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The Effects of Plant Growth Regulators and Explant Types on Callus Induction and Shoot Regeneration In *Tulipa Gesneriana*

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Abstract : (genus *Tulipa*), genus of about 100 species in the Liliaceae family, which are cultured in temperate regions for their showy blooms., tulips are among the most popular of all garden flowers, numerous cultivars and varieties have been developed. The aim of the study is to determine the effect of growth regulators on the amount of callus produced using the technique of plant tissue culture. Callus was induced from the tulip plant using a combination of thidiazuron (TDZ) and benzyladenine (BA) and auxin 2,4-dichlorophenoxyacetic acid (2,4-D) naphthaleneaceticدد acid (NAA). The (seeds) were sterilised with 4% sodium hypochlorite (NaOCI) and sterile distilled water in a 1:1 ratio for 10 minutes. Different parts of the seedling leaf, flowering stem were cultured on MS medium together with a combination of hormones BA and 2,4-D, NAA and TDZ at different concentrations, and significant differences were observed after four weeks of cultivation. The best combinations of plant growth regulators for callus induction rate of 90% from the flowering stem were at a combination of 1.5 TDZ and 1.5 2,4-D mg/L, while the highest number of shoots was achieved with 12 shoots in the media BA 2.0 mg/ L and IBA 0.1 mg/L.

Keywords: Auxin, Callus Induction, Plant Tissue Culture,Thidiazuron ,Tulip.

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Introduction

The (Tulip) *Tulipa Gesneriana* plant belongs to the Liliaceae family. Its origin country is Turkey. It is also called the turban flower because it consists of several layers of colored petals (1, 2). The cultivation of these flowers moved to Europe 400 years ago and spread in it in a very large way, and it found special care in its cultivation, especially in the Netherlands, which has become a symbol and a blanket for her great income. It produces a billion flowers annually and is exported to all countries of the world. The genus of tulips includes about 120 species, some of which are perennial and others are perennial or winter (3). The tulip regeneration methods discussed in existing literature weren't very effective. During the 1980s, 1990s, and early 2000s, a lot of research was done on directly regenerating shoots or micro bulbs from initial plant parts like bulb scales, buds, and flower stems.

Among these, flower stems showed the most potential for regeneration (4,5). To speed up multiplication, using plant tissue culture for *in vitro* propagation is a key alternative to traditional methods. Micropropagation through shoot culture is commonly utilized to uphold clonal fidelity (6, 7). The tulip plant is vegetative propagation by bulb, the number of tulip produced from one bulb is limited when propagated by traditional method (2, 3 bulb) (8). One of the most well-known methods, plant tissue culture has been used to create healthy, diseasefree plants with the right quality at a faster rate of production throughout the year, regardless of the season. Every plant has a unique shape and set of dietary needs. Furthermore, the tissues in each section of the plant have different nutritional needs (9). The technique of tissue culture has made it possible to multiply and genetically enhance economically useful plants. Under controlled conditions, tissue culture has been utilized to research aspects of plant physiology, development, metabolism, reproduction, and nutritional requirements. With little plant tissue, the plantlets are created in a relatively short period of time. The growth of many plants in the absence of seeds or necessary pollinators for the formation of seeds (10). Propagation of plants using the tissue culture technique is also one of the scientific applications of this technique due to its unique characteristics over traditional methods . (11,12). The response and rate of callus regeneration in wheat plants are influenced by several factors, most notably the nutritional composition of the culture medium, as various nutrients play an important role in regulating the processes of cell differentiation and regeneration.(13) Auxins and cytokinins are among the most widely used regulators for this purpose. (14). Induction of callus allows the researcher to obtain large numbers of cells in small space like a Petri dish under controlled conditions, without the interference of the rest environmental (15). To grow explants successfully requires high-quality and dependable culture media with the right nutrients in the perfect balance for optimal growth (16). To induce organ formation, cytokinins, important for cell division and differentiation, are required. Thidiazuron, a cytokinin-like compound, is a potent growth regulator used for this purpose. Additionally, auxins are added to the culture media to facilitate organ formation (5,17). In the present study, a protocol for obtaining callus by *in vitro* technique was developed for tulip (*Tulipa gesneriana*)., and shoot regeneration from this callus.

Materials and Methods

The study was conducted at the Plant Tissue Culture Laboratory at the College of science for women/ University of Baghdad, the tulip seed obtained from nursery of Istanbul / turkey.

