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# Tulip propagation *in vitro* from vegetative bud explants

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**Abstract:** *Tulip propagation in vitro from vegetative bud explants.* In this study, authors attempted to regenerate tulip plants through organogenesis from vegetative bud explants – isolated from unchilled bulbs. For this purpose, apical buds were excised from bulbs in the vegetative stage in July, and axillary buds were excised from bulbs in the generative stage in September. Before the isolation of the buds in July, half of the bulbs were subjected to a preparatory treatment at 34°C for one week, while the remaining bulbs were kept at 20°C. The buds were cultured on a full-strength MS medium containing: 5 μM α-naphthaleneacetic acid (NAA) and 5 μM thidiazuron (TDZ). To obtain adventitious bud regeneration, the shoots developed from the apical and axillary buds were then cut into slices of a thickness of 2–3 mm and subcultured on the same medium for 12 weeks at 20°C. The cultures were also chilled at 5°C for another 12 weeks. Some of the apical and axillary shoot explants incubated at 20°C were exposed to gibberelin (2.89 μM $GA_3$). Regardless of the bulb-treatment temperature, the first adventitious shoot-regeneration was significantly more efficient in the case of the apical buds (isolated in July) compared with the axillary buds (excised in September) – even though only 23.4–26.4% of the explants formed shoots. Following the treatment at 5°C, the maximum percentage of explants forming adventitious shoots was 38.8% for the apical buds, which had first been treated at 20°C and then at 5°C.

*Key words*: *Tulipa*, organogenesis, buds, TDZ, NAA

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## INTRODUCTION

The tulip is an important worldwide cut flower and bedding plant [Taghi et al., 2007]. It is commercially propagated through asexual reproduction by using bulbs, but the efficiency of this process is low [Le Nard and De Hertogh, 1993]. Research using *in vitro* methods to investigate efficient multiplication rates in the tulip has been in progress for many years. Unfortunately, the laboratory techniques employed in its propagation continue to produce low yields [Wilmink et al., 1995, Podwyszyńska and Marasek, 2003, Ptak and Bach, 2007, Maślanka and Bach, 2010].

Organogenesis is a type of plant regeneration that can be used in clonal propagation. In bulbous plants this micropropagation method results in the formation of adventitious shoots or bulbs [Hulscher et al., 1992, Wilmink et al., 1995, Podwyszyńska and Marasek, 2003, Ghaffor et al., 2004]. The organs are formed directly on explants or indirectly via callus tissue [Taghi et al., 2007, Liu and Yang, 2012]. Among geophytes, organogenesis can occur on various explants, including on buds [Sen and Sen, 1995, Arumugan and Gopinath, 2012, Ghaffor et al., 2004]. *In vitro* cultures of the tulip have been initiated

mainly from chilled bulbs; though some experiments have used non-chilled plant material [Ptak and Bach, 2007]. Cytokinins, which are known to affect cell division and cell differentiation [Mok and Mok, 2001], are necessary to induce organogenesis. Thidiazuron, which is a cytokinin-like substance [Mok et al., 1982, Chin-Yi, 1993], is a highly effective growth regulator in tissue cultures [Murthy et al., 1998]. To induce organogenesis, culture media are also supplemented with auxins. In presented study, authors attempted to regenerate tulip plants through organogenesis from vegetative bud explants (apical and axillary buds), isolated from non-chilled bulbs.

## MATERIAL AND METHODS

The experiments were conducted with the tulip cultivar 'Apeldoorn'. The starting material for the initiation of cultures came from vegetative buds. For this purpose, buds were excised from bulbs in the vegetative stage in July and from bulbs in the generative stage in September. The buds isolated in July were apical and those in September were axillary. Axillary vegetative buds were collected in September because apical vegetative buds differentiated into generative buds during the summer. Before the isolation of buds in July, half of the bulbs were subjected to a preparatory treatment at 34°C for one week, while the remaining bulbs were kept at 20°C. The excised buds were laid out in Petri dishes on a full strength MS medium [Murashige and Skoog, 1962] containing: 5 μM NAA, 5 μM TDZ, 3% saccharose (pH 5.8), solidified with a Biocorp gelling agent (0.5%). Regeneration took place in the dark at 20°C. After 12 weeks of culture the development of single shoots (apical, axillary) was evaluated in terms of the percentage of explants developing shoots, and the length of the shoots.

The apical and axillary shoots thus obtained were subsequently cut into slices of a thickness of 2–3 mm and – to allow the formation of adventitious shoots – placed on the same medium as the apical and axillary shoot explants. After 12 weeks, the shoot explants were subcultured on a medium containing 5 μM NAA and 5 μM TDZ (control) or 5 μM NAA, 5 μM TDZ and 2.89 μM $GA_3$, for a further 12 weeks. Some of the shoot explants in the control group were also chilled for 12 weeks at 5°C. During the experiment, an assessment was made of the degree of formation of adventitious shoots and of the number of shoots per replication.

