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Effect of different plant growth regulator on in vitro propagation of endangered plant; yellow tomato (*Lycopersicon esculentum* Mill.)

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Abstract

In the present study, a convinient procedure for in vitro propagation of yellow tomato plant, (*Lycopersicon esculentum* Mill.) under the risk of extinction, was developed. Shoot tip, hypocotyl, cotyledon, leaf, node and internode were excised from sprout seedlings and used for explant sources. In vitro adventitious shoot regeneration was achieved in MS medium supported with the particular concentrations and combinations of plant growth regulators (PGRs) via direct organogenesis. The most successful (100%) adventitious shoot regeneration was provided from node and shoot tip explants in MS medium supported with the particular concentrations and combinationss of 6-Benzylaminopruine (BAP) and Naphtalane acetic acid (NAA). The highest root formation (100%) was achieved on regenerated adventitious shoot in MS medium supported with Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA). The whole seedlings regenerated in vitro were adapted to soil and acclimatized in field.

Key words: Yellow tomato, micropropagation, plant growth regulators

Introduction

Plants and plant products have function in sustaining the human life. Plants as a food source constitute directly 93% of the human diet, and 7% indirectly. Tomatoes (Lycopersicon esculentum Mill.) are a main portion of human nutrition worldwide . It is a dicotyledonous plant in the Solanaceae family and possesses juicy fruits with 6 % of dry matter. Although it includes relatively low concentrations of vitamin C, pro-vitamin A and minerals, compared to other fruit species, it is one of the main food source of human diet because of the consumption in high quantity (McGlasson, 2003).

Plant regeneration in tissue culture is an alternative way for genetic transformation (Park et al., 2003), mass production and coping with plant diseases caused product loss (Moghaieb et al., 1999). The successful in vitro regeneration protocol requires an influential culturing, qualified genotype, suitable explant and optimal incubation (Plana et al., 2005). Tomato is a major food product, on which successful in vitro propagation applications and genetic manipulations have been conducted for a better frut quality (Lindsey, 1992). In vitro propagation has been succeeded in tomato through employing almost all parts of the plant as explant (McCormic et al., 1986; Young et al., 1987; Branca et al., 1990; Compton and Veillux, 1991; VanRoeke et al., 1993; Oktem et al., 1999).

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In the present study, an in vitro tissue culture protocol was intended to provide on yellow tomato grown in Europe and Asia especially in Northern Iraq region and Turkey. The impacts of PGRs and explants on in vitro regeneration of yellow tomato were investigated.

Materials and Methods

Plant material

Yellow tomato seeds (Gooseberry Tomato) were provided from Northern-Iraq. Seeds were germinated in the Laboratory of Molecular Biology and Genetics Department in Van Yuzuncu Yil University. Mature plants with flower and fruit was identifed by plant taxonomist in Biology Department (Davis, 1982).

Sterilization of glassware and equipment

All materials used in this experiment first were cleaned with detergent and water. All metal and glassware were packed in aluminum foil. Then all the equipment were incubated in autoclave at 121 ° C and 1.5 atmosphere. Sterilization was carried out in 25 minutes for media and 1 h for metal and glassware.

Seed sterilization

In this study, most widely used commercial sodium hypochlorite (bleach) was employed. 100% commercial bleach (NaOCl- ACE-Turkey) was purchased and, diluted to 5% and treated with 20 minutes on seeds. Then seeds were rinsed 3 times with sterile double distilled water and incubated in MS and White medium. With such a sterilization methods very few incidence of contamination occurred.

Growth media and culture conditions

Nutritional environment is the most important factors of success in tissue culture studies. MS medium (Murashige & Skoog, 1962), sucrose, agar and Plant Growth Regulators were used for plant regeneration. Media, sugar, agar (gelling agent) and PGRs concentration were arranged according to commercial instruction and literature.Five different explants ;hypocotyl, leaf, cotyledon, node, internode were obtained from germinated seedlings. Plant Growth Regulators (PGRs) were employed in following concentrations; Kinetin (2 mg/L, 4 mg/L), BAP (0.3 mg/L, 0.5 mg/L, 0.6 mg/L, 0.8 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, 4mg/L), NAA (0.5 mg/L, 1 mg/L, 4 mg/L), 2,4-D (0.5 mg/L, 1 mg/L, IAA (0.5 mg--/L, 1 mg/L, 2 mg/L) and IBA (0.1 mg/l, 0.5mg/L, 1 mg/L). pH was regulated to 5.8 with 1 M NaOH and 1 M HCl. Double distilled pure water was used for media and stock solutions. Plant tissue culture media contain all necessary mineral salts, amino acids and vitamins.

