African Journal of Agricultural Research Vol. 6(22), pp. 5133-5138, 12 October, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1156
ISSN 1991-637X ©2011 Academic Journals

*Full Length Research Paper*

# Optimizing seed germination threatened endemic species of the Persian shallot (*Allium hirtifolium* boiss.)

**Etemadi Nematollah, Haghighi Maryam* and Zamani Najmeh**

Department of Horticulture, College of Agriculture, Isfahan University of Technology, 8415683111, Isfahan, Iran.

Accepted 3 September, 2011

**Persian shallot grows as a wild plant in Zagros Mountain. In this experiment, attempt was made to study the different aspects of *Allium hirtifolium* Boiss germination for the first time in controlled laboratory conditions. In the first experiment, treatments were 0, 2, 4, 6 and 8 weeks of stratification. In the second experiment, seeds were imbibed in 0, 500 and 1000 ppm $GA_3$ for 12 h and transferred to an incubator at 5, 10, 15, 25°C. Results showed that an increased stratification time improved Germination percentage after the sixth week in 4°C. The $GA_3$ concentration did not affect the germination percentage. A significant increase in mean germination time (MGT) was observed in treatments where $GA_3$ concentration increased. Maximum germination percentage reached the highest amount (38%) at 10°C. 10°C was the temperature, which allowed the lowest MGT, meaning the greatest germination rate. In general, 10°C temperature after prolonged period of stratification in 4°C were sufficient for germination of Iranian shallot native to the central part of Iran.**

**Key words:** Germination rate, endemic, $GA_3$, stratification, Iranian shallot.

## INTRODUCTION

The genus *Allium* L. includes more than 700 species, which wildly grow in the temperate, semi arid and arid regions of the northern hemisphere and therefore, results in a remarkable polymorphism (Hanelt et al., 1992). The constituents of *Allium* is divided into two main groups: sulfur containing compounds and non-sulfur containing compounds. Most of the medicinal benefits of *Allium* such as reducing total plasma cholesterol, blood pressure and platelet aggregation are attributed to a sulfur compound known as allicin (Schulz et al., 1998). The same benefit was reported for *Allium hirtifolium*. The small and medium quantities of Iranian bulbs for domestic consumption were recommended for the treatment of rheumatic and inflammatory disorders, gout, arthritis, diarrhoea, stomach pain, psoriasis and hemorrhoid. Persian shallot is a nutritive plant with special taste and its dried bulb slices used as an additive to yogurt and pickling mixtures. Its powder used as a tasty additive or spice for foods in Iran. In addition, it has crucial medicinal effects; aqueous extract of Mooseer has shown antibacterial effects (Ashrafi et al., 2004; Asili et al., 2010; Ebrahimi et al., 2009). Persian shallot (*A. hirtifolium* Boiss.), that is, native and endemic to Iran, called ''Mooseer'' is from this family. It grows as a wild plant in the Zagross Mountains in west, south and the central parts of Iran (Rechinger, 1984; Ebrahimi et al., 2009). It is a wild, perennial, herbaceous and aromatic plant. It consists of a naked and erect scape with 80 to 120 cm height. The green leaves are linear and lanceolate with 20 to 30 cm length (Ghahreman, 1984).

There are some morphological differences between common shallots (*Allium ascalonicum* L.) and *A. hirtifolium* Boiss. Bulbs of common shallot are pear-shaped reddish-brown skinned and clustered at the base of the plant and its clusters may contain as many as 15 bulbs. But in Persian shallot is yellow, oval, white skinned and usually consists of a single main bulb or rarely of two bulbs, the weight of each bulb being 8 to 15 times of garlic clove (Rubatzky and Yamaguchi, 1997) (Figure 1). There are some differences in their origin that Persian shallot is originated from cold mountains of Iran, but common shallot is originated from warm regions of west Asia at high elevations and in Iran spread across the Zagross Mountains of different provinces from

*Corresponding author. E-mail: mhaghighi@cc.iut.ac.ir.

**Figure 1.** Morphological differences of Iranian shallot (above figures) and common shallot (below figures) in bulb, inflorescence and bush.