The seed were surface sterilization with 4% sodium hypochlorite for 10 min., seed washed of with sterile distilled water and culture on MS Media (18). Free of growth regulator. Culture subjected to photoperiod 16/8 hours (light/dark) in growth chamber, temperature was set at 25°C. Germination was measured after 14 days. After germination, inter node, flowering stem of tulip were cut at the end into section approximately 0.5 cm in length under aseptic condition and cultured on MS Medium supplemented with TDZ (0,0.5,1,1.5) mg/L, 2,4-D (0,0.5,1,1.5) mg/L, proline 5mg/L and BA (0,0.5,1,1.5) mg/L each treatment in the experiment was 10 replicates. The measurement was included: the percentage of response to callus induction. Shoot regeneration and multiplication medium: Explants which have been planted in initiation media for 1-2 weeks were transferred into regeneration and shoot multiplication media.

The regeneration and multiplication medium were used with MS basal media supplemented different range of BA (0,0 1.0, 2.0, 3.0 mg/L) combined with IBA (0.0, 0.1, 0.2, 0.3 mg/L).

Experimental design and statistical analysis

The Statistical Analysis System-SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means in this study (19).

Results and Discussion

Seed germination

The sterilized seed of tulip were seedling was fully germinated in range 3-4 weeks. (Figure 1)

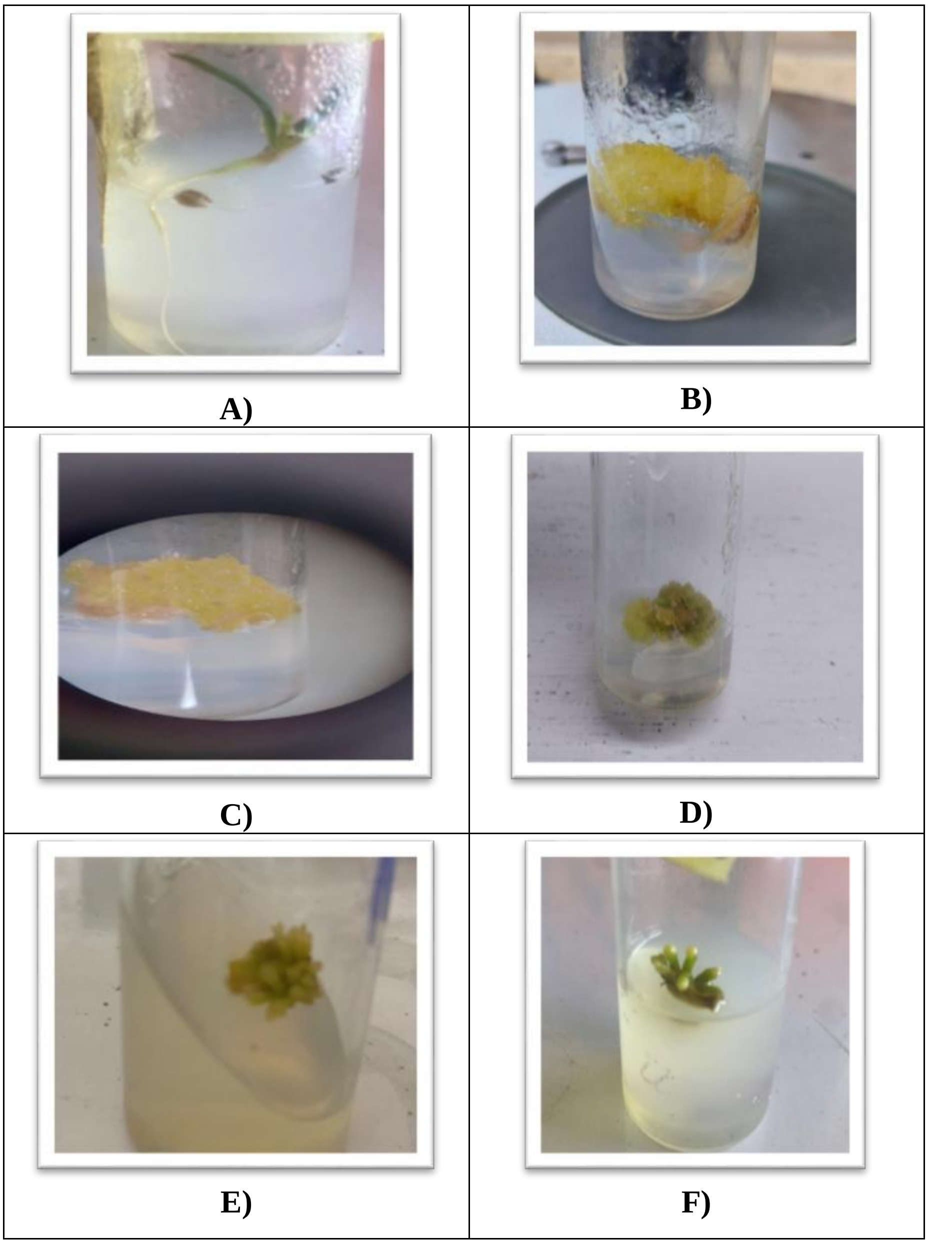

Figure (1): (A) Seedling of tulip grown on MS medium free hormone after 25 days the highest response to callus induction from different explants of seedling with various concentration of hormones, (B) flowering plant stem (1.5mg/L TDZ and 1.5mg/L 2,4-D), (C) leaf (1.5mg/L BA and 1.5mg/L 2,4D), (D) greenish callus from flowering stem on MS media with 0.5 mg/L 2,4D and 1.5 mg/L TDZ, (E) Shoot Regeneration from callus after 3 weeks, (F) regeneration. after subculture on the same media for shoot regeneration.