Seedling culture

The seeds were rinsed three times with sterile distilled water and 5 seed were cultured in petri dish containing 20 ml of solid medium. PGRs free MS medium containing 3% sucrose and 0.6% agar was employed for experiments. The pH was adjusted to 5.8 before autoclaving at 121 °C for 25 min. The cultures were incubated in a growth chamber (Phytotron, Sanyo, Gellenkamp PLC, UK), maintained at 25 ±2° C, and a 16-h photoperiod and 8-h in dark, was provided by cool white fluorescent lamps; 500 micromol^{-2} sec. (Phillips Canada, carborough, Ont.). Explants were excised from the seedlings after 17 days of seed incubation.

Taking explant from germinated shoot

Cotyledons, shoot tip, hypocotyls, leaves, nodes, internodes were excised from 17 days old seedlings and used as explant. Explants were incubated in MS medium supported by PGRs of BAP, NAA, kinetin, 2,4-D, IBA, IAA in different concentrations and combinations. After 3 to 4 weeks of explant incubation adventitious shoots were developed. Then adventitious shoots were transferred to different medium condition for roots proliferation.

Calculation of yield percentage

Yield percentage was calculated by proportioning the total number of cultivated explants to the number of individually shoot, callus and root producing explant. Experiments were repeated at least triplicates.

Results and Discussion

After 17 days of germination period shoot tip, hypocotyl, cotyledon, leaf, node and inter node explants were isolated and incubated in MS supplemented with PGRs for plant regeneration.

The medium and plant growth regulators (PGRs) used in the present study was selected according to cited studies carried out on tomato varieties. The type, concentration and combination of PGRs were optimized according to the response of the explants to PGRs applications. Here, especially MS medium supported with 2 mg/l BAP+1 mg/l NAA and 0.5 mg/l BAP caused 100% shoot proliferation on node explant.

The main aim in the experiment was to produce shoots from explants, but some PGRs types and combinations caused callus formation along with shoot development. MS supplemented with 2 mg/l kinetin caused 83.33% callus on hypocotyl and 75% callus on leaf explants. Interestingly 2 mg/l kinetin also caused 80% shoot on node explant. MS medium supplemented with 2 mg/l BAP+1 mg/l NAA cultured 66.66% callus on hypocotyl (Table 1). The outcomes of the table are; the hypocotyl explant promotes callus formation. Kinetin induces callus on hypocotyl and leaf explant, but promotes shoot formation on node explant at low concentration of BAP alone and in combination with of NAA. High concentrations of cytokinin in combination with low concentrations of auxin, or low concentration of auxin alone increased shoot formation. High concentrations of kinetin also promotes shoot formation on node explant.

Table 1. The Effects of different PGR concentration, combination and explant types on shoot and callus formation.

PGR concentration (mg/L)	Hypocotyl		Leaf		Cotyledon		Node		Inter node	
	Callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %	Callus %	Shoot %
2 (mg/L) kinetin	83.33	0.00	75.00	0.00	16.66	0.00	40.00	80.00	50.00	0.00
2 mg/L BAP+0.5 mg/LNAA	25.00	0.00	0.00	0.00	0.00	0.00	20.00	50.00	N	N
2 mg/L BAP+1mg/L NAA	66.66	0.00	0.00	0.00	0.00	0.00	0.00	100.00	50.00	0.00
3 mg/L BAP	0.00	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00
1mg/L 2,4-D	0.00	0.00	0.00	0.00	0.00	0.00	50.00	0.00	0.00	0.00
0.5mg/L BAP	20.00	0.00	50.00	0.00	N	N	0.00	100.00	42.85	0.00

N = the explant is not used in the tests, 0.00 = callus or shoot is not produced

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Figure 1: The effects of different PGRs concentration and explant types on shoot formation

Table 2. The influence of diverse PGR concentration, combination and explant on shoot formation.