Northwestern to Southern of Iran with the climate of very cold to moderate cold (Ghahreman, 1984; Salunkhe and Kadam, 1998). The main difference in their propagation is that Persian shallot produce more seed than common shallot and it can propagate from seed commercially. Persian shallot grows as a wild plant across the Zagross Mountains at high elevations of different provinces from Northwestern to Southern of Iran with the climate of very cold to moderate cold (Ghahreman, 1984). Shallots can be grown from seed, but usually small bulbs are planted in late fall or early spring. The "mother" bulbs divide to several bulbs.

However, the germination of Persian shallot seed faces certain problems such as the low germination percentage and velocity as well as the slow growth of the subsequent seedlings. Such problems obstruct the use of seeds as a convenient way of propagation and prefer using bulbs. Since Persian shallot (Mooseer) grows as a wild plant only in some mountains of Iran, there are some information on genetic diversity of *A. hirtifolium* ecotypes given by Ebrahimi et al. (2009) and Asili et al. (2010) and its immune characteristics published by Jafarian et al. (2003). Characteristics of its habitat may strongly influence on probability of germination and subsequent survival, so optimizing seed germination requirement is a necessity to disperse its cultivation in other place and prevent its extinction. To the best of our knowledge, there is no previous report on the propagation of *A. hirtifolium* with seed. Taking into consideration the value of shallots as the popular plant in Iran, containing the natural nutraceuticals (allicin), it is necessary to disperse its cultivation in other places and prevent its extinction. The afore-mentioned circumstances encouraged us to investigate the impact of different treatments on collected endemic seeds to report the behavior of seed germination to optimize the best conditions for their cultivation. The introduction of the best conditions for the shallot's cultivation may be meaningful for the industry in commercial seed production.

## MATERIAL AND METHODS

### Seed collection

For the first time, different aspects of *A. hirtifolium* Boiss germination were conducted in controlled laboratory conditions, aimed at optimizing its germination. For this reason, mature seeds of *A. hirtifolium* were collected from the Farsan from a habitat of natural growth in the Chaharmahalo Bakhtyari Province; Latitude 35° 50'N Longitude 16° 32'E, elevation 2020 m above mean sea level (Table 1). Seeds were disinfected in a 3% (v/v) sodium hypochlorite solution for 10 min before starting the germination tests. Seeds were placed in 9-cm diameter Petri dishes (100 seeds each) and covered with a double layer of filter paper type Whatman number 1 filter paper.

The first experiment was conducted in order to determine the effect of stratification duration on germination. Second experiment treatments included different Gibberellic Acid ($GA_3$) concentrations

**Table 1.** Environmental condition of native region of Iranian shallot habitat.

| Temperature | | | Humidity | | Rainfall/year mm |
|---|---|---|---|---|---|
| Min | Mean | Max | Max | Min | |
| 13.6 | 23.2 | 32.8 | 56 | 14 | 599.4 |

**Table 2.** The effect of stratification on G% and MGT of Iranian shallot seeds.

| Stratification | G% | SE | MGT | SE |
|---|---|---|---|---|
| 0 week | 15.792$^{C}$ | 13.1727$^{8}$ | 17.259$^{A}$ | 8.411028 |
| 2 weeks | 20.479$^{B}$ | 9.795325 | 15.959$^{A}$ | 7.398643 |
| 4 weeks | 17.917$^{C}$ | 21.12933 | 10.123$^{B}$ | 7.764551 |
| 6 weeks | 47.021$^{A}$ | 10.79218 | 9.237$^{B}$ | 9.953097 |

Means in each column having similar letters are not significantly different using LSD test at 5% level.

**Table 3.** The effect of stratification on $T_{50}$ of Iranian shallot seeds.

| Stratification | 0 week | 2 weeks | 4 weeks | 6 weeks |
|---|---|---|---|---|
| **$T_{50}$** | 16.52172 | 44.92308 | 11.8507 | 10.61818 |

in different temperature regime for growth. For these reasons, a factorial experiment was established involving four replications per treatment in a completely randomized design. Each experimental plot contained 100 seeds. The results analyzed with variance analysis, and the means compared with the Least Significant Difference (LSD) multiple tests, using the Statistic Analysis System (SAS, version 9).

