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A REVIEW OF *IN VITRO* STUDIES ON *Allium tuncelianum* (Kollman) Ozhatay, Matthew, Siraneci

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Abstract

Allium tuncelianum (Kollman) Ozhatay, Matthew, Siraneci is an endemic plant species only grown in Turkey. Unlike common garlic, it has only one clove bulb and it can also produce fertile flowers and seeds. Due to the similarity of its flavor and taste to Allium sativum, it is called 'tunceli garlic' and 'ovacik garlic' in the region. In recent years, the amount of consumption has increased due to revealing the benefits of biochemical content to human health. For this reason, Allium tuncelianum has been collected from nature for domestic and medical purpose by herbalists and local people. So, it is in danger of extinction due to unconscious and over-exploitation from the nature. In recent years, different strategies have been developed to protect Allium tuncelianum from destruction. Germination problems of its seeds have led researchers to use in vitro techniques. These studies focus to develop an efficient protocol for propagation and conservation of this endemic species. In this review, in vitro studies on Allium tuncelianum were evaluated.

Key words*: Allium tuncelianum, Tunceli garlic, in vitro propagation.*

INTRODUCTION

Allium tuncelianum (Kollman) Ozhatay, Matthew, Siraneci has a limited distribution in eastern Anatolia in the Tunceli and Erzincan areas. It is locally called as 'Tunceli garlic' or 'Ovacik garlic'. *Allium tuncelianum* is originally named as *Allium macrochaetum* Boiss and Haussk subsp. *tuncelianum* Kollmann. It is an important endemic species for Turkey. It was discovered in 1980s. The bulbs and young leaves of *A. tuncelianum* are used as vegetable and spice locally, being very similar in flavor to *Allium sativum* (Ozhatay & Mathew, 1995; Etoh & Simon, 2002; Yanmaz & Ermis, 2005; Kosar et al., 2006; Ipek et al., 2008; Yanmaz et al., 2010; Baktir et al., 2013; Kiralan et al., 2013; Aasim, 2015; Yarali & Yanmaz, 2016).

Consumption of *Allium tuncelianum* has several benefits such as stimulates the body's immune system, lowers the level of sugar and cholesterol in the blood, improve blood circulation thus reduces the risk of heart attack (Agbas et al., 2013; Aasim, 2015; Atila et al., 2017). In addition, *Allium tuncelianum* has a strong antioxidant and antiradical activity than *Allium sativum* L. Because of high amount of p-Coumaric acid content of *Allium tuncelianum*, it has much higher antioxidant activity compared with the *Allium sativum*. And also in terms of fatty acid compositions *Allium tuncelianum* is observed having more effective level of essential omega acids compared to the common garlic (Sehitoglu et al., 2014; 2018).

Picking of endangered geophytes for trade is banned in Turkey for conservation purpose in agreement with the "Convention on the International Trade in Endangered Species (CITES)". However, *Allium tuncelianum* has been collected from nature for domestic and medical purpose by herbalists and local people in Turkey. So, it is in danger of extinction due to unconscious and over-exploitation from the nature (Yanmaz et al., 2010; Aasim, 2015). Conservation efforts can be complemented by development of *in vitro* conservation protocols along with improved agronomic techniques suitable for cultivation of the plant in other areas of Turkey. The application of *in vitro* culture techniques for the protection of

endemic *Allium tuncelianum* is less common than for *Allium sativum*. Therefore, extensive studies are needed to develop tissue culture techniques for Tunceli garlic with significant advantages (Kosar et al., 2006; Yazar, 2006; Kizil et al., 2014; Aasim, 2015). In recent years, different strategies have been developed to protect *Allium tuncelianum* from destruction. However, there are a limited number of studies on *in vitro* conservation of endemic 'Tunceli garlic'. In this review, *in vitro* studies on *Allium tuncelianum* were evaluated.

IN VITRO STUDIES

Unlike *Allium sativum*, *Allium tuncelianum* has only one clove bulb and it can also produce fertile flowers and seeds (Yazar, 2006; Yanmaz et al., 2010; Agbas et al., 2013; Baktir et al., 2013; Kiralan et al., 2013; Aasim, 2015; Kizil & Khawar, 2015; Takim, 2015; Yarali & Yanmaz, 2016; Babacan et al., 2017) (Figures 1, 2 and 3).

