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Improving seed germination and bulb induction of *Allium tuncelianum* kolmann under aseptic conditions

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Abstract

Allium tuncelianum (Kollman) N. Ozhatay, B. Mathew & Siraneci] or Tunceli garlic is endemic to the Eastern Turkish Provinces of Tunceli, Sivas Erzincan and [Munzur mountains]. They are edible and bear attractive deep lilac colored flowers with fertile black deep dormant seeds. Tunceli garlic seeds were collected from field-grown plants and aimed to break seed dormancy to optimize conditions for induction of bulblets, along with their growth, development, and increased bulb diameter. Therefore, these were cultured on MS medium amended with different strengths of KNO_3. They were germinated on MS medium with or without 20 g L^{-1} sucrose followed by their culture on 1, 2, 4 and 6 × KNO_3 (found in MS medium) to increase bulb diameter. Improved seed germination was noted on MS medium with and without sucrose but with variation compared to the previous reports. The bulb formation rate on each of the germinated seeds was not parallel. The results showed 34 and 28.5% bulb induction noted on germinated seeds after 150 and 158 days on MS medium containing 20 g L^{-1} sucrose and no sucrose in the same sequence. The results emphatically noted the role of cold stratification on agar-solidified MS medium supplemented with sucrose to improve seed germination. The best increase in bulb diameter was noted on MS medium containing 1 × KNO_3 (found in MS medium) after 178 days with bulblet diameter and weight of 0.54 cm and 0.048 g, respectively. Consequently, the bulbs induced on sucrose-containing MS medium could be transferred to pots earlier. Increased (>1 × KNO_3 found in MS medium) negatively affected on the growth and development of Tunceli garlic bulbs. The strategy of seed germination and bulblet induction reported in this study could be positively used to conserve and protect this endemic plant species.

Keywords: Tunceli garlic, Seed, dormancy, Bulblets, Bulb growth

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INTRODUCTION

Tunceli garlic [*Allium tuncelianum* (Kollman) N. Ozhatay, B. Mathew & Siraneci] with white-to-purple or lilac colored inflorescences on single-gloved, cream-white bulbs are endemic to Eastern Turkish Province of Tunceli and the surrounding Munzur mountains (Baktir, 2005; Yanmaz and Ermis, 2005). Tunceli garlic bears fertile black seeds that can easily be used for propagation (Arslan et al., 2010); however, they undergo deep seed dormancy soon after maturity. The bulbs are generally produced asexually by bulbil propagules attached to the main body of mother bulbs. Propagation by seed can produce mature plants after 2 – 3 years under ideal conditions of growth and development.

Like all allium species, Tunceli garlic also contains a biologically active organic sulfur compound, allicin (thio2-propene-1-sulphinic acid S-allyl ester). Allicin has been reported to have anti-coagulant, anti-hypertensive, antimicrobial, anti-biotic, anti-parasitic, anti-mycotic, anti-viral, anti-tumor, anti-oxidant, and anti-aging activities (Jacob, 2006; Ozkan et al., 2013). Allicin is also known to detoxify heavy metals, be hypolipidaemic (i.e., lipid-

lowering), anti-carcinogenic, and antimutagenic characteristics (Ozkan et al., 2013; Munchberg and Anwar, 2007; Iciek et al., 2009; Kizil et al., 2014).

Dormancy can be broken with the cold treatment given to garlic before planting it. Garlic growth is slow when it begins its vegetative activity, because the plant needs a cold moist climate, with nocturnal temperatures between 8 and 20 °C and diurnal temperatures of 13 and 24 °C to grow vigorously (Rahman et al., 2003).

The low temperatures that plants receive in the period before bulb induction determine the beginning of vegetative growth which obviously affects yields. With temperature of 0 °C, the plants are no longer able to take nutrients from the soil and remain in dormancy until temperatures rise.

The bulb formation stage in garlic begins when the average soil temperature is around 18 to 20 °C. Better induction and growth of bulbs are observed on long days with high temperatures compared to short days with low temperatures. There is no report on seed germination or seed dormancy break of Tunceli garlic. This situation complicates the cultivation of the plant by seed.

Therefore, the study aimed to break the seed dormancy of Tunceli garlic, induce new bulblet formation and increase bulb diameter by culturing germinated seeds on different strengths of KNO_3 in MS medium.