**Experiment 1: Effect of stratification duration on seed germination of *A. hirtifolium***

Treatments included 0, 2, 4, 6 and 8 weeks of stratification (at 4℃). The seeds allocated to stratification treatments transferred to moist perlit (1:5, v/v) in 1 kg plastic bags and were placed in a refrigerator at 4℃ for stratification. The perlit was thoroughly washed and soaked for 3 h in distilled water before application. Finishing each period of stratification, seeds transferred to an incubator (at 10℃).

**Experiment 2: Effect of hormone application on seed germination of *A. hirtifolium***

For study of the impacts of the external application of $GA_3$, seeds were watered for 24 h then seeds were imbibed in 0, 500 and 1000 ppm $GA_3$ for 12 h. For evaluation of germination, seeds were transferred to an incubator (at 5, 10, 15, 25℃) in the B.O.D. incubator for a 10 h photoperiod of 20 µmol $m^{-2}s^{-2}$, 400 to 700 nm white fluorescent light (10 h of light : 14 h of darkness) (AOSA, 2006). The evaluations of germination were conducted every 24 h until seeds had no more germinated. The final percentage germination calculated the end of germination test. The rate of germination and germination percentage was evaluated using AOSA methods (1983). Seed germination considered successful when the root had emerged and reached out by one mm in length. The evaluations were conducted every 24 h until seeds germination stabled to a constant value. The mean germination time (MGT) measured for seed germination for a certain period after applying each treatment, was calculated by the formula:

$$MGT = {\sum g_i n_i}/{G}$$

Where $g_i$ is the number of seeds germinating on the $n_i^{th}$ day of germination testing and G is the total number of seeds germinating during the test (Etemadi et al., 2010). MGT were scored as the mean of 4 replicates with standard deviation. Time (in days) to obtain 50% germination referred to as $T_{50}$ was calculated using the following formula:

$$T_{50}=[(t_2-t_1)\times 50\%+(p_2 t_1-p_1 t_2)]/(p_2-p_1)$$

Where $t_1$ = time at which the germination percentage is less than 50%, $t_2$ = time at which the germination percentage is more than 50%, and $p_1$ and $p_2$ are the measurements of germination percentage occurring at $t_1$ and $t_2$, respectively (Heydecker and Wainwright, 1976).

## RESULTS

### Effect of stratification on germination

Along with increase of stratification to the sixth week, G% improved (Table 2). Deduction in MGT observed by increasing stratification time and highest MGT reached 44.92 at the second week (Table 3).

### Effect of $GA_3$ concentration on germination

The $GA_3$ application did not affect the G% (Table 4). A significant MGT improvement observed when $GA_3$ concentration increased. Highest MGT was 16.58 at 1000

**Table 4.** The effect of $GA_3$ on G% and MGT of Iranian shallot seeds.

| $GA_3$ | G% | SE | MGT | SE |
|---|---|---|---|---|
| 0 | 24.422$^{A}$ | 19.18159 | 10.846$^{B}$ | 9.748798 |
| 500 | 25.109$^{A}$ | 18.62659 | 12.001$^{AB}$ | 10.23788 |
| 1000 | 26.375$^{A}$ | 18.4418 | 16.586$^{A}$ | 8.775022 |

Means in each column having similar letters are not significantly different using LSD test at 5% level.

**Table 5.** The effect of $GA_3$ on $T_{50}$ of Iranian shallot seeds.

| $GA_3$ | 0 | 500 | 1000 |
|---|---|---|---|
| $T_{50\ (day)}$ | 17.81571 | 22.93042 | 18.67791 |

**Table 6.** The effect of different temperature regime on G% and MGT of Iranian shallot seeds.

| Temperature (℃) | G% | SE | MGT | SE |
|---|---|---|---|---|
| 5 | 34.271$^{B}$ | 11.10459 | 19.496$^{A}$ | 7.764551 |
| 10 | 38.188$^{A}$ | 13.75852 | 7.005$^{C}$ | 9.275134 |
| 15 | 10.021$^{D}$ | 9.795325 | 14.422$^{AB}$ | 8.785032 |
| 25 | 18.729$^{C}$ | 21.12933 | 11.653$^{BC}$ | 7.398643 |

Means in each column having similar letters are not significantly different using LSD test at 5% level.

