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Bulbs Surface-Sterilization Protocol for *Tulipa julia* C. Koch from Turkey

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Abstract

Tulipa julia is an ornamental important plant and a wide geographical distribution eastern of Turkey. However, due to habitat loss and illegal over collection in the wild it is included as a vulnerable species. The development of a protocol for *Tulipa julia* bulblet propagation *in vitro* may be useful for reintroducing plants in their natural habitats, and for germplasm conservation. A difficult problem encountered during the establishment of an *in vitro* culture is explants disinfection, especially when working with endangered species, from which explant availability is restricted. Thus, the establishment of a sterilization protocol is crucial for the initiation and success of bulblets micropropagation system for *Tulipa julia*. This study was to evaluate the effect of sodium hypochlorite concentrations and treatments time in bulbs surface disinfection, tissue sensitivity and development. Sodium hypochlorite solutions (2 or 3 %, 20 or 25 min; 4 or 5 %, 30 or 35 min) were effective in eliminating bulbs superficial contaminants. There was significant difference among the effective sterilization sodium hypochlorite concentrations and treatments time in relation to surface sterilization bulbs of *Tulipa julia*. Also, no damage to bulbs tissues were observed. Surface sterilization of bulbs, for initiation of an *in vitro* culture, required higher concentrations of sodium hypochlorite (4 or 5 % NaCl, 30 or 35 min) for controlling fungal and yeast contamination, compared to bulbs sterilization.

Keywords: *Tulipa julia*, surface sterilization, *in vitro* culture.

1. Introduction

Turkey is one of the richest countries in variability of flora, it has more than 10000 plant taxa about 3000 of which are endemic. 'There are about 600 species of flower bulbs in Anatolia' (Arslan et al., 2002) and many of them are known as ornamental and medicinal plants (Atay, 1996). 'A number of these geophytic taxa have been exported from Turkey for a long time' (Arslan et al., 2002). Tulip (*Tulipa* L.) genus is a Monocotyledona and belongs to the Liliaceae Juss. Family and comprises more than 100 species in the world (Hall, 1940). In Turkey, *Tulipa* was divided into two subgenera and they represented in total 19 taxa (Eker et al., 2014). *Tulipa* L. taxa are among significant plants widely used as ornamentals, they have been originated in Eastern countries and Iran and Turkey were introduced in Europe (Matin, 1998). Tulips are unique representative of plants; their significance has always been exceptional. Tulips are important bedding bulbous ornamental plants that widely used in the park and gardens and widely cultivated in world and in Turkey for cut flower, potted plant, landscaping. The Tien Shan and Pamir-Alay mountain ranges in central Asia are considered the primary gene centers for *Tulipa* species' (Botschantzeva, 1962),

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with the Caucasus as a secondary center. They are popular spring-flowering garden plants; millions of bulbs are sold annually and over 5.000 cultivars are registered (Van Scheepen, 1996).

Several species are in cultivation, but they cover less than 7 % of the total tulip area in the Netherlands. The boundaries between taxa of various ranks are still a subject of dispute (Zonneveld, 2009). The vegetative propagation of *Tulipa* taxa are effective, but creation of new tulip cultivars is especially time-consuming because tulip seedlings begin to blossom only after seven years. Researchs were carried out to make this period shorter, but no positive results were received. The process of creating a new cultivar takes long years because not only the period from sowing till blossom of seedlings is long, but also a long period is needed for bulb propagation till standard extents of industrial production' (Baliūnienė, Juodkaitė, 1991). 'It is commercially propagated through asexual reproduction by using bulbs, but the effciency of this process is low' (Lenard, De Hertogh, 1993).

In vitro storage organ formation (tuberization) has been broadly studied with most of geophytes (Podwyszyn´ska, 2012), like *Scilla siberica* subsp. *armena* (Ozdemir et al., 2016). Most geophytes require certain induction factors for storage organ formation, e.g. *Tulipa* taxa require low temperatures, some onion genotypes require long photoperiods, and potatoes need short photoperiods and low night temperatures. 'However, numerous reports suggest that two factors induce an in vitro storage organ formation in most geophytes, including bulbous plants: a high sucrose concentration in in vitro media and a sharp reduction in endogenous gibberellin levels in response to environmental cues' (Podwyszyn´ska, 2012). The latter has been approved by demonstrations that application of gibberellin biosynthesis inhibitors stimulates bulb formation in garlic (Kim et al., 2003), lily (Kumar et al., 2005) and tulip (Podwyszyn´ska, 2006). Several other plant growth regulators also have extensivel reported stimulatory effects on bulbing, including auxins (Van Aartrijk, Blom-Barnhoorn, 1981), ethylene (Taeb, Alderson, 1990) and jasmonates (Podwysyn´ska, 2006). However, there are conflicting indications of the roles of cytokinins in bulb formation *in vitro*. Benzyladenine stimulated the bulb development in *Lilium longiflorum* (Easter lily, Bermuda lily, trumpet lily) and *Urginea maritima* L. (Baker) (Nhut, 1998) but inhibited bulb formation in *Narcissus jonquilla* L. (Chow et al., 1992). A high cytokinin to auxin ration improved the bulb production in *Hyacinthus* L. (Liliaceae) taxa (Kim et al., 1981) and *Fritillaria fleischeriana* Steudel et Hochst. ex Schultes et Schultes Fil. (Mirici et al., 2005). On the other hand, low exogenous cytokinin to auxin ratio reportedly promote bulb growth of *Hippeastrum* (Amaryllidaceae) taxa (Huang et al., 2005)

