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Using Continuous Immersion System Liquid Media for *In Vitro* Microtuber Potato (*Solanum Tuberosum* L.) Production in Bioreactor

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Abstract

Purpose of this study was to determine effects of *in vitro* continuous immersion culture system, solid media, different doses of Thidiazuron (TDZ) (0.5, 1.0, 1.5 and 2.0 mg/l) and Indole-3-butyric acid (IBA) (0.5, 1.0, 1.5 and 2.0 mg/l) on stem segments, microtuber number per plant. *In vitro* continuous immersion liquid culture media and solid media were used in potato (*Solanum tuberosum* L.) cv. Granola for stem segments and microtuber micropropagation. The stem segments were cultured on 1/2 Murashige and Skoog (MS) medium supplemented with 2 mg/l gibberellic acid, 10 mg/l paclobutrazol, 5.0 g/l activated charcoal, 100 g/l sucrose with or without 3.5 g/l phytagel; and TDZ and IBA *in vitro*. Liquid medium was distributed to the carrier *in vitro* continuous immersion with supporting net liquid culture system containing glass wool + filter paper layer as substrate. *In vitro* continuous immersion liquid culture media was more suitable and stable for organogenesis of potato microtubers than phytagel (3.5 g/l) solid media. After 30-day of incubation, there were 4.76 axillary stem segments formation having 7.21 mm diameter of microtubers weighing 155.74 mg fresh weight. Microtuber formation rate 84.66% and there were 2.06 microtubers per plant at 0.5 mg/l IBA *in vitro* continuous immersion liquid culture media treatment. 1.5 mg/l TDZ treatment had 7.56 axillary stem segments formation having 6.21 mm diameter of microtuber weighing 150.10 mg fresh weight. Microtuber formation rate was 92.55 % and there were 3.82 microtubers per plant. Formation and development of microtubers was lowest at 2.0 mg/l and highest at 1.5 mg/l TDZ concentration. At 1.5 ppm TDZ concentration, microtubers cropped from *in vitro* continuous immersion liquid culture system were bigger and heavier than phytagel solid media.

Key words: tuberization, liquid culture, micropropagation, potato, *Solanum Tuberosum* L.

1. Introduction

The potato (*Solanum tuberosum* L.) belongs to the family Solanaceae. It is assumed that tuber-bearing solanum species were first domesticated [11]. Potato is an annual crop plant, and vegetatively propagated through tubers. Potato is one of the most important agricultural plants all over the world. The crop is grown under different agro-ecological zones. Different pathogens such as fungal (for example *Phytophthora infestans*), bacterial, viral, nematode, wilt diseases, .. etc are also common and affect different parts of the potato plant. If the seed stock is not maintained well or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3 - 4 successive crop seasons resulting in almost half or one third yields [16].
Potato is propagated by tubers to preserve the parental properties and also because the seed setting is usually very poor. The importation of high cost certified seed tubers could become a major constraint to potato production. For example importation of certified seed tubers accounts for 40

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to 60% of the total production costs of a potato crop [27]. In potato, microtuber production with through conventional methods are limitations from the components of the culture environment and to the low photosynthetic ability of the explants or plantlets [25]. Tissue culture techniques are economically feasible to get better quality of crop propagules such as potato which are susceptible to virus and other pathogens. *In vitro* meristem culture in potato is an established possible method of eliminating viruses and micropropagation [26; 7]. Workers separate the newly formed buds and shoots by hand and reculture them to produce more buds. Thus, both shoot and microtuber production systems are still less competitive and economical when compared with *in vitro* rapid multiplication. In *in vitro* propagated potato plantlets and microtuber is largely applied both in laboratory experiments and in commercial potato microtuber production. Stem cuttings from *in vitro* produced plantlets or produced from *in vitro* derived plantlets are also used *in vitro* produced microtubers [20]. An *in vitro* mass-tuberization procedure which was reported at the many researchers have described techniques for mass microtuber propagation from stem cuttings, stem segments and microtubers and its potential use for potato seed tuber production [21; 28]. To develop an *in vitro* mass microtuberization methods for potato, microtubers became advantageous. Stem shoot layering, which enables stem segments and microtuber formation in a nutrient medium solution with the hydroponic layer culture method were reported by some researchers [3; 9; 21]. Leclerc et al. [17] reported that after 56-day under an inductive environment the microtuber weight from layered shoots was 3-5 times greater than for nodal cuttings.

