J. Agric. Food. Tech., 2(3)56-60, 2012

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

Seed Dormancy Breaking Evaluation Of Two Subspecies *Seashore Iris*, Iranian Native, Through *In Vitro* Culture

Golrokh Esmaili Lashkarian[1], S. Kalatehjari[2], M. Khosroshahli[3]
[1]M.S. Student, Department of Horticultural sciences group, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Horticultural sciences group, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[3]Department of Agricultural Biotechnology group, Science and Research Branch, Islamic Azad University, Tehran, Iran.

ABSTRACT

Iris spuria is a herbaceous and perennial plant with long and drawn leaves which is surrounded by purple flowers. Sometimes the thin bearded form of iris is called "seashore iris" which in terms of the distribution, this type has a broad geographic area and has a variety of color and size.
Present project has been done as a case study on surveying the effects of various mechanical and chemical treatments alone and in combination on eliminating the seed recession of the two subspecies (*Iris spuria* L. subsp. *Musulaminica* (Fomin) Takht and *Iris Supria* L. subsp. *Halophila*) in vitro. After washing by distilled water, outer shell of mature seeds of two subspecies removed physically. Then the seeds with and without scratches were treated with different concentrations of ethylene gas and GA3 in the natural environment with natural light of 40-45 micro molecule per square meters in growth module with 25 ± 2 centigrade degree. The project results will indicate that in hormone free environments with seed soaking and scraping treatments and also in ethylene seed treatment without scratches no germination would be seen. The assumption is that the best results would be obtained from GA3 $^{1mg}/_{l}$ hormonal treatment of *I. musulmanica* genotype and *I. halophila* genotype respectively over 60% and 50%. In present project, diagrams have been done by software and data analysis also has been done by SAS software.
KEY WORDS: Iridaceae, iris spuria, I. musulmanica, I. halophila, GA3, ethylene.

INTRODUCTION

Seeds often sprout with warm weather in spring and some even start to sprout in the summer and fall. Iris seeds as most species of temperate climate have dormancy and their germination is possible when germination inhibitors such as chemicals are removed and receive needed light and nutrient.

ABA and GA3 levels of seeds are the dormancy and germination controllers and using of them in order to study seed dormancy in vitro has been reported many times such as Qi-he Yaung reports in 2007.

Iridaceae Iris contains more than 300 species with different values of medicinal and horticultural. The distribution scopes of this plant are various and they are distributed from mountainous regions to grassy hillsides and meadows and also they are found along rivers in Europe, Middle East, North Africa, Asia and North America. Iran also has various species of iris generally found in northern areas, Gorgan, Qazvin, Kerman and Lorestan. *Iris spuria* is divided to different subspecies based on distribution and has a wide geographical area. In the west, Iran, a subspecies under the name of musulmanica can be seen which is somewhat taller and have larger flowers than Khorasan's subspecies named halophila. Halophila plant in Iran doesn't have yellowish flowers and despite most Iranian irises both mentioned subspecies grow in wet and salty meadows and along the rivers (4,9).
Iris supuria subsp. *musulmanica* usually germinates at about 30-545 days and may be in ideal conditions no sprout may be found. Seeds are planted at a depth of 6 mm in a metal container at a temperature of about 15-20 ° C (7).

In this project the aim of using various mechanical and chemical treatments in the study of seed dormancy has been done on two *Iris spuria* subspecies named:
Iris spuria L. subsp. *Musulmanica* (Fomin) Takht, *Iris spuria* L. subsp. *halophila* (Pall) .

MATERIALS AND METHODS

Mature seeds of iris was collected from Joghagard an environ of Fereydounshahr in Isfahan with a height of 1700 meters over sea level and they were transported to the laboratory in order to test their seed dormancy. Then in the

***Corresponding Author**: Golrokh Esmaili Lashkarian, M.S. Student, Department of Horticultural sciences group, Science and Research Branch, Islamic Azad University, Tehran, Iran.

preparation stage, the seeds were soaked in distilled water for 24 hours and later a sandpaper was used to give a mechanical abrasion to seeds.