Investgation using *in vitro* methods to examine efficient multiplication rates in the *Tulipa* taxa has been in progress for many years. 'Unfortunately, the laboratory techniques employed in its propagation continue to produce low yields' (Maglanka, Bach, 2010). Organogenesis is a type of plant regeneration that can be used in clonal propagation. In bulbous plants like tulips this micropropagation method results in the formation of adventitious shoots or bulbs (Ghaffor et al., 2004). 'The organs are formed directly on explants or indirectly via callus tissue' (Liu, Yang, 2012). Among geophytes, organogenesis can occur on various explants, including on buds, pedicels, seeds (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, Bach, 2007).

2. Relevance

The present investigation was undertaken to ensure that large numbers of clean explants should survive sterilization. In the present study two concentration of sodium hypochlorite % 2-3 and % 4-5 (NaCl) were used.

3. Material and methods

The bulbs of *Tulipa julia* were collected natural habitats, during within field work project (BAP –TBMYO.2016.00.001) from Bingol-Solhan province by taxonomist O. Kılıç. All of using this study bulbs washed under running tap water for 30 min. before from the study. Different concentrations of sodium hypochlorite (2 %, 3 %, 4 %, 5 %) were used for 20, 25, 30, 35 min. inside in the laminar flow air cabine, and a final wash with autoclaved distilled water 5 times. All *Tulipa julia* bulbs cultivated in MS (Murashige, Skoog, 1962) medium. Cultures were incubated in the

controlled conditions of temperature (24±1 °C) and light intensity (2000-2500 lux for 16 h); the experiments were replicates 3 times.

4. Discussion

It is always a big challenge to avoid contamination and establishment of aseptic cultures from the field grown plants which are always at high risk of internal and external contamination. The present investigation was carried out to optimize sterilization protocol for fast multiplication of *Tulipa julia*. There was significant difference among the effective sterilization sodium hypochlorite concentrations and treatments time in relation to surface sterilization bulbs of *Tulipa julia*. At lower concentrations and little treatments time (2 or 3 %, 20 or 25 min.) of sodium hypochlorite when used showed less in sterilizing the *Tulipa julia* bulbs but higher concentrations and high treatments time (4 or 5 % NaCl, 30 or 35 min) of sodium hypochlorite when used showed effective in the sterilizing of bulbs. Also, no damage to bulbs tissues were observed. Surface sterilization of bulbs, for initiation of an *in vitro* culture, required higher concentrations and treatments time of sodium hypochlorite (4 or 5 % NaOCl, 30 or 35 min) for controlling fungal and yeast contamination, compared to bulbs sterilization.

Comparing salt types, NaCl proved to be superior compared to other salts for bulbous growth as used in this study. 'NaCl is normally expected to have an adverse effect on plant growth and development due to suppressed cell division and restricted growth activities' (Bohnert, Jensen, 1996). Accumulation of Na+ and Cl– in tissues led to toxicity (Karimi et al., 2009) in the cells' cytoplasm, which affected distinct biochemical and physiological processes (Jampeetong, Brix, 2009). Our results revealed that bulblets tolerated a concentration of 4 % or 5 % NaOCl, 30 or 35 min, which ultimately promoted the bulbous growth more efficiently. The positive response of bulblets to a specific salt concentration might be due to higher tolerance showed by plants at maturity, or might depend on the type of organ (Jenks et al., 2007) used in the study. Similarly, negative effects of a higher KCl concentration lead to salt stress and may affect plant growth and development by causing callus induction, necrosis, and shoot regeneration, in line with the findings of Zahid et al. (2014). The results clearly show that the addition of salts at low concentrations for a specific time can be used to increase bulblet size and to harden the bulblets. 'Acclimatization of in vitro regenerated bulblets is the most challenging task due to smaller size and dormancy found in the in vitro regenerated bulblets' (Petric et al., 2011). Therefore, optimum bulblet size with adequate rooting is a prerequisite for successful acclimatization. Researchers adopted different approaches in order to increase bulblet size prior to acclimatization.