Figure 1. Bulbs of *Allium tuncelianum* (Photographed by: Faika YARALI KARAKAN)

Figure 2. Inflorescence of *Allium tuncelianum* Photographed by: Faika YARALI KARAKAN)

Figure 3. Seeds of *Allium tuncelianum* (Photographed by: Faika YARALI KARAKAN)

Allium tuncelianum multiplies naturally by seed or vegetative by newly regenerated bulb attached to mother bulbs. However, the percentage of new regenerated bulbs and the number of plantlets per bulb is too low for practical regeneration purposes. In addition, seeds have germination problem (Yanmaz et al., 2010; Kizil et al., 2014). *In vitro* techniques *are* useful tool to develop propagation methods for *Allium tuncelianum* (Yanmaz et al., 2010). For this reason, researchers aimed to develop *in vitro* protocols by using *in vitro* techniques, such as shoot and root culture, leaf culture, bulb culture, *in vitro* seed germination and gynogenesis.

Root and shoot culture

In vitro root and shoot tip culture methods used to propagation of *Allium tuncelianum* by Yazar (2006). Murashige and Skoog (MS) basal medium supplemented with BA (0.0, 0.1 and 1.0 mg/l), 2,4-D (0.0, 1.0 and 2.0 mg/l), NAA (0.0, 1.0 and 2.0 mg/l) were used in root tip culture. Root tip explants prepared from root tissues are planted in petri dishes then cultured at 25 ± 10°C under dark conditions and taken 1 month later under fluorescent light to 16/8 h. In shoot tip culture, bulbs were opened under a binocular microscope then explants having 0.5-1.0 cm long leaflets were prepared after bulbs were disinfected. They were cultured in MS basal medium supplemented with BA (0.0, 0.05 and 0.1 mg/l); 2,4-D (0.0, 0.1 and 0.5 mg/l); NAA (0.0, 0.1 and 0.5 mg/l). As a result of the research, it was stated that callus formation couldn't be provided in root tip culture

experiments. But in the shoot tip culture experiment, shoot formation started approximately 1 month after shoot tip planting. After 4 sub-cultures, it was determined that MS medium containing IAA gave out better results than NAA with regard to the number of shoots. The highest shoot rate was obtained from the medium supplemented with 0.05 mg/l BA+0.1 mg/l IAA with 76%. This was followed by medium supplemented with 0.1 mg/l BA, 0.1 mg/l BA + 0.1 mg/l NAA with 67% and 0.05 mg/l BA + 0.5 mg/l IAA with 65%, 0.05 mg/l BA with 56%. In a similar study, Yanmaz et al. (2010) aimed to develop a novel micropropagation method for *in vitro* propagation of *Allium tuncelianum* by root tip and shoot culture techniques. Root tips were obtained from 18 days old *in vitro* plantlets. To determine the best combinations of the growth regulators; 2,4-D and NAA (0, 1.0, 2.0 mg/l) and BA (0, 0.1, 1.0 mg/l) were used in MS medium. According to results, the root tip culture was not found as a proper method for shoot proliferation. On the other hand, shoot culture was found effective on shoot formation. As an average, 1 or 2 shoots were obtained per explant. The researchers stated that *Allium tuncelianum* could be propagated at lower doses of plant growth regulators such as IAA and BA (0.1 mg/l, 0.1 mg/l) via *in vitro* shoot culture. Contrary to these findings Kizil et al. (2014) suggested that root tip explants were most suitable for bulblet regeneration of *Allium tuncelianum*. They used MS medium supplemented with 1.0, 2.0, 3.0, 4.0, 5.0 mg/l 2,4- D and 1.0, 2.0, 3.0, 4.0, 5.0 mg/l BAP and 0.5 mg/l NAA. The results indicated that root tip explants were most suitable for bulblet regeneration on MS medium containing 5.0 mg/l BAP and 0.5 mg/l NAA. Similarly, Icgil (2012) stated that root explants showed bulblet regeneration on root tips on MS medium containing various concentrations of BAP and NAA. The bulblet regeneration rate from root explants ranged from 13.33% to 100%. When the effect of MS medium containing different concentration of BAP and 0,5 mg/l NAA on the bulblet regeneration rate was examined, it was seen that the bulblet regeneration rate and the number of bulblets per explant decreased as the concentration of BAP increased in the media containing 1, 2 and 3 mg/l BAP + 0.5 mg/l NAA. The bulblet regeneration rate increased to 60% at a concentration of 4 mg/l BAP + 0.5 mg/l NAA and reached 100% at a concentration of 5 mg/l BAP + 0.5 mg/l NAA. For obtaining virus-free plant, Taskin et al. (2013) aimed to combine meristem culture technique by shoot tip culture technique. They used two different culture media (Medium 1; MS + 0.5 mg/l 2-IP + 0.2 mg/l NAA + 30 g/l sucrose and Medium 2: MS + 2 mg/l BA + 0.5 mg/l IBA + 30 g/l sucrose) and two garlic species, *Allium sativum* and *Allium tuncelianum*. They stated that Medium 2 was found more effective in term of number of shoots than Medium 1. In the first propagation, 14.10 shoots/plant and 4.63 shoots/plant were obtained from Medium 2 and Medium1, respectively. And also Medium 2 has been successful at subculture. It was obtained 13.27 shoots per plant from Medium 2. Similar results were obtained in shoot tip culture. 11.37 and 2.41 shoots per plant were obtained from Medium 2 and Medium 1 in the first propagation, respectively. Considering the explant types, meristem explants were found to be more successful compared to shoot tip explants for both *Allium sativum* and *Allium tuncelianum*. Real-time PCR analysis revealed that *in vitro* plants obtained from meristem culture do not have any onion yellow dwarf virus (OYDV) and leek yellow stripe virus (LYSV). Contrary to these findings OYDV and LYSV viruses were detected in plants obtained via shoot tip culture.