Then after disinfectant treatment, these seeds transferred to the medium of $^{1}/_{2}\,ms$ containing GA3 (0,1,2 $^{mg}/_{l}$) and the medium without hormone of $^{1}/_{2}\,ms$. Also it is noteworthy that numbers of these seeds without scratches and others with scratches were put in a closed container containing ethylene gas of 100ppm. After one week they were transferred to a medium without hormone. Ambient condition and concentration of two parameters are in growth regulation and are visible according to Table 1. The stock solutions of medium of $^{1}/_{2}\ ms$ and growth regulators were prepared and were kept in the refrigerator. It is noteworthy that in order to prepare one liter of the medium, a certain proportion of mineral and organic solutions, sucrose and agar were mixed together and then they were sterilized through autoclave at 121˚C and 15 psi pressure in 20 minutes. Then the mediums were transferred to 9cm Petri dishes and after solidification were kept in refrigerators. In the next stage after 24 hours soaking in distilled water, seeds were scratched by sandpaper and after removing the outer shell they were kept in 95% ethanol for 60 seconds. After leaching in order to become disinfected they were put in a concentration of 2% NaOCl solution for 10 minutes with few drops of Twin 20 to increase exposure to disinfectant. Then they were washed with sterilized distilled water several times. After seed disinfection process, seeds were transferred to Petri dishes containing culture medium and hormone treatments and put in the culture chamber with natural light of 40-45 micro molecules on square meters per second and the temperature was 25 ± 2 centigrade degree (1).

Proprietary data in this study were factorial with 5 replicates and analyzed with a random basis design.

RESULTS AND DISCUSSION

Over a four week period of testing, seed dormancy was broken and germination began to start. The results obtained from variance table (tabel 2.) of treatment effects on seed dormancy showed that all qualities were significant in 1% and 5% levels.

1. After checking genotypes in removing seed dormancy, 21/53% of genotype seeds of *I. musulmanica* sprouted and genotype seeds of *I. halophila* sprouted about 17%. According to the chart reviews (Diagram1.), the result was that they have significant differences statically. Results showed that removal of seed dormancy depends on plant genotype (6) in a way that musulminica showed a better response to the treatment of dormancy removing than halophila .
2. After reviewing treatments implemented, it is concluded that control treatment which is free from any growth regulator and ethylene treated seed without scratches has not been effective in removing seed dormancy and no sprout was seen on such under treatment seeds. Among other treatments, hormone treatments could effectively resolve the seed dormancy. The best treatment in removing dormancy was the treatment including GA3 $1mg/l$ with the rate of 66/33% and after treatments containing GA3 $2mg/l$ and ethylene gas with mechanical abrasion respectively 28/33% and 6/5% of seeds started to sprout (Diagram2.). Also according to the (Diagram.3) in reviewing the interaction of genotype and hormonal treatment on the sprout percentage, the best results were obtained from hormone treatment of GA3 $1mg/l$ from *I. musulminica* and after that *I. halophila* genotype respectively 66/33% and 56/66% which statically were obtained at 5% level of significance.
3. In different surveys it can be obtained that seed dormancy in iris species under study only with mechanical abrasions or hormone treatments will not be removed and for removing their dormancy, hormonal treatments with conjugated mechanical abrasion should be used. Hormonal treatment of gibberellic acid was more effective than ethylene in removing dormancy.
4. In comparison with similar projects it was found that the hard shell of iris is one of the dormancy factors and should proceed to fix it. Mechanical abrasion can be an effective aid and its combination with GA3 hormone would have a more effective rule in removing dormancy than ethylene.

Samarah *et* al., 2009 (11) reported that by using GA3 they could remove dormancy of black iris seeds and 87% of seeds sprouted in this treatment.

GA3 can be a substitute for the seeds which need cold weather and most of iris species that need to spend a period in a wet and cold weather(12). GA3 in concentration of $1mg/l$ with mechanical abarsion of the outer shell can be effective in dormancy removing of the seeds under study (2). Ethylene is a germination stimulant and will increase that indirectly(3).

In some references(5,8) ethylene hormone was used in the form of gas to remove dormancy of corm and bulbous plants.

It is suggested that ethylene production in the stage of before germination of oat seed will increase cytokinesis which cause germination. In present study using ethylene without mechanical abrasion was not effective on removing seed dormancy and using of ethylene with mechanical abrasion was less effective than treatment with gibberellin. Also best results of hormonal treatment of GA3 $1 mg/l$ from genotype *I.musulmanica* and then genotype *I.halophila* respectively obtained with 66/33% and 56/66% which statically were significant in 5% level. Germination percentage of ethylene treatment with abrasion in iris *I.musulmanica* and *I.halophila* was respectively 9% and 4%.

Table1. treatments, components of media and light regimes used in this study

	Treatments	Component	Light regime
1	Soaking + Scrashing	1/2MS without growth regulation	Medium light intensity[1]
2	Soaking + scrashing + ethylen	1/2MS without growth regulation	Medium light intensity
3	Soaking +scrashing	1/2MS + 2mg/l GA3	Medium light intensity
4	Soaking +scrashing	1/2MS + 2mg/2 GA3	Medium light intensity
5	Soaking + ethylen	1/2MS without growth regulation	Medium light intensity

[1] 40-45 µmol $m^{-2}s^{-1}$

Table 2. analysis variance for effect of treatments on breacking seed dormancy

Sources	Free degree	Germination%
genotype	1	154.13**
treatment	4	4156.88 **
Genotype*treatment	4	29.88*
error	20	12.73
Corrected total	29	

* *: significantly different ($p<0.01$) *: significantly different ($p<0.05$).

