength. It is also very helpful in healing wounds because of the antibiotic properties found in the ripe fruits. It is an essential ingredient of most of the vegetarian and non-vegetarian diet (Kalyani and Rao, 2014). The successful application of plant tissue culture presupposes the establishment of an efficient culture system, consisting of a competent genotype and explant source as well as optimal culture conditions. The tissue culture regeneration system of tomato is influenced by genotype, type of explants, hormones, and other factors (Rai *et al*., 2012, 2013). Many studies demonstrate that variety characteristics are the key factors of tomato regeneration, so it is important to choose the appropriate acceptor. Selecting the appropriate varieties, explants and the additives of medium can increase the frequency of transformation of tomato. Plant tissue culture has contributed to the advancement of agricultural sciences (García-Gonzáles *et al*., 2010). It is an invaluable tool for solving basic problems applied to plant biology; since employing this technique a strict control is obtained as the material is confined in an aseptic microenvironment, occupying very little space and, by clonally propagating, it allows the maintenance of the genotype (Slack S., 1980; Plana *et al*., 2005). As an application of plant tissue culture. In vitro micropropagation can be mentioned, which in recent decades has gained great importance as an alternative for the mass production of plants with agronomic characteristics of interest, resulting in benefits in horticulture, being used to increase or replace the vegetative propagation techniques used until today (Vinoth *et al*., 2012; Vikram *et al*., 2012; Chyi and Phillips, 1987). Development of protocols independent of exogenous plant growth regulators could help standardize techniques for different species and cultivars, thereby, reducing problems of regeneration efficiency and elongation of regenerated and abnormal shoots (Cano *et al*., 1998). Tomato is a self-pollinated plant, the process of hybrid seed production involves hand pollination, whole process of hybrid seed production is done manually under field conditions, and undesirable weather conditions. So all these factors lead to increasing cost of tomato hybrid seeds. Tissue culture technique can help reduce the price of hybrid seeds and increase mass propagation of high quality seeds (Bhatia and Ashwath, 2004). Several protocols have been published for in vitro plant regeneration of Lycopersicon species. Methods previously reported are in general tedious and

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time consuming, with variable efficiencies and high production costs. In all cases, regeneration systems involved media containing growth regulators. Although tomato adventitious shoot regeneration is not considered a real problem, the interest to obtain more efficient, reliable, simple, rapid and universal methods for genetic engineering is well documented in literature (Pozueta *et al*., 2001; Olodakoon *et al.* 1995; Torelli *et al*., 1996; Cano *et al*., 1998; Chyi and Phillips, 1987). The purpose of our work was to propagate some tomato with high price hybrid seeds and to study the effect of Nodal explants and shoot tips using several concentrations of BA and IAA on shoot regeneration in vitro in tomato using tissue culture technique.

MATERIALS AND METHODS

This work was carried out at Plant Tissue Culture Lab., Biotechnology Research Center (BTRC), in Tripoli, Libya in 2009 to produce transplants of two hybrids of tomato (hybrid HH-56 – hybrid AZHAR F1) through tissue culture technique.

Seeds Sterilization

Seeds were surface-sterilized by washing with running tap water. Seeds were then immersed in 70% ethanol for one minute and were rinsed three times with distilled water, followed by 20% Clorox (sodium hypochlorite) for 20 minutes. Sterilized seeds were then rinsed three times with sterilized distilled water.

Seeds Germination

Seeds of tomato hybrids were cultured in jars containing MS basal medium without hormones, (Cortina *et al*., 2004). Ten seeds were cultured in jars and were kept for 25 days to get sterilized seedlings as a source of explants for multiplication stage.

Media Conditions

Explants (Nodal explants and shoot tips) were inoculated onto solid nutrient medium MS (Murashige and Skoog's, 1962) with a range of BA (0, 0.5, 1, 1.5, 2 mg/l BA + 0.5 mg/l IAA). All cultured jars were incubated under 16 hours/day and 8 hours/night photoperiod conditions for four weeks, and all explants were kept in a room with temperature of 25–27°C (Dahanayake *et al*., 2010).

Multiplication Stage

The explants, about 2-3 cm from the seedlings were cut. Nodal explants and shoot tips of seedlings were placed into jars with MS medium. The cultures were incubated in normal growth room conditions (16/8 light/dark regime) having the same light intensity and temperature as above for three weeks. Number of shoots per explant, shoot length and number of leaves per shoot were determined.

Rooting Stage

Newly formed shoots were transferred into MS nutrient medium supplemented with different concentration of growth regulators, naphthalene acetic acid (0, 0.5, 1, 2 mg/l NAA) for further development and rooting. The number of shoots that produced roots were recorded after two weeks of incubation. Rooting percentage, number of roots per plantlet, root length, shoot length were recorded after 2 weeks.

Acclimatization Stage

The aim of this stage was to adapt tomato plantlets before transferring to the open field. Rooted plantlets were taken from vessels and washed with sterile distilled water. All Rooted plantlets of about 6 cm in length were transplanted in cups, then covered with polyethylene bags to maintain high humidity (70 to 80%) around plantlets. The cups containing 1:1 peat/soil were kept in growth room.

Design and Statistics Analysis

The experiments were designed as a completely randomized factorial with two factors (type of hybrid X regulator rate) with five replicates for each treatment. Data relative to the number of regenerated shoots and number of rooted and acclimatized plantlets were analyzed with ANOVA using Mstat software were compared with the least significant difference (LSD). (Toothaker *et al*., 1993).

