

BREAKING OF SEED DORMANCY IN *Iris suaveolens* Boiss. et Reuter UNDER *in vitro* CONDITIONS

Mortaza Hajyzadeh^{1✉}, Mehmet Ugur Yildirim², Sam Mokhtarzadeh³,
Ercument Osman Sarihan², Khalid Mahmood Khawar⁴

¹ Central Laboratory, Mehmet Emin Acar Campus 73000, Sirnak University Rectorship Building, Yeni Mahalle, Sirnak, Turkey

² Department of Field Crops, Faculty of Agriculture and Natural Sciences, Usak University, 64200, Eylül Campus, Izmir Yolu, Usak, Turkey

³ Department of Field Crops, Faculty of Agriculture, Bingol University, Selahaddin-i Eyyubi Mah., 12000, Bingol, Turkey

⁴ Department of Field Crops, Faculty of Agriculture, Ankara University, 1 Fatih Street, 06110, Ankara, Turkey

ABSTRACT

Iris species have great economic importance for their use in ornamental and pharmaceutical industry. Flora of Turkey reports about 43 local taxons of which 16 are endemic. *Iris suaveolens* Boiss. et Reuter is an endemic species with high seed dormancy and has high tolerance to cold and drought. This study focused on seed dormancy break of *I. suaveolens* under *in vitro* conditions. In the first experiment, the seeds were given stratification treatment on MS medium containing different concentrations of BAP with or without NAA. The 2nd experiment reports effects of alternative combinations of cold (at 4°C, 24 h dark) – warm (16 h light photoperiod) conditions on medium containing different concentrations of plant growth regulators on agar solidified MS medium. Both experiments showed about 8.33% seed germination against 64.5% seed viability as confirmed by tetrazolium test. All germinating seeds were abnormal and very weak. In the third experiment, 10 minutes acid scarified seeds cultured at 24°C in 16 h light photo period on MS medium achieved 60.0% germination. Similar treatment under cold + dark condition was inhibitory and failed to show identical results. This underlines the importance of acid scarification, photoperiod and warm treatments of *I. suaveolens* seeds to break seed dormancy. All germinated seeds showed normal growth and development under field conditions.

Key words: seeds, breaking dormancy, ornamental, medicinal, *Iris suaveolens*

INTRODUCTION

Genus *Iris*, family *Iridaceae* has a vital place among rhizomatous, perennials and is widely available in the temperate regions of the North Hemisphere. Many species of genus *Iris* are cultivated for their use in ornamental, pharmaceutical, medicinal, perfume and cosmetic industries [Atak et al. 2014]. There are around 43 species and taxons (found in Turkey) in this genus that are well elaborated in Flora of Turkey and Tubives [2017]. Sixteen (16) species of them are endemic [Tubives 2017].

Iris suaveolens Boiss. et Reuter is an important geophyte from this family that is very tolerant to chilly, dry spell and develops well under Mediterranean climatic conditions of Turkey [Kaššák 2012].

It is an annual plant that develops during early spring to autumn and spreads from the Balkans to Marmara region of Turkey in the bluff and woods of the rough mountains with purple and yellow blooms [Mathew 1984]. This species is highly desired for its economic value, but is neglected and untouched for

✉ m.hajyzadeh@gmail.com

number of reasons. Generally, the species is vegetatively propagated by extension of rhizomes or rhizome splits under favorable environmental conditions.

It is imperative to protect local species population by reintroduction, or by developing vegetative and sexual methods of propagation to re-establish them in their natural environment or under cultured conditions [Khawar et al. 2005, Parmaksiz and Khawar 2006, Ozel et al. 2008, Soyler et al. 2012]. It is notable that induction of *in vitro* morphogenetic response from the seeds is very limited. The seeds of this iris species most often fail to germinate, apparently because the seed coats prevent cell differentiation [Arditti and Pray 1969]. There are various studies on seed dormancy break in different plant species including iris species [Morgan 1990, Blumenthal 1986, Bell et al. 1995, Liu et al. 1998, Finch-Savage and Leubner-Metzger 2006, Sun et al. 2006, Rajjou et al. 2012].

Natural seed propagation of *I. suaveolens* is inefficient and very slow. There is no scientific study to propagate the species under *in vitro* or *ex vitro* conditions and evaluate it for large scale commercial propagation.

The seeds of numerous species (belonging to family *Iridaceae*) often develop dormancy [Arditti and Pray 1969, Tillich 2003] and the dormant seeds are difficult to germinate. This restricts establishment of new plants to maintain population size at desired levels.

Solution to seed dormancy release can be helpful to conserve the species under unpredictable natural conditions. Most outstanding types of seed dormancy incorporate mechanical [Blumenthal et al. 1986], morphological, morphophysiological [Coops and Veld 1995] and physiological dormancy [Arditti and Pray 1969, Morgan 1990], singly or in combination. This

conserves species to survive their sprouts under alluring conditions [Tillich 2003]. It is vital to break high seed dormancy among this species for reclamation of their plantings at places, where their population is under threat.