Automated micropropagated culture systems that could increase the volume and decrease the unit cost. In order to improve tuber quality and number of microtubers produced per plant several semi-automated systems have been developed, based on submersion as well as bioreactors, without and with forced ventilation [9; 23; 29]. Several reports have evaluated the effects of continuous submersion culture systems using bioreactor fermenter techniques on microtuberization [22; 24]. A semi-automated systems (temporary immersion systems) also allow the reduction of intensive manual handling and hence increase productivity and decrease the costs of production [5]. Akita and Takayama [2] suggested that the culture efficiency can be improved by continuous submersion of shoots at a scale-up culture using jar fermenter techniques. They reported that shoots were cultured under the semi-continuous medium in which the medium surface level was raised or lowered throughout the culture period. Tubers were induced and developed in every area in this jar fermenter system. The adaptation of bubble column, EFBR, and temporary immersion bioreactors for propagation of budclusters and shoots has provided a workable means for improving the number of tubers per plant and tuber quality [23]. Some researchers reported that potato microtubers could be propagated efficiently by using an EFBR system with a culture vessel in 18 weeks [1; 12]. McCown and Joyce [29] reported that a similar method as EFBR type of culture system in which a slow rotating cylinder was employed has been used for mass propagation of potato microtubers. Rapid stem segments proliferation from excised stem shoot tips enhanced by support of air-lift, ebb and flow-type bioreactor (EFBR) and flood system. The increased leaf surface area available for the perception of the photoperiodic stimuli and surface area in contact with the medium may have contributed to the increased microtuber production. *In vitro* continuous immersion liquid culture system is a plant culture technique, which enables plant growth in a nutrient medium solution with the glass wool layer as substrate support of ebb and flood system. Application of continuous immersion culture technique can be considered as an alternative approach for large-scale potato microtuber and stem segments production of some desired and valuable cultivars in *in vitro* conditions [12].

Work on microtuberization in potato has mainly focused on the use of phytohormone [4; 10; 14; 21]. Although growth regulator free MS medium has been used satisfactorily in supporting repeated subculturing and development of nodal explants of potato [13] but some researchers [3] found that the multiplication potential of axillary buds declined when no auxin was added at the subcultures. This loss in regeneration competence could be eliminated by enriching the medium with some auxin and cytokinin. The level of endogenous cytokinins was high in the induced tissue and also during the later stage of tuber growth. No tuber initiation in cultures without cytokinin in the medium was observed. Gopal et al. [8] reported that under short-day and low temperature conditions the addition of BA increased microtuber yield and average microtuber weight. Liljana et al. [18] reported that sprouts and nodal explants of potato, cultivars Agrija and Andrea, were cultured on MS medium, the addition of 4 mg/l Kin for sprouts as an initial explants and 4mg/l Kin + 1mg/l IAA and MS + 2 mg/l BAP + 1 mg/l NAA for nodal explants were increased microtuber formation and growth of potato on *in vitro*. Also the cultivar Agrija showed greater ability for *in vitro* propagation, with 2.14 tubers per shoot and 13.33% microtuber formation.
Improvements in the stem segments and microtuber production from stem segments are still required since most current systems have problems obtaining sufficiently large microtubers for planting materials (seed potato micro-macrotuber) and; a hormone-free or with hormone medium is also desirable to avoid off-types in the progeny of micro-macrotubers as a result of culture-induced genetic variants. In this paper, aspects of potato microtuber formation and stem segments proliferation (axillary stem segments formation) in relation to effects of different doses of Thidiazuron (TDZ) and Indole-3-butyric acid (IBA) and, a novel model version system of a low-cost, *in vitro* continuous immersion liquid culture system (continuous immersion culture system) constructed by using a simple cylindered transparent plexiglass capped bioreactor consisting of glass wool + filter paper layer as substrate was investigated. To further optimize the performance of the proposed continuous immersion culture system used as carrier liquid medium were also tested.