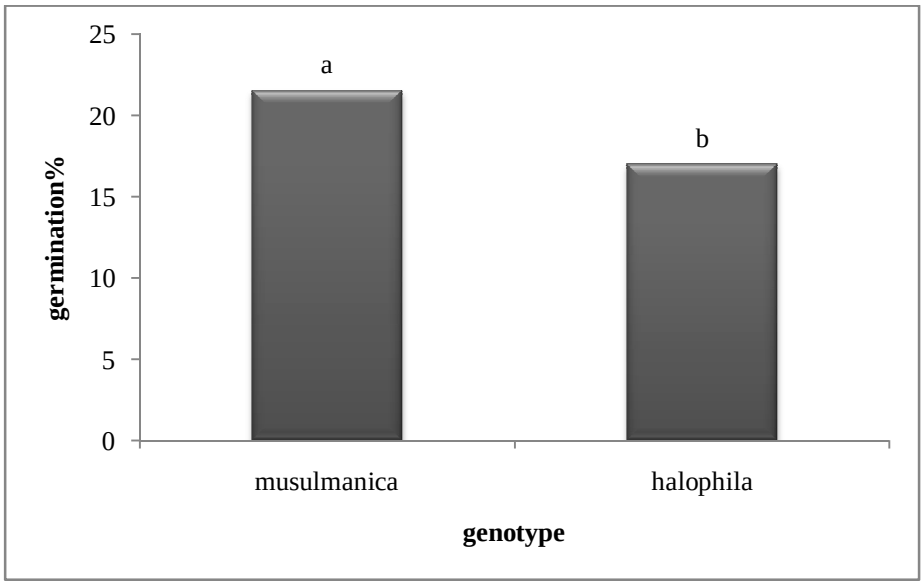

Diagram 1.
Effect of genotype on germination percentage

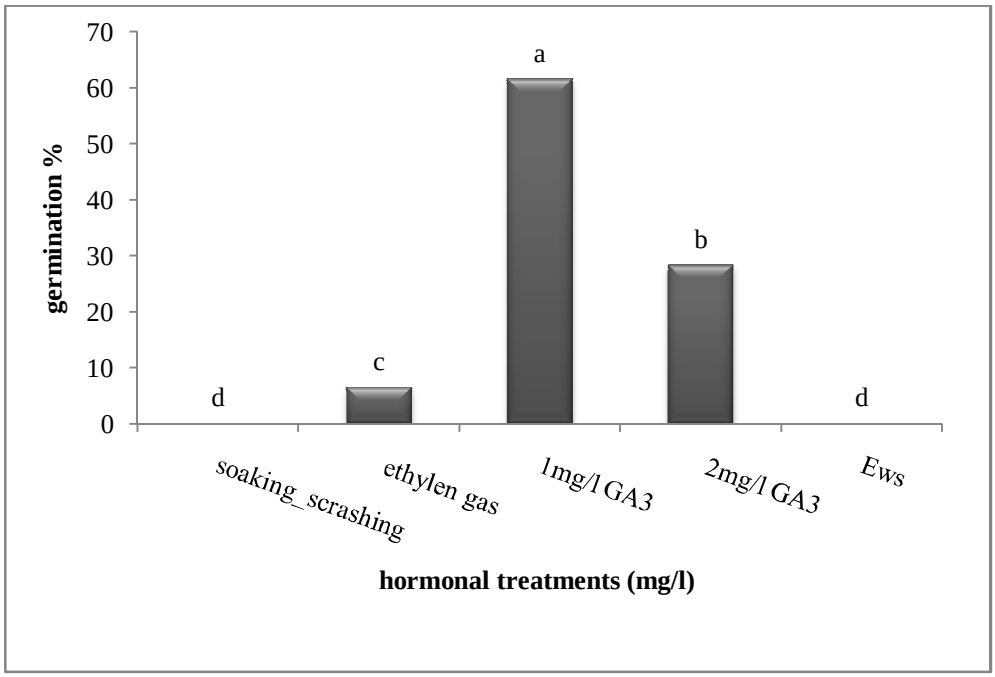

Diagram 2.
Effect of hormonal treatments on germination percentage (EWS: Ethylen Without Scrashing)