Callus induction

Callus initiation from flowering stem occurred much faster than leave explant. Callus proliferation from flowering stem was also more extensive and scoring was possible after 4 weeks.

In this stage the, flowering stem, leaf was used from seedling as explant for callus induction. the explant were used and cultivated on MS media contain BA, 2,4-D, NAA and TDZ at a various concentration (0, 0.5,1.5) mg/L, after 4 weeks of culture .

In vitro callus cultures of *T. gesneriana* were initiated from flower stem slices on MS media, which contained different concentrations of auxins and cytokinins. Increase in auxin concentrations, especially 2,4-D, cause dan intensive enlargement of explant, After 4 weeks of culture callus developed. The callus color and structure varied depending on the growth regulators used. Explants cultured on media containing 2,4-D (1.5 mg/L) with addition of TDZ (1.5 mg/L) gave rise to nodular callus, which was yellow and looser structure as seen in picture B and C, whereas the picture D show the greenish callus developed on media enriched in lower 2,4-D concentration and high TDZ. Effect of AB and 2,4-D (mg/L) and interaction between them on the percentage callus induction from the flower stem after 30 days of culture the results in (table 1) the addition of 1.5 mg/L BA and 1.5 mg/L 2,4-D was the highest response to callus induction was 80% compared to control which gave any response to callus induction and the lower response when add only BA 1.5 mg/L was 10%, this result due to Due to the hormonal balance between auxin and cytokines, it gives the best response rate to callus induction (6, 15). Table (2) show the effect of AB and 2,4-D and interaction between them on the percentage callus induction from the leaf after 4 weeks of culture , the addition of 1.5 mg/L BA and 1.5 mg/L 2,4-D was the highest response to callus induction was 50% compared to other, while the control treatment give no response to callus induction, due to the lack of auxin and cytokines necessary for callus induction and the lower response was addition only BA 0.5 mg/L and 1 mg/L was 10% it is well known that cytokinins especially BA has many positive roles in enhance tissue culture by the increase the levels of cell division , growth and differentiation (20). In Table 3 and 4 seen the effect of TDZ and 2,4-D (mg/L) and interaction between them on the percentage callus induction from the flower stem and leave after 4 weeks, addition 1.5 mg/L TDZ and 1.5 mg/L 2,4-D was the highest response to callus induction was 90% for flowering stem compared to high percentage callus induced from leave which gave 40%, while the control treatment give no response to callus induction and the lower response was addition only TDZ 0.5 mg/L and 1 mg/L was 10% for two explants.

Table (1): Effect of AB and 2,4-D (mg/L) and interaction between them on the percentage callus induction from the flower stem after 4 weeks of culture.

BA mg/L / 2,4-D mg/L	0	0.5	1	1.5	Mean
0	0	30	20	10	15.0
0.5	30	30	30	20	27.5
1	30	40	50	50	42.5
1.5	40	40	50	80	52.5
Mean	25.0	35.0	37.5	40.0	---
LSD value: 2,4-D: 6.49 * , BA: 6.49 * , 2,4-D x BA: 10.95 * .					

Table (2): Effect of AB and 2,4-D (mg/L) and interaction between them on the percentage callus induction from the leaf after 4 weeks of culture.

BA / 2,4-D	0	0.5	1	1.5	Mean
0	0	10	10	10	7.5
0.5	10	20	30	20	20.0
1	20	20	30	20	22.5
1.5	20	30	30	50	32.5
Mean	12.5	20.0	25.0	25.0	---
LSD value: 2,4-D: 7.41 * , BA: 7.41 * , 2,4-D x BA: 12.63 * .					

Table (3): Effect of 2,4-D and TDZ (mg/L) and interaction between them on the percentage callus induction from the Flowering plant stem after 4 weeks of culture.

TDZ / 2,4-D	0	0.5	1	1.5	Mean
0	0	10	10	20	10.0
0.5	20	30	40	40	32.5
1	20	60	60	70	52.5
1.5	30	60	70	90	62.5
Mean	17.5	40.0	45.0	55.0	---
LSD value: 2,4-D: 6.91 * , TDZ: 6.91 * , 2,4-D x TDZ: 11.07 * .					

Table (4): Effect of 2,4-D and TDZ (mg/L) and interaction between them on the percentage callus induction from the leaf after 4 weeks of culture.