Losses due to contamination under *in vitro* conditions average between 3 and 15 % at every subculture in the majority of commercial and scientific plant tissue culture laboratories, the majority of which is caused by fungal and bacterial contaminant (Leifert et al., 1989). Therefore, to ensure the reduction of the contaminants as well as high survival rate of explants, it requires efficient aseptic techniques in tandem with effective sterilization methods before subjecting them for tissue culture study (Srivastava et al., 2010). Sodium hypochlorite is a very effective sterilant and extensively used to stimulate reduce contamination in cultures (Nongalleima et al., 2014).

5. Conclusion

In the present investigation NaCl (4 or 5 %) for 30 or 35 min was found more effective for sterilization and further *in vitro* response of bulbs. The previous research also suggests that NaCl is an effective sterilizing agent (Chengalrayan et al., 2005). Our finding suggests the use of sodium hypochlorite for higher time period to obtain aseptic culture of *Tulipa julia* bulbs. The findings will provide a good base for effective and quick sterilization of *Tulipa julia* bulbs especially when they are procured from field grown plants.

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References

Arslan et al., 2002 – *Arslan, N., Gürbüz B., Gümüşçü, A., Özcan, S., Mirici, S., Khawar, KM.* (2002). Cultivation of Sternbergia fisheriana (Herbert) Rupr. and a study on its morphological charecteristics. Pakistan Journal of Botany. 34: 411–418.

Atay, 1996 – *Atay, S.* (1996). Soğanlı Bitkiler. Türkiye'den İhracatı Yapılan Türlerin Tanıtım ve Üretim Rehberi. İstanbul.

Hall, 1940 – *Hall, AD.* (1940). The genus *Tulipa*. The Royal Horticultural Society, London

Eker et al., 2014 – *Eker, İ., Babaç, MT., Koyuncu, M.* (2014). Revision of the genus *Tulipa* L. (Liliaceae) in Turkey. Phytotaxa. 157: 001–112.

Matin, 1998 – *Matin, F.* (1998). Tulips of Iran. Publication of Agricultural Research, Education and Development. Tehran, Iran.

Botschantzeva, 1962 – *Botschantzeva, ZP.* (1962). Tulips: taxonomy, morphology, cytology, phytogeography, and physiology. English translated edition by HQ Varekamp. Balkema, Rotterdam.

Van Scheepen, 1996 – *Van Scheepen, J.* (1996). Classified list and international register of tulip names. KAVB, Hillegom, the Netherlands.

Zonneveld, 2009 – *Zonneveld, B.J.M.* (2009). The systematic value of nuclear genome size for "all" species of *Tulipa* L. (Liliaceae). Plant Systematic Evoluation. 281: 217–245.

Baliūnienė, Juodkaitė, 1991 – Baliūnienė A, Juodkaitė R. Tulpės. Vilnius, 1991.

Lenard, De Hertogh, 1993 – *Lenard, 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.

Podwyszyn´ska, 2012 – *Podwyszyn´ska, M.* (2012). The mechanisms of in vitro storage organ formation in ornamental geophytes, Flor Ornament Biotechnology. Glob Sci Books. 6: 9–23

Ozdemir et al., 2016 – *Ozdemir, F.A., Yildirim, M.U., Kahriz, M.P., Kılıc, O.* (2016). In Vitro Bulblet Regeneration From Scilla siberica subsp. armena (Liliaceae) Peduncle. Propagation of Ornamental Plants. 16: 14–18.

Kim et al., 2003 – *Kim, EK., Hahn, EJ., Murthy, HN., Peak, KY.* (2003). High frequency of shoot multiplication and bulblet formation of garlic in liquid cultures. Plant Cell Tissue Organ Culture. 73: 231–236.

Kumar et al., 2005 – *Kumar, S., Kashyap, M., Sharma, DR.* (2005). In vitro regeneration and bulblet growth from lily bulbscale explants as affected by retardants, sucrose and irradiance. Biology Plant. 49:629–632.

Podwyszyn´ska, 2006 – *Podwyszyn´ska, M.* (2006). Improvement of bulb formation in micropropagated tulips by treatment with NAA and paclobutrazol or ancymidol. Acta Horticulture. 725: 679–684.

Van Aartrijk, Blom-Barnhoorn, 1981 – *Van Aartrijk, J., Blom-Barnhoorn, GJ.* (1981). Growth regulator requirements for adventitious regeneration from Lilium bulbscale tissue in vitro, in relation to duration of bulb storage and cultivar. Sci Horticulture. 14: 193–197.

Taeb, Alderson, 1990 – *Taeb, AG., Alderson, PG.* (1990). Shoot production and bulbing of tulip in vitro related to ethylene. Journal Horticulture Science. 65: 199–204.