RESULTS AND DISCUSSION

In vitro culture was used in tomato in different biotechnological applications, production of virus free plants (Moghaieb *et al*., 1999), genetic transformation (Ling *et al*., 1998) and in many fundamental researcher programs (Arriliaga *et al*., 2000). Nodal explants and shoot tips were used from seedling of a high frequency and quick regeneration in two hybrids of tomato (hybrid HH-56 – hybrid AZHAR F1).

Seed Germination

Seeds were inoculated on MS medium to observe the behavior of hybrids for in vitro seed germination. HH-56 was 95% and AZHAR F1 showed 78% growth on MS plane medium (Table 1). The results showed that HH-56 gave the maximum percentage of seed germination. The reason behind the difference in germination rate might be due to the genotypes of the hybrids (JaeBok *et al*., 2001). This result confirmed that the germination rate depends upon the genetic makeup of the varieties.

Table 1: Germination percentage of the tow hybrids of Tomato on MS plain medium

Hybrids	Total number of Seeds	Germinated Seeds	Percentage germination
AZHAR F1	100	78	78%
HH-56	100	95	95%

Effect of Tomato Hybrids

Results in (Table 2) show that there were no significant differences between HH-56 and AZHAR F1 hybrids with

respect to shoot length and number of shoots/ explant. HH-56 hybrid was regarded the highest value (4.54) for the number of leaves/ shoot.

Table 2: Effect of tomato hybrids on shoot characteristics during multiplication stage

Hybrids	No. of leaves	Shoot length (cm)	No. of shoots
AZHAR F1	3.71 b	3.04 a	1.62 a
HH-56	4.54 a	3.60 a	1.65 a

Means having the same letters within each column are not significantly differed at 0.05 level

Effect of BA and IAA Concentrations

There were no significant differences among BA and IAA concentrations and control regarding to the number of leaves/ shoot (Table 3). In addition, there were significant differences among BA and IAA concentrations with control in shoot length and number of shoots/ explant. (Arkita *et al*., 2013) came to similar results on tomato. The average number of shoot was observed when BA and IAA were combined together. However, BA at 0.5 mg/l + 0.5 mg/l IAA gave the highest value of shoots number/ explant and shoot length. In this connection, (Mohamed *et al*., 2010), came to similar results. It was illustrated that the BA levels were associated with increasing the tomato shoots number and shoot length through tissue culture technique.

Table 3: Effect of growth regulators on shoot characteristics of tomato hybrids during multiplication stage

Treatment	Code	No. of leaves	Shoot length (cm)	No. of shoots
MS (control)	M0	3.55 a	2.01 b	1.00 c
0.5 mg/L BA+0.5 mg/L IAA	M1	4.48 a	3.89 a	1.90 a
1.0 mg/L BA+0.5 mg/L IAA	M2	3.86 a	3.49 a	1.69 ab
1.5 mg/L BA+0.5 mg/L IAA	M3	4.38 a	3.88 a	1.36 bc
2.0 mg/L BA+0.5 mg/L IAA	M4	4.44 a	3.59 a	1.56 ab

Means having the same letters within each column are not significantly differed at 0.05 level

Effect of IAA

Results presented in Table 3 show that supplementing MS media with 0.5 mg/l IAA increased the number of shoot and Shoot length compared to control (MS media without hormons). These results could be explained by the promotive effect of auxins on number of shoot, as noticed by (Deklerk *et al*., 1999).

Rooting Stage

Tomato hybrids and Hybrid HH-56 gave the highest number of roots and Shoot length 9.54, 9.11 respectively compared to AZHAR F1 hybrid. Root length showed no significant differences between hybrids (Table 4). Rooting percentage was 100% for both hybrids. However, the best result for the number of leaves for hybrids were regarded the highest values at MS + 1.0 mg/l NAA, HH-56 gave 9.85 and AZHAR F1 was regarded 8.30 on number of leaves (Table 5).

Table 4: Effect of tomato hybrids on rooting percentage of plantlet

Hybrids	No. of leaves	Shoot length (cm)	No. of shoots
AZHAR F1	3.71 b	3.04 a	1.62 a
HH-56	4.54 a	3.60 a	1.65 a

Means having the same letters within each column are not significantly differed at 0.05 level

Table 5: Effect of the interaction between tomato hybrids and growth regulators on growth characteristic and rooting.

Hybrid	Treatment	Shoot length (cm)	No. of roots/ Plantlet	Root length (cm)
AZHAR F1	MS Control	4.03 c	3.80 c	6.33 b
	MS + 0.5 mg/l NAA	7.22 ab	4.44 c	5.87 bc
	MS + 1.0 mg/l NAA	9.11 a	8.30 a	8.11 a
	MS + 2.0 mg/l NAA	8.22 a	4.55 c	4.21 c
HH-56	MS Control	7.04 ab	7.95 b	8.03 a
	MS + 0.5 mg/l NAA	7.51 ab	8.35 a	7.50 ab
	MS + 1.0 mg/l NAA	9.94 a	9.85 a	7.92 a
	MS + 2.0 mg/l NAA	7.43 ab	7.66 b	7.88 a

Means having the same letters within each column are not significantly differed at 0.05 level

CONCLUSION

It is concluded that supplementing 0.5 mg/L BA + 0.5 mg/L IAA to MS media gave the highest value of shoot length and number of shoots of HH-56 hybrid. On the other hand, supplementing NAA at 1 mg/l to MS media were the best treatments for rooting stage of HH-56 and AZHAR F1 Tomato hybrids.

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