**Table 7.** The effect of different temperature regime on $T_{50}$ of Iranian shallot seeds.

| Temperature (℃) | 5 | 10 | 15 | 25 |
|---|---|---|---|---|
| $T_{50\ (day)}$ | 33.97003 | 8.797997 | 6.555353 | 27.24424 |

ppm $GA_3$. The highest $T_{50}$ observed in 500 ppm of $GA_3$ (Table 5).

## Effect of temperature regime on germination

G% was low in all temperature regime and Maximum G% reached the highest amount (38%) at 10℃ (Table 6). The lowest MGT observed at 10℃, concluding the greatest germination rate in this temperature (Table 6). The highest $T_{50}$ is indicated at 5 and 25℃ (Table 7). Comparing $T_{50}$ and MGT results with the highest G%, it can be concluded that MGT was a better indicator for germination rate and got the higher G% earlier.

## DISCUSSION

The plants occur in the Irano-Turanian floristic region of Central Asia, characterized by hot dry summers, after seed maturation, and cold and snowy winters, when seeds lie in the wet soil (Kamenetsky and Yitzchak, 2000). It is therefore evident that *Allium* species from different taxonomic groups and habitats have differing responses to environmental conditions, and a variety of germination mechanisms. Germination of *Allium* species from the subgenus *Melanocrommyum* from Central Asia was examined in the present study. In order to optimize germination, attempts were made to eliminate possible dormancy inhibitors by taking patterns from the native habitat, using several pretreatments. Iranian shallot grow in the cold winter climate of its native mountains, Therefore, it clearly appears that stratification during winter improves germination in the native mountains of its growth, that it may involve in breaking seed dormancy. The results from the laboratory conditions confirm the effect of stratification on seed germination of Iranian shallot, as the G% increased and MGT decreased by extending the stratification time to 6 weeks. During the stratification, some biochemicals and phytohormone were changed to be able to germinate dormant seeds. By decreasing abscisic acid (ABA) and increasing GAs, the seeds, with embryo dormancy residing in the embryo, can germinate (Hurtman et al., 1997). Prechiling increases

GA biosynthesis, which decreases the accumulation of proteins that control dormancy (Nicolas et al., 1996; Nicolas et al., 1997).

Each of the *Allium* species is characterized by a specific morphology, life cycle, and bulb periodicity. Some species are adapted to a prolonged dry period followed by a cold wet season; others resume growth following a single wet period (McNeal and Ownbey, 1973). Iranian shallot according to native growth habit need to spend almost 6 weeks even more cold condition to germinate properly. Highly specialized *Allium* species from the subgenus *Melanocrommyum* require prolonged stratification at 4 to 5℃ during the winter (Zimmer and Weckeck, 1989; De Hertogh and Zimmer, 1993; Gutterman et al., 1995). Short stratification period at 4℃ (30 days for *Allium suworowii* and 45 days for *Allium altissimum*) were insufficient to initiate germination (Kamenetsky and Yitzchak, 2000). *Melanocrommyum* species, which inhabit the region with cool winters, require a prolonged period of wetting at 4℃ in order to germinate well. Seed germination at 4 and 15℃ was tested for six *Allium* species from the subgenus *Melanocrommyum* from the Central Asian part of the Irano-Turanian floristic region. Typical natural habitats of these plants are characterized by cold and snowy winters and dry hot summers (Hanelt et al., 1992). The genotypic and ecological background of natural habitats, and phenotypic influences such as the time of seed harvest, seem to play predominant roles in the germination mechanism. Thus, differing germination dynamics were observed in Iranian shallot with common shallot similar result was obtain for *A. altissimum* and *Allium sewerzowii* (Gutterman et al., 1995) And *Allium aschersonianum* (Kamenetsky, 1992).