MATERIALS AND METHODS

Tunceli garlic seeds were obtained from the Experimental Gardens of the Department of Field Crops, Dicle University, Diyarbakir, Turkey. They were washed in slow - flowing tap water to remove all dirt and soil. The seeds were tested for seed viability before seed germination test using 2,3,5-Triphenyl-tetrazolium chloride. The 1000 seed weight of Tunceli garlic was determined as 2.13 g. Then, the seeds were surface sterilized using 100 % (v/v) bleach (containing 5 % (v/v) NaOCl) for 20 min. The seed viability of Tunceli garlic was tested using 1 mg ml^{-1} 2,3,5 - triphenyl tetrazolium chloride, by penetration and immersion of living tissues of longitudinally cut seed embryos in the dark at 24 °C. The seed viability was noted after 24 hours by observing the formation of insoluble reddish formazan.

Thereafter, the seeds were rinsed 3 × 3 min in distilled sterilized water. The seeds were cultured on MS medium (Murashige and Skoog, 1962) supplemented with and without 2.0 % (w/w) sucrose solidified with 0.62% (w/w) agar (Duchefa – Haarlem, The Netherlands) at 4 ±1°C (Table 1).

The germinated seeds were cultured on MS medium containing 1, 2, 4 and 6 × KNO_3 (found in MS medium) supplemented with 30 g L^{-1} sucrose to increase bulb diameter. After 8 weeks in culture, the rooted bulblets were transferred to pots containing peat moss. The bulblets in pots were initially grown under controlled environmental conditions for 10 d in a growth chamber to acclimatize them followed by their transfer to the greenhouse.

The pH of all cultures was adjusted to 5.7±0.1 using 0.1 M NaOH or 0.1 M HC1 before autoclaving. All media were autoclaved at 104 kPa pressure and 121 °C for 20 min. All cultures were incubated in the refrigerator at 4 ± 1 °C in the dark during seed germination in a growth chamber (Fitotron SGC 120-United Kingdom) at 24 ± 1ºC with a 16 h light photoperiod during bulblet induction.

RESULTS AND DISCUSSIONS

Seed viability of Tunceli garlic was made using 2,3,5 triphenyl tetrazolium chloride, a test developed for rapid seed viability testing. A penetration of 2,3,5 triphenyl tetrazolium chloride in living tissues of longitudinally cut-through embryos and cotyledons resulted in the reduction of hydrogen ions released by enzymes involved with the respiration process. This resulted in the formation of an insoluble reddish formazan compound after 24 hours of incubation at 24 °C in agreement with Youngblood (2008), Sosnoskie and Cardina (2009), and Miller and Peters (2010).

The result showed that 60.0% of seeds (240/ 400 seeds) were viable; as their embryonic axes and the cotyledon tissue were fully stained red by masking their dirty white color.

Surface sterilized seeds germinated on MS medium with and without 20 g L^{-1} sucrose at 4 ±1°C in dark showed average seed germination of 56.5% and 51.5% (Table 1; Figure 1a) respectively. Similarly, seed germination on MS medium with and without 20 g L^{-1}sucrose started after 81 and 85 days of culture, which continued till 110 and 117 days, respectively (Table 1 and 2).

The bulb formation rate on each of the germinated seed was not parallel to the seed germination rate. A total of 34% seeds (with 138 seeds that converted to bulbs) (Figure 1b) and 28.5% (with 94 seeds that converted to bulbs) on MS medium with and without 20 g L^{-1} sucrose respectively (Table 2). Previous studies suggest that storage at low temperatures has sharp and significant effects on breaking seed dormancy of garlic (Cantwell et al., 2003; Vazquez et al., 2006). Similarly, Arguello et al. (2001) also suggested that garlic seeds treated at a 4°C and Gibberellic Acid (GA_3) were helpful in seed dormancy break. It is important to know that storage at 4°C results in the hydrolysis of seed starch resulting in carbohydrate mobility in *Allium sp*. (Fulton et al., 2001). This results in the conversion and transport of macro carbohydrate molecules into sucrose, glucose, and fructose, with accumulation of these macromolecules ends up into energy that is utilized during metabolism of cells and energy required for the growth of plants (Hapkins, 1999; Langens et al., 2003).