The experiment was performed in five replications with five explants each. The results were subjected to an analysis of variance. With the aid of the Statistica 9 software package, the mean values were compared using Tukey test at a confidence level of $\alpha = 0.05$.

## RESULTS AND DISCUSSION

Harvested tulip bulbs are usually stored at 20°C. In horticultural practice, bulbs are often prepared, that is, treated at 34°C for one week to control flowering. Subsequently, the tulip bulbs need to be chilled for about 12 weeks at 5°C in the autumn [Le Nard and De Hertogh, 1993].

In presented experiment, all of the bud explants had begun to swell and enlarge their diameter within a few days of the initiation of the culture – after which the buds developed into apical and axillary shoots. The percentage of the bud explants developing shoots was within the range 94–100% (Table 1). It was found that, when applied to the tulip bulbs in the vegetative stage of the tulip, a temperature of 34°C enhanced the development of the shoots. The highest shoot-length, 27.4 mm, was observed in the apical buds (isolated in July), following treatment at 34°C. These shoots were significantly longer than the shoots derived from the other apical and axillary buds (isolated in September). According to Le Nard and De Hertogh [1993], preparatory treatment at 34°C has a positive influence on leaf primordia forming inside the bulbs.

TABLE 1. The effect of the treatment temperature of tulip bulbs and the time of bud isolation on apical and axillary shoot development

| Time of bud isolation | Treatment temperature (°C) | Explants developing shoots (%) | Length of shoots (mm) |
|---|---|---|---|
| July | 34 | 100.0 b* | 27.4 c |
| | 20 | 97.0 ab | 19.2 b |
| September | 20 | 94.0 a | 5.6 a |

* Means marked with the same letter within columns do not differ significantly at the significance level of $\alpha = 0.05$.

The apical and axillary shoot explants, which were incubated on the same medium, had formed adventitious shoots within four weeks of culture. At the end of culture, the maximum percentage of explants forming adventitious shoots was 26.4% (Table 2). Irrespective of the treatment temperature, the apical shoot explants proved to be more efficient (2.2–2.8 shoots) than the axillary shoot explants (0.3 shoots). Callus tissue had appeared on the surface of some axillary shoot explants (17.2%) derived from the buds isolated in September. Having been separated and laid out on the initial medium, the callus hardly proliferated, forming only single adventitious shoots (data not shown).

TABLE 2. The post-treatment effect of the temperature treatment and time of bud isolation on adventitious shoot formation from apical and axillary shoot explants

| Time of bud isolation | Treatment temperature (°C) | Explants forming adventitious shoots (%) | Number of adventitious shoots (pcs) |
|---|---|---|---|
| July | 34 | 23.4 b* | 2.8 b |
| | 20 | 26.4 b | 2.2 b |
| September | 20 | 4.5 a | 0.3 a |

* Means marked with the same letter within columns do not differ significantly at the significance level $\alpha = 0.05$.

The type of growth regulators and their concentration are responsible for the morphogenetic response of explants [Gaspar et al., 2003]. Although it is completely different from them chemically, TDZ can serve as a substitute for cytokinins, and even auxins [Singh et al., 2003, Guo et al., 2011]. It has also been found to increase the regeneration potential of the tulip [Podwyszyńska and Rojek, 2000, Podwyszyńska and Marasek, 2003] and of the garlic [Myers and Simon, 1999], and to be more efficient than BAP in the organogenesis of the gloriosa lily [Kozak, 2006]. NAA, meanwhile, has had a positive impact on

the formation of shoots from the bud explants of tulip [Ghaffor et al., 2004] and has been used as a supplement in an optimum regeneration medium for axillary bud explants of *Dianthus* and *Vanilla* [Miller et al., 1991, George and Ravishankar, 1997]. Finally, shoot multiplication has been obtained in the gloriosa lily under the joint influence of NAA and BAP [Sayeed Hassan and Roy, 2005].

on the formation of adventitious shoots between the control group, $GA_3$ treatment and chilling (Table 3). The time of bud isolation was found to be relevant only in the case of chilled apical and axillary shoot explants. In this case 31.4% and 38.8% (treatment temperatures of 34°C and 20°C respectively) of the apical shoot explants formed adventitious shoots and 5.7% of the axillary shoot

TABLE 3. The effect of $GA_3$ and chilling on adventitious shoot formation from apical and axillary shoot explants, depending on the temperature of the prior treatment and the time of bud isolation

| Time of bud isolation | Treatment temperature (°C) | Explants forming adventitious shoots (%) | | | Number of adventitious shoots (pcs) | | |
|---|---|---|---|---|---|---|---|
| | | Control | $GA_3$ | Chilling | Control | $GA_3$ | Chilling |
| July | 34 | 35.1 b* | 28.2 ab | 31.4 b | 2.9 ab | 2.7 ab | 3.9 b |
| | 20 | 27.5 ab | 20.0 ab | 38.8 b | 2.9 ab | 2.4 ab | 3.6 b |
| September | 20 | 17.7 ab | 14.6 ab | 5.7 a | 1.2 ab | 1.0 ab | 0.4 a |

* Means marked with the same letter within columns and rows do not differ significantly at the significance level $\alpha = 0.05$.