PGR Concentration mg/l	Shoot Tip	Node	Leaf	Hypocotyl
	Shoot formation %	Shoot formation %	Shoot formation %	Shoot formation %
2 mg/L 2,4-D	88.88	85.00	0.00	0.00
2mg/L BAP+4mg/L NAA	100.00	37.50	0.00	0.00
0.8 mg/LBAP+0.5mg/L 2,4-D	100.00	66.66	0.00	8.33
2mg/L IAA+4mg/L NAA+ 4 mg/L BAP+4mg/LKinetin	75.00	90.00	0.00	0.00
0.5mg/L IAA	0.00	25.00	0.00	0.00

ST = Shoot tip, 0.00 = shoot formation is not occurred

Data presented in Table 2 was provided from the experiment designed for shoot production on explants by analysing the data obtained from the Table 1. Particularly shoot tip and node explants gave good results in shoot formation at different PGRs concentrations and combinations. MS medium supported with the combination of 4 mg/l NAA + 2 mg/l BAP and 0.5 mg/l 2,4-D + 0.8 mg/l BAP resulted in shoot formation at 100% on shoot tip. MS medium supplemented with 0.5 mg/l 2,4-D + 0.8 mg/l BAP produced 66.66% shoot on node explant. MS medium supplemented with 2 mg/l 2,4-D caused 88.88% shoot production on shoot tip and 85% on node explant. The combination of 2 mg/l IAA + 4 mg/l NAA + 4 mg/l BAP + 4 mg/l Kinetin in MS medium resulted in 75% shoot growth on shoot tip and 90% shoot growth on node explant.

Table 3. The effects of different PGR concentrations, combinations and explants on shoot and root formation.

PGR concentration mg/l	ST Explant		Hypocotyl Explant		Node Explant		Leaf Explant	
	Shoot %	Root %	Shoot %	Root %	Shoot %	Root %	Shoot %	Root %
1 mg/LBAP + 0.5 mg/L IBA	80.00	100.00	0.00	0.00	50.00	25.00	0.00	25.00
0.6 mg/L BAP+ 0.1 mg/LIBA	85.00	50.00	0.00	0.00	75.00	0.00	0.00	0.00
0.6 mg/L BAP + 0.5 IBA mg/L	100.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
0.3 mg/L BAP + 0.5 mg/L IBA	75.00	50.00	0.00	0.00	75.00	50.00	0.00	0.00

ST = Shoot tip, 0.00 = shoots or roots is not produced.

Simultaneously shoot and root developmental potential of explants were evaluated. The data were presented in Table 3. In the experiment low concentrations of auxin and cytokinin combinations were applied on explants. MS medium supplemented with higher cytokinin and lower auxin concentration produced 75-100% shoot growth on shoot tip and node explant. However root formation was also observed on all the explants incubated except for hypocotyl where shoot formation was also not seen depending on PGRs used.