2. Materials and Method

Potato tubers of Granola cultivar were washed several times with detergent followed by several times rinsing with distilled water, dried and placed in dark room for one month untill sprouting started. The one-week old sprouts were surface sterilized by treating with 0.5% acaricide, 2 drop Tween-20 for 10 minutes was rinsed with tap water. it was washed for 30-second with 70% alcohol, for 10 minutes, later it was washed with 10% commercial sodium hypochlorite (NaOCl) and later it was 5 times rinsed with autoclaved distilled water for 4 minutes.
In general, there are two basic steps in microtubers propagation. The first step aims to produce vegetative growth and the second stage to induce microtuberization and allow enlargement of the microtuberlets. But this work, in order to obtain the plant material three steps were followed:
Fist step (Meristem culture): For meristem culture of potato 'Granola', explants derived from stems cutting of 3 cm of potato tubers one-week old sprouts which had been cultured on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l gibberellic acid (GA_3), 10 mg/l putrescine, 0.5 g/l activated charcoal, 30 g/l sucrose with 3.5 g/l phytagel (gelrite) without phtohormon in test tubes (one explants and 10 ml media per tube) *in vitro* were used. The pH of the medium was adjusted to 5.0 before autoclaving (at 121°C and 1.2 kg cm^{-2} pressure for 20 min).

Second step (microshout propagation): After six weeks of meristem culture, single node cuttings were cut aseptically from *in vitro* shoots from growing of meristems at first step and then stem cuttings cultured on liquid 1/2MS medium supplemented [21] with 1 mg/l GA_3, 1 mg/l NAA, 5 mg/l BAP, 50 mg/l coumarine, 0.5 g/l activated charcoal, 30 g/l sucrose and antibiotic in Meganta box (50 stem cuttings and 15 ml media per Meganta vessel) were obtained. In first and second steps, the stem-nodal segment cuttings were inoculated in cylindrical bioreactor and in Meganta vessel for multiplication of the stock stem segments for four weeks.

Third step (shoot proliferation and microtuber formation): After six-week of culture at the second step, single node cuttings were cut at aseptically conditions from *in vitro* stem segments and, 1.5 cm length single node cuttings (nodal explants with one leaf) were used for the explants at this experiment. Explants were placed on solid media and on glass wool + filter paper layer with liquid media. In this experiment, single node cuttings were excised from *in vitro* stem segments derived from 3 cm. Explants were cultured on 1/2 MS medium supplemented with 100 mg/l inositol, 2 mg/l GA_3, 10 mg/l paclobutrazol, 0.5 g/l activated charcoal, 100 g/l sucrose with or without 3.5 g/l phytagel; and different doses of TDZ (0.5, 1.0, 1.5 and 2.0 mg/l) and IBA (0.5, 1.0, 1.5 and 2.0 mg/l) *in vitro*. In all experiments for testing the effects of TDZ , IBA doses, *in vitro* continuous immersion liquid culture media (continuous immersion culture system) and phytagel, solid media were used for the shoot and root proliferation, microtuber formation and their morphological characteristics.

The *in vitro* continuous immersion culture system [15] used in this study consists of: (1) a cylindered transparent plexiglass capped bioreactor used as culture vessel; served as a medium container (glass wool+filter paper layer as substrate) for potato plantlets' development helping fix the carrier sheets and keep it above the liquid medium level. *In vitro* continuous immersion culture system in cylindrical bioreactor were set up by: (a) containing 2500 ml of water box in the cylindered transparent plexiglass bioreactor; (b) one-story stainless fine sieve were placed at the bottom sheet of the inside of bioreactor; (c) the capillary glass wool + filter paper layer of the inside of bioreactor was finally placed at the upper sheet of the inside of bioreactor from the top to the bottom through a hole at the edge of each story; (d) 150 stem-nodal segment cuttings (1.5 cm in length) were placed on this story to provide that substrate (150 ml media per bioreactor) the necessary nutrient for development of stem-nodal segment per cuttings in bioreactor. To finish, the cylindrical bioreactor were capped to keep the system in the aseptic condition and then were placed at the growth room. The Liquid medium was distributed to the carrier *in vitro* continuous immersion (ebb and flood) with supporting net liquid culture system containing glass wool + filter paper layer as substrate. The volume of input air was adjusted to 0.1 vvm (air volume/culture volume, min). The ebb and flood system was programmed to immerse the plantlets in the medium 80 times per day and 2 min each time. The 3.5 g/l phytagel was added in control treatment in which explants were cultivated in solid media.