In presented study, as in other experiments [Miller et al., 1991, Wilmink et al., 1995], adventitious shoots appeared on the explants through direct organogenesis, although shoot differentiation via the callus tissue phase has also been observed [Arumugan and Gopinath, 2012].

Due to the low efficiency of the formation of adventitious shoots, the apical and axillary shoot explants were chilled at 5°C or treated with 2.89 μM $GA_3$. Chilling is required for proper growth and flowering in tulips [Rietveld et al., 2000] and $GA_3$ can be used as a partial substitute for cold treatment [Le Nard and De Hertogh, 1993].

Regardless of the temperature treatment beforehand, the results obtained did not reveal any significant influence

explants did the same. Similar observations were made with regard to the number of adventitious shoots. The largest numbers of these (3.6 and 3.9) appeared following chilling of apical shoot explants. Meanwhile, a significantly lower result of 0.4 adventitious shoots was obtained following the chilling of axillary shoot explants.

## CONCLUSIONS

The results indicated that the time of bud isolation had a significant influence on the development of both apical and axillary buds, as well as on the formation of the first adventitious shoots. The lowest number of adventitious shoots (0.3) appeared in the case of axillary shoot explants and the highest (2.2 and 2.8) in

apical shoot explants. After subjecting the shoot explants to chilling, the differences between the results for origins of the buds were also very distinct: the number of adventitious shoots obtained from the axillary shoot explants (0.4) was almost ten times lower when compared with that of the apical shoot explants (3.6 and 3.9). The application of $GA_3$ did not produce significant effects on shoot formation. Presented results demonstrate that there remains a need for further investigation of the culture conditions that will best enhance tulip proliferation and shoot formation.

## REFERENCES


ARUMUGAN A., GOPINATH K. (2012). *In vitro* micropropagation using corm bud explants: an endangered medicinal plant of *Gloriosa superba* L. Asian J. Biotechnol. 4: 120–128.

CHIN-YI L. (1993). The use of thidiazuron in tissue culture. In Vitro Cell. Dev. Biol. Plant 29: 92–96.

GASPAR T.H., KEVERS C., FAIVRE-RAMPANT O., CREVECOEUR M., PENEL C.L., GREPPIN H., GEORGE P.S., RAVISHANKAR G.A. (1997). In vitro multiplication of *Vanilla planifolia* using axillary bud explants. Plant Cell Rep. 16: 490–494.

GHAFFOR A., MAQBOOL I., WASEEM K., QURAISHI A. (2004). In vitro response of tulips (*Tulipa gesnerina* L.) to various growth regulators. Int. J. Agr. Biol. 6: 1168–1169.

GUO B., ABBASI B.H., ZEB A., XU L.L., WEI Y.H. (2011). Thidiazuron: A multi-dimentional plant growth regulator. Afr. J. Biotechnol. 10 (44): 8984–9000.

HULSCHER M., KRIJGSHELD H.T., Van der LINDE P.G. (1992). Propagation of shoots and bulb growth of tulip in vitro. Acta Hortic. 325: 441–446.

KOZAK D. (2006). Wpływ BA i TDZ na regenerację pędów gloriozy (*Gloriosa rothschildiana* O'Brien) *in vitro*. Zesz. Probl. Post. Nauk Rol. 510: 289–294.

LIU X., YANG G. (2012). Adventitious shoot regeneration of oriental lily (*Lilium orientalis*) and genetic stability evaluation based on ISSR marker variation. In Vitro Cell. Dev. Biol. Plant 48(2): 172–179.

MAŚLANKA M., BACH A. (2010). The effect of abscisic acid, ethylene and inhibitors of their biosynthesis (fluridone and salicylic acid) on somatic embryos conversion in tulips. Ecol. Chem. Eng. S. 17: 1135–1139.

MILLER R.M., KAUL V., HUTCHINSON J.F., RICHARDS D. (1991). Adventitious Shoot Regeneration in Carnation (*Dianthus caryophyllus*) from Axillary Bud Explants. *Ann. Bot.* 67: 35–42.

MOK M.C., MOK D.W.S. (2001). Cytokinin metabolism and action. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52: 89-118.

MOK M.C., MOK D.W.S., ARMSTRONG D.J., SHUDO K., ISOGAI Y., OKAMOTO T. (1982). Cytokinin activity of N-phenyl-n'--1,2,3-thiadiazol-5-yl urea (thidiazuron). Phytochem. 21: 1509–1511.