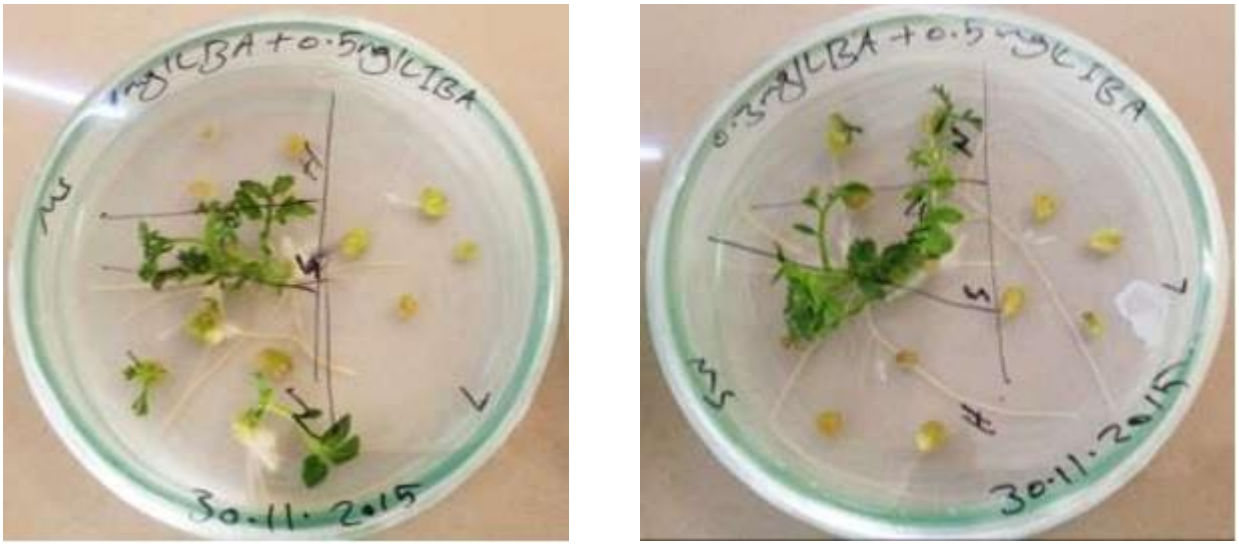

Figure 2: The effects of PGR type and concentration on shoot and root formation.

The shoots obtained from experiments were separated from each other and made independent and transferred to rooting medium. Shoots regenerated from shoot tip and node explants were exposed to 0.5-1mg/l of auxin combinations for rooting. In particular, the combination of 0.5mg/l IBA + 0.5mg/l IAA resulted in 100% rooting. It was observed that the auxin alone caused low rooting. MS medium supplemented with 1mg/l IBA+2 mg/l NAA caused 62.50% of root on shoot regenerated from node explant.

Table 4. The effects of different auxin concentrations and combination on root genesis.

PGR concentration mg/L	ST Explant	Node Explant
	Root Formation %	Root Formation %
0.5mg/L IAA	50.00	50.00
1 mg/L IAA	0.00	40.00
1 m g / L NAA	0.00	22.00
0.5 mg/L IBA + 0.5 mg/L IAA	100.00	100.00
1mg/L IBA + 2mg/L NAA	0.00	62.50

N = the explant is not used. 0.00 = root is not produced

Figure 3: Root formation in different hormone concentrations.

After shoot and root regeneration from different explants, plantlets were transferred into the pots containing 1/3 sand and 2/3 soil and covered by polyethylene to remain the moisture in the high level for acclimatization to the different field condition. After two-three weeks plantlets were acclimatized to the field conditions, and 60.00 % of plants were remain alive and grown ambient natural conditions.

In the present study, the effect of different PGRs concentrations and combinations and various explants on adventitious shoot and roots regeneration of yellow tomato plant were investigated. A convinient *in vitro* propagation protocol was developed for yellow tomato plant. Seeds were germinated *in vitro* and explants were excised from *in vitro* regenerated seedlings. Establishing a successful *in vitro* regeneration protocol is a very difficult process because it requires optimization of many factors affecting the regeneration capacity. MS medium was preferred in the experiments. Because, most of the plants respond favorably to MS medium, since it contains all the essential components required for *in vitro* regeneration.

Literature reports successful results on *in vitro* regeneration of tomato plant from hypocotyl and cotyledon explants (Locy, 1981; Motte *et al*., 2013). Unlike the studies in the literature, the most successful results were obtained from the shoot tip and nodal segment explants in our study (Table 1, 2, 3). The reason of the different results could be because of the genotype, the induction period of the shoot growth and the endogenous hormone concentration balance of the explant (Schween and Schwenkel, 2003) and the age of the explant (Locy, 1981). The levels of endogenous oxine and cytokine and their ratio one to another may be induce genes that are effective in cell proliferation and differentiation mechanisms (Henry *et al*., 1994).

In our findings hypocotyl and cotyledone were less shoot producing explants. The reason may be because of the level of endogenous phytohormones present in the explant (Schween and Schwenkel, 2003).