The pH of media were adjusted to 5.0 at liquid and solid medium, prior to the addition of phytagel. The pH of the system was controlled every 7 days to examine the stability of the liquid system. At the continuous immersion culture system, media were distributed on *in vitro* continuous immersion (ebb and flood) supporting net culture system with liquid media in aseptic condition after autoclaving at 121°C and 1.2 kg cm^{-2} pressure for 45 min. The parts of bioreactor were sterilized separately and culture system were set up under aseptic conditions.

The explants were incubated under 5000 lux lighting with flouressent LED lamps, 20-22°C temperature and 70-80% relative humidity *in vitro* in a growth room. After 90-day (21-day 16h/8h

photoperiod + 42-day in a dark room + 27-day 16h/8h photoperiod application) data were recorded for shoot proliferation (shoot height, node number), root proliferation (root length), number of axillary stem segments formation, diameter of microtuber, average microtuber fresh weight, microtuber formation rate (microtuber diameter >3 mm and microtuber number per plant. The experiment was conducted in a factorial experiment based on completely randomized design with three replications. Statistical analysis, experiments were repeated three times with bioreactors (150 stem cuttings per bioreactor) and with Meganta culture vessels (50 stem cuttings per Meganta) per treatment. Data obtained from this study were analyzed by using SPSS software Ver.16. The means of treatments were compared by using Duncan's multiple range tests at 5% probably level (Duncan, 1995).

3. Results

Continuous immersion culture system gave better results than phytagel solid media in terms of shoot height, node number, root length and axillary number (Table 1).

Among the hormon doses, 0.5 ppm IBA and 1.5 ppm TDZ doses were the most suitable doses in terms of shoot height, node number, rooth length and axillary number. While IBA treatment gave better results than TDZ in terms of shoot height and root length, TDZ gave better results than IBA treatment in terms of node number and axillary number (Table 1). While 0.5 ppm IBA treatment gave better results than 1.5 ppm TDZ in terms of shoot height and root length, TDZ gave better results than IBA treatment in terms of node number and axillary number. IBA doses used significantly affected and showed differences in terms of the shoot height and root length. At the 0.5 IBA dose had significantly shoot height and root length than the control. Shoot height, node number, root length and axillary number decreased as IBA doses increased. Since a high 2 ppm TDZ dose is not desirable in the micropropagation of potato. Because; shoot height, node number, root length and axillary number increased until the dose of 2 ppm TDZ after which they decreased (Table 1).

The highest shoot height was obtained from 1.5 ppm TDZ dose in continuous immersion culture system (7.30) than in the phytagel solid media (6.26) while (continuous immersion culture system 3.53, phytagel solid media 3.01 in the control . The highest shoot height was obtained (7.91 cm) from 0.5 ppm IBA dose, but the shoot height in the control treatment was 5.18 cm in the continuous immersion culture system (Table 1). These results are supported by Liljana et al. (2012). The highest node number was obtained from 1.5 ppm TDZ dose in continuous immersion culture system (7.87) than in the phytagel solid media (7.75) while continuous immersion culture system 4.11, phytagel solid media 3.81 in the control. The data suggest that TDZ 1.5 ppm treatment and IBA 0.5 ppm is suitable for the micropropagation of potato (Table 1). These results are supported by Etienne and Berthouly [5] and Yu et al. [29]. At the 0.5 ppm IBA dose, the highest node number was obtained from in the continuous immersion culture system (7.36) than in phytagel solid media (5.39) (Table 1). These results are supported by Badoni and Chauhan [3]. The highest number of axillary stem segments formation was obtained from 1.5 ppm TDZ dose in continuous immersion culture system (7.56) than in the phytagel solid media (6.67). As for IBA, the number of axillary stem segments formation was obtained from 0.5 ppm IBA dose in the continuous immersion culture system (4.76) than in the phytagel solid media (3.91) (Tablo 1).

Continuous immersion culture system gave better results than phytagel solid media in terms of microtuber diameter, average microtuber fresh weight and average microtuber number per plant,

however phytagel solid media gave better results than continuous immersion culture system in terms of average microtuber formation rate (microtuber diameter > 3 mm; Table 2).

TDZ treatment gave better results than IBA treatment in terms of diameter of microtuber, microtuber formation rate and microtuber number per plant, however IBA treatment gave better results than TDZ treatment in terms of average microtuber fresh weight (Table 2).