MURASHIGE T., SKOOG F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473–497.

MURTHY B.N.S., MURCH S.J., SAXENA K. PRAVEEN (1998). Thidiazuron: a potent regulator of *in vitro* plant morphogenesis. In Vitro Cell. Dev. Biol. Plant 34: 267–275.

MYERS J.M., SIMON P.W. (1999). Regeneration of garlic callus as affected by clonal variation, plant growth regulators and culture conditions over time. Plant Cell Rep. 19: 32–36.

Le NARD M., De HERTOGH A.A. (1993). Tulipa. In: A.A. De Hertogh, M. Le Nard (Eds) The physiology of Flower Bulbs. Elsevier Science Publishers, The Netherlands Amsterdam – London – New York – Tokyo: 617–682.

PODWYSZYŃSKA M., MARASEK A. (2003). Effects of thidiazuron and paclobutrazol on regeneration potential of tulip flower stalk explants in vitro and subsequent shoot multiplication. Acta Soci. Bot. Pol. 72: 181–190.

PODWYSZYŃSKA M., ROJEK A. (2000). Effect of light quality and darkness on tulip (*Tulipa gesneriana* L.) in vitro regeneration. Zesz. Nauk. ISiK w Skierniewicach 7: 130–137.

PTAK A., BACH A. (2007). Somatic embryogenesis in tulip (*Tulipa gesneriana* L.) flower stem cultures. In Vitro Cell. Dev. Biol. Plant 43: 35–39.

RIETVELD P.L., WILKINSON C., FRANSSEN H.M., BALK P.A., PLAS L.H.W., WEISBEEK P.J., BOER A.D. (2000). Low temperature sensing in tulip (*Tulipa gesneriana* L.) is mediated through an increased response to auxin. J. Exp. Bot. 344: 587–594.

SAYEED HASSAN A.K.M., ROY S.K. (2005). Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. Plant Tissue Cult. 15: 67–74.

SEN J., SEN S. (1995). Two-step bud culture technique for a high frequency regeneration of *Gladiolus* corms. Sci. Hort. 64: 133–138.

SINGH N.D., SAHOO L., SARIN N.B., JAIWAL P.K. (2003). The effect of TDZ on organogenesis and somatic embryogenesis in pigeonpea (*Cajanus cajan* L. Millsp.). Plant Sci. 164: 341–347.

TAGHI Z.M., BABALAR M., ZAMANI Z.A., NADERI R., ASKARI M.A. (2007). Direct and indirect regeneration of adventitious shoots in ornamental *Tulipa gesneriana* L. 'Apeldoorn' by using *in vitro* culture method. Iran J. Agr. Sci. 37(6): 1031–1039.

WILMINK A., VAN DE VEN B.C.E., CUSTERS J.B.M., NÖLEEN Y., DONS J.J.M. (1995). Adventitious shoot formation in tulip: histological analysis and response to selective agents. Plant Sci. 110: 155–164.

**Streszczenie:** *Rozmnażanie tulipana in vitro z pąków wegetatywnych*. W niniejszej pracy opisano próbę mikrorozmnażania tulipana drogą organogenezy z pąków wegetatywnych, wyizolowanych z niechłodzonych cebul. W tym celu pobierano pąki wierzchołkowe w lipcu – z cebul w fazie wegetatywnej, oraz pąki boczne we wrześniu – z cebul w fazie generatywnej. Przed izolacją pąków w lipcu, połowę cebul poddano zabiegowi preparowania – działaniu temperatury 34°C przez jeden tydzień. Pozostałe cebule przechowywane były w 20°C. Pąki wyłożono do szalek Petriego na pożywki MS zawierające 5 μM NAA i 5 μM TDZ. W celu regeneracji pąków przybyszowych, otrzymane z pąków wierzchołkowych i bocznych pędy zostały pocięte na 2–3-milimetrowe fragmenty i wyłożone na tą samą pożywkę, na 12 tygodni w 20°C. Kultury były także poddane chłodzeniu przez kolejne 12 tygodni. Część wierzchołkowych i bocznych eksplantatów pędowych z 20°C poddano działaniu gibereliny (2.89 μM $GA_3$). Niezależnie od działającej na cebule temperatury, istotnie więcej pędów przybyszowych formowały pąki wierzchołkowe izolowane w lipcu, w porównaniu z pąkami bocznymi, izolowanymi we wrześniu, chociaż obserwowano je tylko u 23,4–26,4% eksplantatów. Po zastosowaniu 5°C, maksymalny procent eksplantatów formujących pędy przybyszowe wyniósł 38,8% pąków wierzchołkowych, poddanych najpierw działaniu 20°C, a następnie 5°C.