Nhut, 1998 – *Nhut, DT.* (1998). Micropropagation of lily (*Lilium longiflorum*) via in vitro stem node and pseudo-bulblet culture. Plant Cell Reproduction. 17:913–916.

Chow et al., 1992 – *Chow, YN., Selby, C., Harvey, BMR.* (1992). Stimulation by sucrose Narcissus bulbil formation in vitro. Journal Horticulture Science. 67:289–293.

Kim et al., 1981 – *Kim, Y.J., Hasegawa, PM., Bressan, RA.* (1981). In vitro propagation of hyacinth. HortScience. 16: 645–647.

Mirici et al., 2005 – *Mirici, S., Parmaksı,z I., Ozcan, S., Sancak, C., Uranbey, S., Sarıhan, EO., Gumuscu, A., Gurbuz, B., Arslan, N.* (2005). Efficient in vitro bulblet regeneration from immature embryos of endangered Sternbergia fischeriana. Plant Cell Tissue Organ Culture. 80: 239–246.

Huang et al., 2005 – *Huang, CL., Chang, KC., Okubo, H.* (2005). In vitro morphogenesis from pedicels of Hippeastrum 9 hybridum. Journal Faculty Agriculture Kyushu University. 50: 27–33.

Maĝlanka, Bach, 2010 – Maĝlanka, M., BACH, A. (2010). The effect of abscisic acid, ethylene and inhibitors of their biosynthesis (À uridone and salicylic acid) on somatic embryos conversion in tulips. Ecology Chemistry Engineering. 17: 1135–1139.

Ghaffor et al., 2004 – *Ghaffor, A., Maqbool, I., Waseem, K., Quraishi, A.* (2004). In vitro response of tulips (*Tulipa gesnerina* L.) to various growth regulators. International Journal Agricultural Biology. 6: 1168–1169.

Liu, Yang, 2012 – *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 Development Biological Plant. 48: 172–179.

Ptak, Bach, 2007 – *Ptak, A., Bach, A.* (2007). Somatic embryogenesis in tulip (*Tulipa gesneriana* L.) À ower stem cultures. In Vitro Cell Development Biological Plant. 43: 35–39.

Murashige, Skoog, 1962 – *Murashige ,T., Skoog, F.* (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15: 473-497.

Bohnert, Jensen, 1996 – *Bohnert, HJ., Jensen, RG.* (1996). Metabolic engineering for increased salt tolerance – the next step. Funct Plant Biology. 23: 661-667.

Karimi et al., 2009 – *Karimi, E., Abdolzadeh, A., Sadeghipour, HR.* (2009). Increasing salt tolerance in Olive, Olea europaea L. plants by supplemental potassium nutrition involves changes in ion accumulation and anatomical attributes. International Journal Plant Production. 3: 49–60.

Jampeetong, Brix, 2009 – *Jampeetong, A., Brix, H.* (2009). Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of *Salvinia natans*. Aquatic Botny. 91: 181–186.

Jenks et al., 2007 – *Jenks, MA., Hasegawa, PM., Jain, SM., Foolad, M.* (2007). Advances in molecular breeding toward drought and salt tolerant crops. Amsterdam, the Netherlands: Springer.

Zahid et al., 2014 – *Zahid, MN., Hasan, M., Adil, M., Hossain, MM., Mian, MAK.* (2014). In vitro screening for salt tolerance in aromatic rice genotypes. Open Science Journal of Bioscience and Bioengineering. 1: 28–32.

Petric et al., 2011 – *Petric, M., Subotic, A., Jevremovic, S., Milana, T., Momcilov, M.* (2011). Somatic embryogenesis and bulblet regeneration in snakehead fritillary (*Fritillaria meleagris* L.). African Journal Biotechnology. 10: 16181–16188.

Leifert et al., 1989 – *Leifert, C.W.M., Waites, J., Nicholas, R.* (1989). Bacterial contaminats of micropropagated plant cultures. Journal of Applied Bacteriology. 67: 353–361.

Srivastava et al., 2010 – *Srivastava, N., Kamal, B., Sharma, V., Negi, YK., Dobriyal, AK., Gupta, S., Jadon, VS.* (2010). Standardization of sterilization protocol for micropropagation of *Aconitum heterophyllum*. Academia Arena. 2: 37–42.

Nongalleima et al., 2014 – *Nongalleima, Kh., Dikash, Singh, Th., Amitabha, Dey., Lokesh, Deb., Devi Sunitibala, H. (*2014). Optimization of surface sterilization protocol, induction of axillary shoots regeneration in *Zingiber zerumbet* (L.) Sm. as affected by season. Biological Rhythm Research. 45: 317–324

Chengalrayan et al., 2005 – *Chengalrayan, K.A., Abouzid, M., Meagher, G.* (2005). İn vitro regeneration of plants from sugarcane seed derived callus. In Vitro Cell Dev Biology Plant. 41: 477–482.