1.5 ppm TDZ and 0.5 ppm IBA doses were most suitable doses in terms of diameter of microtuber, microtuber formation rate and microtuber number per plant, however 0.5 ppm IBA and 1.5 ppm TDZ doses were most suitable doses in terms of average microtuber fresh weight. Among these doses, 1.5 ppm TDZ gave better results in terms of diameter of microtuber, microtuber formation rate ve microtuber number per plant whereas 0.5 ppm IBA gave better results in terms of average microtuber fresh weight (Table 2).

Diameter of microtuber, microtuber formation rate, average microtuber fresh weight and microtuber number per plant decreased as IBA doses increased.

Diameter of microtuber, microtuber formation rate, average microtuber fresh weight, and microtuber number per plant increased until the dose of 2 ppm TDZ after which they decreased (Table 2).

Table 1. Effect of continuous immersion culture system and solid media and, TDZ and IBA concentrations on the on shoot height, node number, and root length of cv. Granola after 30 days in the growing room (Kepenek 2017).

Treatments	**Shoot proliferation**				**Root proliferation**		**Number of axillary stem segmentt formation**	
	Shoot height (cm)		**Node number**		**Root length (cm)**		**Continuous immersion culture system**	**Phytagel solid media**
	Continuous immersion culture system	**Phytagel solid media**	**Continuous immersion culture system**	**Phytagel solid media**	**Continuous immersion culture system**	**Phytagel solid media**		
IBA Treatments (ppm)								
0.0	5,18^{s}±0.2	4,01g±0.6	3,91^{p}±0.5	3,04±0.3	9,12^{c}±0.4	7,69^{d}±0.4	3,65^{e}±0.3	3,01^{e}±0.6
0.5	7,91^{a}±0.2	6,44^{a}±0.5	7,36^{a}±0.1	5,39^{a}±0.4	10,39^{a}±0.4	9,57^{a}±0.5	4,76^{a}±0.6	3,91^{a}±0.4
1.0	7,71^{a}±0.4	6,41^{a}±0.1	6,98^{b}±0.2	5,05^{b}±0.5	9,67^{b}±0.2	8,52^{c}±0.7	4,42^{b}±0.7	3,53^{bc}±0.5
1.5	6,83^{c}±0.5	6,35^{a}±0.2	6,16^{d}±0.3	4,60^{c}±0.2	8,90^{c}±0.6	7,67^{de}±0.8	4,06^{c}±0.4	3,26^{d}±0.2
2.0	5,90^{f}±0.3	5,67^{c}±0.3	5,69^{e}±0.4	4,11^{e}±0.7	8,74^{d}±0.3	7,55^{e}±0.4	3,89^{d}±0.5	3,41^{c}±0.7
	6,70	**5,77**	**6,02**	**4,43**	**9,36**	**8,2**	**4,15**	**3,42**
TDZ Treatments (ppm)								
0.0	3,53^{j}±0.6	3,01^{k}±0.8	4,11^{l}±0.5	3,81^{k}±0.3	6,16^{a}±0.4	5,67^{a}±0.5	4,90^{g}±0.9	3,86^{l}±0.5
0.5	6,91^{ab}±0.7	5,31^{g}±0.4	7,06^{c}±0.4	7,14^{b}±0.4	4,71^{ef}±0.7	4,05^{f}±0.2	7,45^{a}±0.5	6,35^{ab}±0.3
1.0	6,52^{c}±0.5	6,49^{d}±0.7	6,72^{c}±0.8	7,58^{a}±0.7	4,61^{f}±0.3	4,10^{f}±0.3	6,97^{b}±0.8	6,14^{b}±0.5
1.5	7,30^{a}±0.5	6,26^{d}±0.3	7,87^{a}±0.5	7,75^{a}±0.5	5,86^{a}±0.8	4,82^{c}±0.8	7,56^{a}±0.4	6,67^{a}±0.4
2.0	5,40^{f}±0.5	7,81^{a}±0.9	6,15^{e}±0.4	6,36^{d}±0.4	5,45^{c}±0.7	3,16^{l}±0.7	6,32^{d}±0.3	5,10^{e}±0.6
	5,93	**5,77**	**6,38**	**6,52**	**5,35**	**4,36**	**6,64**	**5,62**

Different letters within a column indicate significant difference at a = 0.05 according to Duncan's multiple range tests.