Temperature regime is a principal factor affecting dormancy release and seed germination of the six *Melanocrommyum* species tested by Kamenetsky and Gutterman (2000). They showed that Germination at 15℃ was rather low (except for *Allium decipiens*, which achieved a germination percentage of about 15% after 250 days) while at 4℃ after 60 days and more, all the seed stocks (except June-harvested *A. suworowii*), demonstrated relatively high germination rates. Same results of other studies were along with them (Aoba, 1967; Dalezkaya and Nikiforova, 1984; Specht and Keller, 1997) and indicate that the germination mechanism of this group is an adaptation to the climatic conditions of its natural habitats. The best G% of Iranian shallot obtained in 10℃ and the fastest germination was at 5℃ that is along with the aforestated studies. A minimum of 60 days at low temperature was required for massive germination of *A. suworowii*. *A. altissimum* seemed to demand a longer period at low temperatures for successful germination: at least 70 days were required for massive germination of this species (Kamenetsky and Yitzchak, 2000) and Iranian shallot after 6 weeks in 4℃ showed the highest germination, although it might germinate better in the longest stratification time. Kamenetsky and Gutterman (2000) revealed that for both species, transferring seeds to 15℃ after a cold period accelerated their germination rates, when cold requirements were fulfilled. Iranian shallot after 6 weeks in 4℃ and transferring in 5 to 10℃ germinate well.

These researchers concluded that in natural populations many *Allium* species germinate during early spring, that is why *A. suworowii* and *A. altissimum* is enhanced by low temperatures, but that after fulfillment of cold requirements, seeds can germinate in a wide range of temperatures. Accordingly, Iranian shallot, which is native to cold region, accelerates germination in 5℃, properly.

Longest time to 50% of germination was in insufficient stratification (2 weeks). Lowest time to reach at more than half (50%) G% between treatments was for 5℃; so it can be said that temperature is more critical factor for Iranian shallot seed germination than $GA_3$ after fulfilled stratification. The best conditions for the germination of common shallot have not previously been documented, so the comparison of these two varieties is not possible but according to its native growth, it seems that both shallot can endure low temperature of germination.

Application of $GA_3$ did not significantly affect germination, at the concentrations that were used; the same effect of $GA_3$ has been shown for seeds of *Fagus sylvatica*, *Picea sitchensis and Kelussia odoratissima* (Mortensen and Eriksen, 2004; Etemadi et al., 2010). However, our results cannot weaken the role of GAs in germination of *K. odoratissima* and Iranian shallot revealed previous publication (Etemadi et al., 2010). The Jones and Stoddar (1997) hypothesized that sufficient GAs required for radical protrusion, which produced during moist chilling, the same as proved for both endemic plant of Iran, *K. odoratissima* and Iranian shallot, too. So, addition of exogenous GA did not have a greater effect on their germination and it appears that no dormancy existed after stratification for *K. odoratissima* (Etemadi et al., 2010), Iranian shallot and *F. sylvatica* (Mortensen and Eriksen, 2004). $GA_3$ can accelerate germination of Iranian shallot and the lowest T50 get in 500 ppm of $GA_3$ application.

## Conclusion

In the presented study, it can be concluded that warm temperature (10℃) after prolonged period of stratification in 4℃, even more as shown earlier, were sufficient for germination of Iranian shallot that is native to the central part of Iran.

### REFERENCES

Aoba T (1967). Effects of different temperatures on seed germination of garden ornamentals in Allium. J. Jpn. Soc. Hortic. Sci., 36: 71-76.

AOSA (1983). Seed vigor testing handbook. Contribution No. 32. Association of official seed analysis. In: Valdiva, CB. Sanchez-Urdaneta, AB. Aguirre, JR. Trejo, C. Cardenas, E. and Villegasm A. 2006. Temperature and mechanical scarification on seed germination of maguey (*Agave salmiana* otto ex Salm-Dyck). Seed Sci. Technol., 34: 47-56.

Ashrafi F, Akhavan SA, Kazemzadeh A (2004). Effect of aqueous extract of shallot (*Allium ascalonicum*) on inhibition of growth of Pseudomonas aeroginosa. Iranian J. Pharm. Res., 2: 71.

Asili A, Behravan J, Naghavi MR, Asili J (2010). Genetic diversity of Persian shallot (*Allium hirtifolium*) ecotypes based on morphological traits, allicin content and RAPD markers. J. Medic. Aromat. Plants, 1(1): 1-6.

Dalezkaya TV, Nikiforova VN (1984). Study of germination of some onion species. Ecological Problems of Seed-growing of Introduced Plants, Riga, pp. 24-25 (in Russian).

De Hertogh AA, Zimmer K (1993). Allium—ornamental species. In: De Hertogh, A.A. and Le Nard, M. (Eds), The Physiology of Flowering Bulbs. 187–200 pp. Amsterdam: Elsevier, p. 811.