Leaf culture

Kizil et al. (2014) used leaf tips, the middle portions of leaves and leaf bases explants and MS medium supplemented with different concentrations of 2,4- D, BAP and NAA. They stated that regeneration or callusing was not observed on day 28 of culture on leaf tips or on the middle portion of leaves on MS medium containing 0.5-1.0 mg/l BAP + 0.5 mg/l NAA (five combinations). In addition, no bulblet regeneration was induced from leaf bases on MS medium supplemented with 1.0 mg/l BAP plus 0.5 mg/l NAA, or 5.0 mg/l BAP plus 0.5 mg/l NAA. A maximum of 13.3% regeneration was recorded on MS medium supplemented with 1.0 mg/l BAP plus 0.5 mg/l NAA. All

other culture media showed low regeneration percentages (6.7% each). Mean values of 1.0, 1.0 and 0.7 bulblets per leaf base were recorded on MS medium containing 2.0 mg/l BAP plus 0.5 mg/l NAA, 3.0 mg/l BAP plus 0.5 mg/l NAA, or 4.0 mg/l BAP plus 0.5 mg/l NAA, respectively. Similarly, İcgil (2012) stated that no regeneration was recorded on leaf tip explant from MS medium with different concentrations of BAP-NAA, 2,4-D. Contrary to these findings, the highest regeneration rate (13.3%) on petiole explants was obtained from MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA.

Bulb culture

Icgil (2012) investigated the effects of different concentrations of BAP, NAA, 2,4-D on different bulb explants such as; longitudinally sectioned ½ and ¼ bulb explants. It was determined that plant growth regulators substances and explant types were effective on the shoot formation. While the highest shoot number per explant (83.33%) was obtained from MS medium containing 2 mg/l 2,4-D and no shoot formation was observed from MS medium containing 1 mg/l 2,4-D from longitudinally sectioned 1/2 bulb explants. While the highest shoot number per explant (3) was obtained from MS medium supplemented with 1 mg/l BAP+ 0.5 mg/l NAA, the lowest shoot number per explant (0.67) was obtained from MS medium containing 2 and 3 mg/l BAP+ 0.5 mg/l NAA from longitudinally sectioned 1/4 bulb explants. Contrary to these positive findings, Kizil et al. (2014) stated that vertically-sectioned half or quartered bulb or both horizontally-sectioned upper and lower half-bulb explants were unsuitable for the regeneration of new bulblets. They cultured all these explants on MS medium supplemented with different concentrations of 2,4-D, BAP and NAA. Their results showed that no bulblet regeneration was obtained from any vertically-sectioned half or quartered bulb and both horizontally-sectioned upper and lower half-bulb explant.