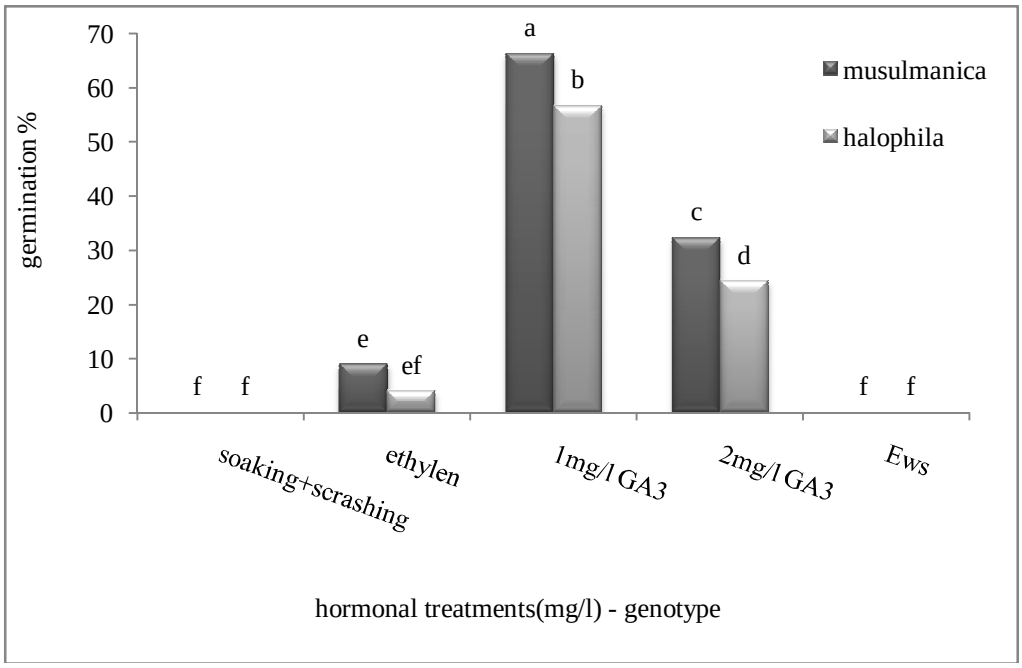

Diagram 3.
Interaction effect of hormonal treatments - genotype on germination percentage (EWS: Ethylen Without Scrashing)

REFERENCES

1. Baskin, J. M., and C. C. Baskin, 2004. A classification system for seed dormancy. Seed Sci. Res. 14: 1-16.
2. Bayat, H., M. Arab, M. Khosh-khui, R. Heidarihaee and V. Rahimi, 2009. Study of Scrafication and GA_3 treatment for breaking dormancy in Iranian Iris (Iris songarica L.) in vitro culture. 6^{Th} iranian horticultural science congress-12-15 july 2009. Rasht. iran.
3. Derek Bewley, J., 1997. Seed germination and dormancy. The Plant Cell.9:1055-1066.
4. Ghahraman, A., 1982. Flora of iran. research institute of Forests and Rangelands publishers. No 3, pp:363.
5. Imanishi, H., E. J. Fortanier, 2003. Effect of exposing freesia corms to ethylene or to smoke on dormancy-breaking and flowering. 18(4): 381-389.
6. Kresa, S., A. Mihovilovic, M. C-Perica, B. Mitic, M.Baric, I.Vresk and S. Marchetti, 2009. In vitro regeneration of the croatian endemic species iris adriatica Trinajstic ex mitic. Acta biological cracoviensia series botanica 51/2:7-12.
7. Lenz, L. W., 1978. Iris classification. In warburton B (Ed) the world of iris. The american iris society, Wichita, Kansas. USA: 1-42.
8. Masuda, M., T. Asahira, 1980. Effect of ethylene on breaking dormancy of freesia corms. Scientia Horticulturae, 13(1): 85-92.
9. Mazhary, N., 1999. Flora of iran. research institute of Forests and Rangelands publishers. No 31.
10. Qi-He, Y., W. Ye and X. Yin, 2007. Dormancy and germination of Areca triandra seeds. Sci. Hort., 113(1) :107-111.
11. Samarah, N. H., S. A. Qurashi, N. S. Karam and R. A. Shibli, 2009. In vivo and in vitro seed germination in black iris: a potential new floricultural crop from jordan. Acta hort. (ISHS) 813:113-120.
12. Sun, Y. C., Y.J. Zhang and K. Wang, 2006. NaOH scrafication and stratification improve germination of *iris lactea* var. *chinensis* seed. HortSci., 41(3): 773-774.