TDZ / 2,4-D	0	0.5	1	1.5	Mean
0	0	10	0	10	5.0
0.5	20	20	20	30	22.5
1	10	30	40	40	30.0
1.5	10	40	40	40	32.5
Mean	10.0	25.0	25.0	30.0	---
LSD value: 2,4-D: 6.47 * , TDZ: 6.47 * , 2,4-D x TDZ: 10.96 * .					

TDZ concentrations it observed that the imitation of callus formation from leaf explant was delayed when the hormones were used in combination (21).

In another experiments we notice the effect of TDZ and NAA (mg/L) and interaction between them on the percentage callus induction from the flower stem after 4 weeks of culture, the results in (Table 5 and 6) illustrate that addition of (1, 1.5) mg/L TDZ and 1.5 mg/L NAA gave highest response to callus induction 40% compared to leave explant which record 30% percentage in callus induction. TDZ it has been found to increase the regeneration potential of the tulip and to be more efficient than AB in the organogenesis of the gloriosa lily (22) NAA has had positive impact on increase the meristemic areas (23, 17).

Table (5): Effect of NAA and TDZ (mg/L) and interaction between them on the percentage callus induction from the Flowering plant stem after 4 weeks of culture.

TDZ / NAA	0	0.5	1	1.5	Mean
0	0	0	10	10	5.0
0.5	0	20	20	20	15.0
1	10	20	30	30	22.5
1.5	10	30	40	40	30.0
Mean	5.0	17.5	25.0	25.0	---
LSD value: NAA: 5.37 * , TDZ: 5.37 * , NAA x TDZ: 9.84 * .					

Table (6): Effect of NAA and TDZ (mg/L) and interaction between them on the percentage callus induction from the leaves after 4 weeks of culture.

TDZ / NAA	0	0.5	1	1.5	Mean
0	0	0	0	0	0.0
0.5	0	20	10	20	12.5
1	0	10	10	10	7.5
1.5	10	20	20	30	20.0
Mean	2.5	12.5	10.0	15.0	---
LSD value: NAA: 4.81 * , TDZ: 4.81 * , NAA x TDZ: 7.18 * .					

The present study reports data on the initiation callus from flowering stem and from leaves explants. The results show that the type of explant and plant growth regulators affected the frequency of callus induction, callus proliferation, the initiation of different callus types. In Tulipa, callus initiation was observed on MS medium with different combinations of growth regulators; i.e., MS with NAA and BA, 2,4-D and kinetin, 2,4D and BA (24, 25). In the present study, callus initiation was observed on MS medium with 2,4-D or NAA with TDZ. Initiation from flowering stem and leaf segments, was observed on medium with 2,4-D or NAA in different percent of induction callus. The results also indicated that callus induction and the initiation observed on a basic MS medium with an auxin in combination with or without a cytokinin (13, 26).

Shoot regeneration and multiplication

The highest number of shoots per cluster (16) was obtained on the MS medium supplemented by using BA 1.0 mg/L and IBA 0.1 mg/L. The lowest number of shoots (14) was achieved on the MS medium supplemented by 10 mg/L BA and IBA 0.1 mg/L as appeared in table (7). It is worth noting that, replacing the cytokinin concentrations 1.0 with 2.0 resulted in an almost twice increase in the number of shoots obtained. The obtained results concerning the effects of selected PGRs added to the MS medium on the in vitro shoot multiplication of the tulip indicate that the optimal medium is a medium enriched with BA 1.0 mg/L in combination with auxin IBA 0.1 mg/L. This may be linked to the composition of endogenous growth regulators (9). Mention for the tulips ‘Recreado’ and ‘Christmas Marvel’, IBA has been proven to be the auxin with the best effect on shoot proliferation, but only when it interacted with cytokinin BA, while (28) found that Plant regeneration was induced by transferring the callus to MS solid medium containing 5µ M BA and 0.5 µM NAA

Table (7): Effect of BA and IBA on shoot regeneration and multiplication.

BA \ IBA	0	0.1	0.2	0.3	Mean
0	0	0	0	0	0.00
1	0	5	5	7	4.25
2	0	12	10	10	8.00
3	0	8	9	8	6.25
Mean	0.00	6.25	6.00	6.25	---
L.S.D. value	BA: 2.942 * , IBA: 2.942 * , BA x IBA: 5.177 * .				

Conclusion

This study demonstrated that the combination and concentration of plant growth regulators significantly influence callus induction and shoot formation in tulip plant tissue culture. The highest callus induction rate (90%) was achieved from the flowering stem explants using TDZ and 2,4-D. Additionally, the optimal shoot regeneration (12 shoots) occurred in the medium supplemented with BA and. These findings highlight the effectiveness of specific growth regulator combinations in enhancing in vitro propagation of tulips

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