Over wintered ‘Tunceli garlic’ bulbs had a potential to produce bigger bulbs than unwintered materials. These bulbs had a significant effect on uniform plant formation. This is very important for cultivation of *Allium tuncelianum* (Yanmaz et al., 2010). Aasim (2015) used wintered and unwintered half cloves. Bulb explants were cultured at MS medium supplemented with 0.25, 0.50 and 1.0 mg/l BA and 0.25, 0.50 and 1.0 mg/l of KNAA for regeneration. As a result, the study determined that unwintered and wintered upper and lower half clove explants failed to regenerate new bulblets. However, proximal half clove of the wintered bulbs was evaluated as the best explant for regeneration on MS medium supplemented with 0.50 mg/l BA with 0.50 mg/l KNAA. The rooted bulbs were acclimatized and transferred to pots and fields. It was concluded that the protocol could be safely used to conserve this plant.

Seed germination

Allium tuncelianum has fertile black seed that can easily be used for propagation. But they undergo deep seed dormancy soon after maturity. For this reason, the aim of *in vitro* studies is to eliminate seed dormancy. Dormancy can be broken with the cold treatment given to garlic before planting. Kizil et al., (2017), aimed to break seed dormancy of *Allium tuncelianum* and determine the conditions for induction of bulblets on these seeds. They collected ‘Tunceli garlic’ seeds from field grown plants. After being surface sterilized, seeds were germinated on MS medium with or without 20 g/l sucrose followed by their culture on 1 × 1900 mg/l, 2 × 1900 mg/l, 4 ×1900 mg/l and 6 × 1900 mg/l KNO_3 to increase bulb diameter. At the end of the study, it was reported that bulb formation rate on each of the germinated seeds was not parallel to seed germination rate. A total of 34% seeds (with 138 seeds that converted to bulbs) and 28.5% (with 94 seeds that converted to bulbs) on MS medium with and without 20 g/l sucrose, respectively. The results showed that MS medium containing sucrose had significantly positive effect on seed germination and bulb induction and vegetative growth. The best increase in bulb diameter was noted on MS medium containing 1×1900 mg/l KNO_3 after 178 days with bulblet diameter and bulblet weight of 0.54 cm and 0.048 g, respectively.

Gynogenesis

There have been conducted out several studies for *in vitro* propagation of *Allium tuncelianum* but breeding studies were not carried out. Today, biotechnological breeding methods offer great benefits in breeding. Using dihaploidization techniques provides great advantages in obtaining the inbreed lines used in hybrid breeding in a short time. Different methods have been improved for *in vitro* haploid production, but only gynogenesis has been reported to be successful in *Alliums*. Yarali and Yanmaz (2016) aimed to ensure optimization technique for *Allium tuncelianum* which were used successfully for other *Allium* species. It is the first research about determining of gynogenic induction frequency of *Allium tuncelianum* via flower bud culture. They used BDS medium supplemented with 0, 1 and 2 mg/l of 2,4-D and BAP and their combinations to determine the effect of plant growth regulators on gynogenic embryo induction. They stated that BDS medium supplemented with different combinations of auxin (2,4-D) and cytokinin (BAP) were effective on callus development on explants. The highest callus formation rate was obtained from BDS medium supplemented with 2+1 mg/l 2,4-D+BAP and 2+2 mg/l 2,4-D+BAP. In this research, callus development was provided on flower buds at 55.28% but plantlets could not be achieved from callus. This study is important due to the guidance for future studies about haploid plant production on *Allium tuncelianum*.

CONCLUSIONS

In vitro studies on *Allium tuncelianum* were carried out by several researchers. This subject is important, considering that it is an endemic species and has biochemical content, important for human health. Researches have been aimed to reveal the well-established protocols for *in vitro* propagation and conservation, and in part, some successful results have been achieved. Efforts have been done to regenerate plants under *in vitro* conditions. But, there is need for doing extensive work for development of comparatively more efficient and reproducible *in vitro* protocols for propagation, breeding and conservation purposes of *Allium tuncelianum*.

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