Table 1. Observation of *A. tuncelianum* seeds under *in vitro* conditions supplemented with different sucrose amounts

Details	MS medium with sucrose	MS medium without sucrose
Seed germination percentage (%)	56.5	51.5
Number of germinated seeds	226	206
Start of *in vitro* seed germination (days)	81	85
End of *in vitro* germination (days)	110	117
Bulb formation (days)	150	158
Bulb formation (%)	34	28.5
Number of bulbs transferred to pots	136	94.0
Bulb transfer to pots (days)	178	196

Table 2. Effect of KNO_3 concentration on bulb growth of *Allium tuncelianum* bulblets

Strength (×) of KNO_3 in MS medium g L^{-1}	Average bulb diameter (mm)*	Average bulb weight (g)	Root induction
1 ×	0.54 a	0.048	+
2 ×	0.47 b	0.036	+
4 ×	0.41c	0.038	+
6 ×	0.35d	0.028	-

* Each figure is mean of 10 × 3 bulbs

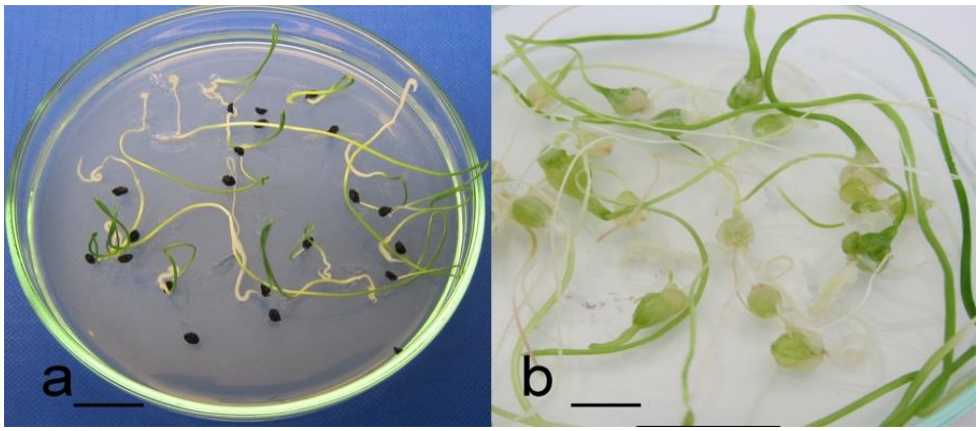

Figure 1. Seed germination of Tunceli garlic under *In vitro* conditions (a) surface sterilized seeds germinated on MS medium (b) bulb formation on 20 g L^{-1} sucrose at 4 ±1°C in dark, bar of Fig 1a=1.5 cm , Bar of Figure 1b=0.75 cm

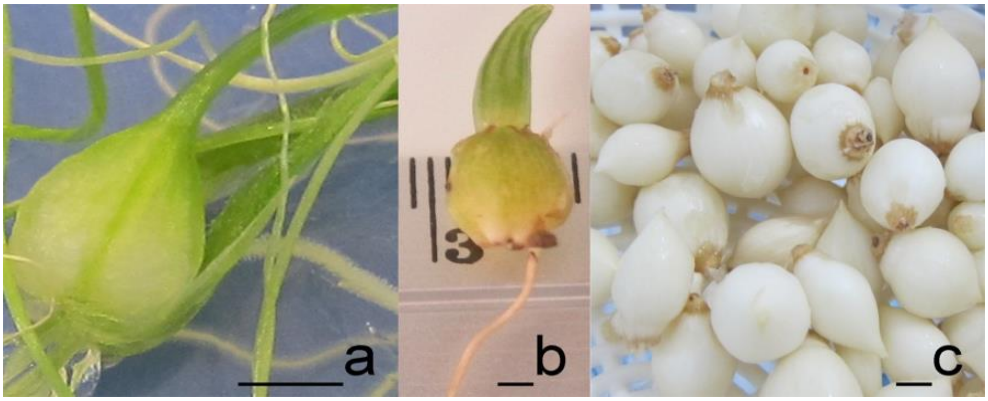

Figure 2. Tunceli garlic bulblets under *In vitro* conditions (a) increase in bulb diameter (b) the rooted bulbs induced on MS medium with sucrose after 178 days on culture before transfer to pots and nature bulbs of *A. tuncalianum*; Bar a, b=1 cm, c= 0.4 cm, d=0.7 cm

The bulbs induced on MS medium with sucrose took 178 days in their transfer from in vitro conditions to pots (Figure 2a, b). The bulbs that were not induced on 20 g L^{-1}sucrose took a longer time and could be transferred to pots in 196 days only. This showed that MS medium containing sucrose had a significantly positive effect on seed germination, bulb induction and vegetative growth.