Table 2. Effect of continuous immersion culture system and solid media and, TDZ and IBA concentrations on the microtuber formation and their morphological characteristics of cv. Granola after 30 days in the growing room (Kepenek 2017).

Treatments	**Diameter of microtuber (mm)**		**Average microtuber fresh weight (mg)**		**Microtuber formation rate (%) (Microtuber diameter > 3 mm)**		**Microtuber number per plant**	
	Continuous immersion culture system	**Phytagel solid media**	**Continuous immersion culture system**	**Phytagel solid media**	**Continuous immersion culture system**	**Phytagel solid media**	**Continuous immersion culture system**	**Phytagel solid media**
TDZ Treatments (ppm)								
0.0	5,48^{c}±1.6	4,03^{b}±1.3	132,09^{c}±0.4	114,80^{b}±0.2	86,45^{b}±1.6	89,83^{b}±1.4	1,95^{k}±0.7	1,12^{l}±0.7
0.5	6,18^{a}±1.2	4,1^{ab}±1.4	143,86^{a}±0.5	122,07^{ab}±0.4	90,14^{ab}±1.3	93,17^{ab}±1.1	3,73^{ab}±0.7	2,31^{e}±0.9
1.0	6,03^{a}±1.3	4,20^{a}±1.5	144,98^{a}±0.4	123,13^{a}±0.7	92,46^{a}±1.2	96,03^{a}±1.3	3,54^{b}±0.5	2,82^{b}±0.6
1.5	6,21^{a}±1.4	4,27^{a}±1.7	150,10^{a}±0.1	127,09^{a}±0.1	92,55^{a}±1.4	96,08^{a}±1.2	3,82^{a}±0.9	2,97^{a}±0.6
2.0	6,08^{a}±1.1	4,02^{b}±1.4	142,46^{a}±0.5	118,94^{b}±0.6	91,03^{a}±1.6	94,92^{a}±1.5	2,97^{e}±0.6	2,19^{f}±0.8
	5,99	**4,12**	**142,69**	**121,20**	**90,52**	**94,00**	**3,20**	**2,28**
IBA Treatments (ppm)								
0.0	4,85^{g}±1.3	3,69^{f}±1.2	133,41^{c}±0.5	111,60^{c}±0.4	78,27^{b}±1.3	85,54^{b}±1.6	1,45^{f}±0.6	1,09^{e}±0.5
0.5	7,21^{a}±1.3	5,02^{a}±1.2	155,74^{a}±0.1	131,38 ^{a}±0.5	84,66^{a}±0.7	91,03^{a}±0.5	2,06^{a}±0.9	1,43^{a}±0.6
1.0	6,59^{b}±1.4	4,45^{c}±1.2	148,72^{ab}±0.5	130,29 ^{a}±0.3	81,17^{ab}±1.6	87,48^{ab}±1.2	1,83^{c}±0.6	1,11^{e}±0.8
1.5	6,05^{d}±1.1	4,77^{a}±1.3	143,64^{b}±0.2	129,46^{a} ±0.3	79,13^{b}±1.4	82,93^{bc}±1.2	1,71^{d}±0.8	1,09^{e}±0.9
2.0	5,16^{f}±1.4	4,10^{d}±1.7	137,80^{c}±0.5	121,74^{b}±0.5	77,16^{b}±1.8	79,61^{c}±1.9	1,16^{i}±0.7	1,03^{f}±0.5
	5,97	**4,40**	**143,86**	**124,89**	**80,07**	**85,31**	**1,64**	**1,15**

Different letters within a column indicate significant difference at a = 0.05 according to Duncan's multiple range tests.

4. Conclusions

An *in vitro* micropropagation system was developed for apical shoot tips explant of potato. Adventitious shoots were successfully produced on potato cultivar in the continuous immersion culture system: 1.5 ppmTDZ. TDZ was more effective than IBA for shoot proliferation *in vitro* from potato Granola cultivar. The root induction in presence of IBA in the culture media. However it needs further research in order to increase regeneration. We described a method for micropropagation of potato, by using various TDZ and IBA doses, and different culture systems (continuous immersion culture system and phytagel solid media) in *in vitro* conditions. If successfully applied, this methodology can lead to the masspropapatin of potato cultivars within a relatively short period of time.

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