Ebrahimi R, Zamani Z, Kashi A (2009). Genetic diversity evaluation of wild Persian shallot (*Allium hirtifolium* Boiss.) using morphological and RAPD markers. Sci. Hortic., 119: 345–435.

Etemadi N, Haghighi M, Nikbakht A, Zamani N (2010). Methods to promote germination of *Kelussia odoratissima* Mozaff., Iranian endemic medicinal plant. *Herba polinica,* p. 2.

Ghahreman A (1984). Color Atlas of Iranian Plants. Institute of Forestries and Grasslands, Botany Division, 5: 512.

Gutterman Y, Kamenetsky R, Van Rooyen MA (1995). Comparative study of seed germination of two *Allium* species from different habitats in the Negev Desert highlands. J. Arid Environ., 29: 303-315.

Hanelt P, Schultze-Motel J, Fritsch R, Kruse J, Maas HI, Ohle H, Pistrick K (1992). Infrageneric Grouping of *Allium* – the Gatersleben Approach. In The Genus *Allium* – Taxonomic Problems and Genetic Resources (Eds. Hanelt, P., Hammer, K., and Knupffer, H.), Gatersleben: IPK, pp. 107-123.

Heydecker W, Wainwright H (1976). More rapid uniform germination of *Cyclamen persicum* L, Sci. Hort., pp. 183-189.

Hurtman H, Kester D, Davis F, Geneve R (1997). Plant propagation: Principles and Propagationes. 6th edition.Prentice-Hall Inc. New Jersey. USA, p. 770.

Jafarian A, Ghannadi A, Elyasi A (2003). The effects of *Allium hirtifolium* Boiss. on cell-mediated immune response in mice. Iranian J. Pharm. Res., 2: 51-55.

Kamenetsky R, Yitzchak G (2000). Germination strategies of some Allium species of the subgenus *Melanocrommuyum* from arid zone of Central Asia. J. Arid Environ., 45: 61-71.

Kamenetsky R (1991). Morphological types and root systems as indicators of evolutionary pathways in the genus *Allium*. In: Hanelt, P., Hammer, K. and Knupffer H. (Eds), The Genus Allium - Taxonomic Problems and Genetic Resources (1992), pp. 129-135. Proceedings of an International Symposium held at Gatersleben, Germany, p. 359.

McNeal DW, Ownbey M (1973). Bulb morphology in some western North American species of Allium. Madrono, 22: 10-24.

Mortensen LC, Eriksen EN (2004). The effect of Gibberelic acid, paclobutrazol and ethephon on the germination of Fagus *silvatica* and *Picea sitchensis* seeds exposed to varying durations of moist chilling. Seed Sci. Technol., 32: 21-33.

Nicolas C, Nicolas G, Rodriguez D (1996). Antagonistic effect of Abscisic acid and Gibberellic acid of the breaking dormancy of *Faguse salvatica* seeds. Physiol. Plant., 96: 244-250.

Nicolas C, Rodriguez D, Poulsen F, Eriksen V, Nicolas G (1997). The expression Of Abscisic acid-responsive glycin-rich protein coincides with the level of seed dormancy in *Faguse salvatica*. Plant and Cell Biol., pp. 1303-1310.

Rechinger KH (1984). Flora Iranica, Alliaceae. Akademische Druck, Univ. Verlagsanstalt Graz, Austria, 76: 85.

Rubatzky VE, Yamaguchi M (1997). World Vegetables, Principles, Production and Nutritive Values, Second ed. Chapman and Hall/International Thompson Publishing.

Salunkhe DK, Kadam SS (1998). Handbook of Vegetable Science and Technology.Marcel Dekker, Inc. New York, p. 721.

Schulz V, Hansel R, Tyler VE (1998). Rational Phytotherapy: A Physician’s Guide to Herbal Medicine. Berlin: Springer–Verlag.

Specht CE, Keller ERJ (1997). Temperature requirements for seed germination in the species of the genus *Allium* L. Genet. Resour. Crop Evol., 44: 509-517.

Zimmer K, Weckeck K (1989). Effect of temperature on some ornamental Alliums. Acta Hortic., 246: 131-134.