Bulbs induced on MS medium were transferred to 1, 2, 4 × and 6 × KNO_3 in MS medium (Table 2) supplemented with 30 g L^{-1} sucrose. A positive increase in bulb diameter and weight in the range of 0.35 to 0.54 cm and 0.28 to 0.48 g was noted on different strengths of KNO3-supplemented media. Each increase in the strength of KNO_3 was inhibitory and affected bulb diameter negatively. Maximum increase in bulb diameter was only noted on supplementing 1× KNO_3 in MS medium. The results showed both reductions in bulb diameter and weight on increased 6 × KNO_3 (found in MS medium) and consequently, no rooting was noted on bulbs cultured on MS medium supplemented with 6 × KNO_3 (found in MS medium). Bulb diameter in the range of 0.41-0.54 cm and bulb weight in range of 0.038 - 0.48 cm was favorable for rooting (Fig 1d). Rooting was noted on all bulbs cultured on MS medium supplemented with 1, 2 × and 4 × KNO_3 (found in MS medium). Volk (2009) suggests that garlic (*A. sativum*) cultures grown under diverse conditions are affected by soil potassium levels. Potassium levels are positively correlated with bulb circumference and fresh weight. Similarly, Arguello et al. (2001) suggested that garlic micro-bulblets physiologically mature and are able to sprout after 90 days of storage. Cloves exposed to 5°C for 15–30 days before sowing accelerated the maturity of bulbs compared with cloves cultured at room temperature or 20°C Rahim and Fordham, 2001). Dufoo-Hurtado et al. (2013) emphasize that pre planting cold storage at 5°C for 5 weeks resulted in significant improvement in maturity and purple color enhancement of garlic bulbs.

Each of the induced bulbs was cultured on 0, 30, 60 and 90 g L^{-1} sucrose to increase bulb diameter. No statistical differences were noted among the number of shoots per bulb, and shoot length due to changing concentrations of sucrose. Maximum bulb diameter was noted on 30 g L^{-1} sucrose. Whereas, maximum bulb weight was noted on 90 g L^{-1} sucrose. All concentrations of sucrose induced variable number of roots. Kumar et al (2005) noted that bulbscales of Star Gazer hybrid induced bulblets on MS medium with growth retardants, and several sucrose concentrations, and exposure to light or darkness. Alar, Cycocel, and Paclobutrazol increased number of bulblets but decreased with increased sucrose additives, and none of them produced leaves in continuous dark.

Subsequent culture of these bulblets (the bulblets cultured on 30 g L^{-1} sucrose) on MS medium using 30 g L^{-1} sucrose for 20 – 22 weeks showed further improved the morphological parameters. Each bulb developed average number of 0.7 shoots with a shoot length of 2.30 cm, bulb diameter of 0.63 cm, bulb weight of 0.21 g, and rooting percentage of 83.3%.

The results are in partial agreement with Pooler and Simon (1994), who reported that garlic seeds, stored at 3ºC for 1–2 months, had a germination rate of 10% only. However, the seeds, harvested in spring, showed 80% germination when they were stratified at 0–3 ºC for 2 weeks in cool and humid conditions. They transferred seeds to 22°C temperature under 16 h long light conditions after seed germination. The results show improvement over previous reports by Etoh and Simon (2002) and are in partial agreement with them. They reported 20% seed germination by storing the seeds at 3ºC for 3–6 months followed by transfer to 5ºC. They noted treatment with of phytohormones was not suitable for seed germination. They confirmed that garlic seeds need stratification at low temperatures. Yanmaz and Ermis (2005) tried to break seed dormancy in Tunceli garlic using variants of GA_3 (0.5, 1.0 and 2 mg L^{-1}) for 30 to 90 d at 0 - 5°C and achieved 20% seed germination only. They emphasize that stratification under moist conditions had an edge over GA_3 treatments in seed germination.

CONCLUSIONS

- ✓ The seed germination rate was higher under both conditions mentioned in the experiment compared to the previous reports.
- ✓ Cold stratification on agar-solidified MS medium was needed to improve seed germination.
- ✓ Supplementation of sucrose in the germination medium reduced the seed germination to 81-110 days after culture.
- ✓ No supplementation of sucrose in the germination medium delayed the seed germination to 85-117 days after culture. The seed germination rate was parallel to the seed viability rate.
- ✓ Bulbs induced on sucrose-containing MS medium were transferred after 178 d of culture to pots.
- ✓ Bulbs induced on non-sucrose-containing MS medium were transferred only after 196 d of culture to pots.
- ✓ The best bulblet diameter and weight induction was noted on MS medium containing 1 × KNO_3 (in MS medium) + 30 g L^{-1} sucrose.
- ✓ The study describes a simple procedure for the conservation of this significantly important plant species.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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