The explant type and genotype is also important factors for *in vitro* plant regeneration (Kumar and Reddy, 2010). Explant is a critical parameter when optimizing tissue culture methods (Kumar *et al*., 2011). Therefore, choice of appropriate explants is an important determinant of *in vitro* plant regeneration (Takashina *et al*., 1998). Jamous and Abu-Qaoud (2015) reported effective adventitious shoot proliferation from shoot tip followed by hypocotyl, leaf, and cotyledon. Many sucessful protocol has been repoted on *in vitro* tomato regeneration from different explant sources (Gubis *et al*., 2004; Liza *et al*., 2013). Shoot development is a complicated process. Some candidate genes thought to be related to shoot development at the molecular level are likely to increase shoot growth capacity (Motte *et al*., 2013). Key genes related to shoot development are belived to be connected to hormone biosynthesis, transport, signal transduction and hormonal interaction (Su and Zhang, 2014).

One of the factors of *in vitro* plant regeneration achievement is the use of seddling germinated *in vitro* as the explant source (Teng, 1999). The fact that these explants are more hygine, not exposed to pre-sterilization process, and possess the adaptation ability *in vitro* regeneration media. In this study, seedling grown from seeds *in vitro* were used as explant source. *In vitro* morphogenesis of plants is organized by the reciprocal influence and balance between the exogenous growth regulators and the compunds synthesized endogenously (George *et al*., 2008).

Auxines and cytokinins were employed alone in different concentrations and also together in different combinations. 2 mg/l KIN caused 80% adventitious shoot proliferation on nodal segment. 0.5 mg/l BAP also produced 100% shoots on nodal segments. 2 mg/l 2,4-D caused 89% shoot proliferation on shoot tip and 85% in nodal segment. 0.5 mg/l IAA also produced 25% shoots on nodal segment (Table 2). Here the independent auxines in high and the cytokinins in both high and low concentration have been found to be efficient in adventitious shoot proliferation on meristematic tissues both on shoot tip and nodal segments of yellow tomato plant. Similar results was reported by Durrani *et al*., (2017).

Adventitious Root and shoot growth is dependent on the proportion of auxin and cytokinin augamented to the medium. If auxine is higher than cytokinin root is developed. If cytokinin is higher than auxine shoot is developed (Taiz and Zeiger, 1991). While these hormones demonstrate the morphogenetic effects of independent treatments, their combinations have yielded more successful results in shoot and root development. PGR balance is dependent on the type and stage of *in vitro* culture medium (George *et al*., 2008).

Higher cytokinin and lower auxine combinations resulted in efficient adventitious shoot proliferation. Two different cytokinins and auxin co-administration resulted in 90% adventitious shoot proliferation on nodal segment and 75% on shoot tip. The combination of low auxine and cytokinin (0.6 mg/l BAP+0.5 mg/l IBA) resulted in 100% shoot proliferation on shoot tip and nodal segment (Table 2, 3).

The response of growth hormones in the culture media differs within genotype and explant (Slater *et al*., 2003). Particular explants incubated in different media and PGRs had distinctive response to regeneration (Kaur *et al*., 2011).

In the rooting experiments from *in vitro* regenerated shoot, auxines were applied. 0.5 mg/l IAA+0.5 mg/l IBA combination was found to be more efficient (100%) than other auxine combinations and independent treatments. In rooting experiment, the shoots regenerated from nodal segment explant were detrmined more successful (100 %) as the case in adventitious shoot production. 1mg/l IBA+2mg/l NAA also caused 62.50% root production from adventitious shoot regenerated nodal segment (Table 4).

In rooting phase, cytokine is not always necessary. The auxine can perform rooting alone or in combination with another auxin. However, it has been reported that different oxine combinations are more successful than single oxine administration (Ouyang *et al*., 2003).

In micropropagation, adventitious root induction is a critical and complex process and is affected by the type and concentration of auxin in the first degree (Hatzilazarou *et al*., 2006). Cytokinins suppress rooting (Feito *et al*., 1996) and it has been reported that it complicates the acclimatization process (Valero-Aracama *et al*., 2010).

Conclusion

Acclimatization of *in vitro* regenerated plants is an important step. Thus, the plants are grown in field and can be commercialized. *In vitro* plant regeneration can shorten the growth and improving period of the plant compared to the natural growth period.

Conflict of Interest

The authors declare that there is no conflict of interest.

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