Some experiments have accounted to explore the impacts of treatment to remove the integument at the micropylar end in iris seed germination [Blumenthal et al. 1986]. However, factors affecting the seed dormancy may vary from species to species in relation to environmental and developmental conditions. It is well established that plant growth regulators play an important role during the seed germination [Ritchie and Gilroy 1998] and affect the physiological processes like growth and differentiation of plant cells [Ritchie and Gilroy 1998, Nadjafia et al. 2006]. Plant growth regulators like BAP (with cytokinin-type activity) and NAA (with auxin type activity) are also known to promote seed germination and break seed dormancy [Greipsson 2001, Nadjafia et al. 2006, Soyler and Khawar 2006]. It is well known that GAs also promote enzyme synthesis by converting stored starch nutrients to sugars. This, in turn, helps seed germination through reduction of abscisic acid and improved [Manz et al. 2005, Nadjafia et al. 2006] rapid cell respiration. However, changing environmental conditions also have bearing on germination behaviour of the seeds.

Variability in light-gibberellin and nitrate requirement of *Arabidopsis thaliana* seeds due to harvest time and conditions of dry storage effects seed germination is significant [Derkx et al. 1993a]. Comparative positive effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine, *Arbor vitae* and cappariss were

Table 1. Alternate dark and light treatments to seeds at 4°C in dark and 24 ±1°C in 16 h light photoperiod

Treatment No.	Dark treatment in Weeks at 4°C	16 h light photoperiod at 24 ±1°C in weeks
1	8	0
2	6	2
3	4	4
4	2	6
5	0	8

noted [Soyler and Khawar 2006, 2007]. It has also been reported that arabidopsis seed dormancy can be broken by treatments like light, cold stratification and growth regulators or chemicals like gibberellins and KNO_3 [Derkx and Karssen 1993a, b, Ali-Rachedi et al. 2004], NO – nitric oxide [Bethke et al. 2007], singly or in combination. Changing sensitivity to light and nitrate, but not to gibberellins, regulate seasonal dormancy patterns in *Sisymbrium officinale* seeds [Derkx and Karssen 1993b]. The external factors for seed germination could substitute or replace each other in complimentary or supplementary way [Cone and Spruit 1983] depending on genotypes, and the length of treatment time before or after ripening that could be determined by empirical testing [Finch-Savage et al. 2007].

Along these alluring lines, the aim of the study was to develop a protocol for breaking seed dormancy of highly interesting and priced ornamental *I. suaveolens* from Turkey.

MATERIAL AND METHODS

Plant material. Two plants of *I. suaveolens* with healthy and matured seed capsules were gathered in May 2016 from wilds of Uşak in western Turkey (Latitude: $38^{\circ}40'24''\text{N}$, Longitude: $29^{\circ}24'20''\text{E}$ at altitude of 910 m). Dry seeds of wild plants were collected in mid June 2016, after 4 weeks of anthesis, when they turned black and developed hard exterior cover. The capsules were cut and opened under sterilised conditions to obtain ripened seeds. The voucher samples of plants are saved at The Herbarium of the Istanbul University, Turkey vide No. ISTE 38966.

Tetrazolium test. Fresh, *I. suaveolens* seeds were used to evaluate the viability of the seeds (black colored, sub-globose seeds – about 0.4–0.5 cm in length and 0.3–0.35 cm in width). Three samples of 50 seeds each were carefully given vertical cuts avoiding damage to embryos using sharp scalpel blades to remove seed coats. These were soaked in 1 mg/ml 2, 3, 5 tetrazolium chloride solution in falcon tubes for 24 hours at $24 \pm 1^{\circ}\text{C}$.

All seed embryo tissues transforming bright transparent solution to formazan (red color) by reduction of tetrazolium chloride were accepted as viable. Whereas, variably red stained (light red coloured or mosaic red colored) embryos were counted weakly viable.

The seeds with (i) light red colored, mosaic red colored or (ii) non stained cotyledon tissues including embryos (plumule, embryonic axis and radicle) were considered dead [AOSA/SCST (2010)].

Seeds surface sterilization. The intact and non-infected seeds were precisely selected and surface was sterilized using 30% commercial bleach (3% NaOCl Ace, Turkey) and rinsed 3×3 min with sterilized distilled water to remove traces of bleach.

Three separate experiments were conducted to break seed dormancy of *I. suaveolens*.

Treating seeds with plant growth regulators on MS medium. The first experiment consisted of the seeds cultured on MS and MS medium containing diverse concentrations of 0, 0.3, 0.9, 1.5, 2 mg L^{-1} BAP + 0.6 mg L^{-1} NAA supplemented with 20 g L^{-1} sucrose. All cultures were maintained at 24°C under 16 h light photoperiod and $35 \text{ mol m}^{-2}\text{s}^{-1}$ light intensity. Germination was recorded following eight weeks.

Combined alternate cold-warm treatments on MS medium. In the first experiment, the seeds were placed on MS medium containing diverse concentrations of 0, 0.3, 0.9, 1.5, 2 mg L^{-1} BAP + 0.6 mg L^{-1} NAA supplemented with 20 g L^{-1} sucrose. The seeds in the first level were given alternate cold treatment at $4 \pm 1^{\circ}\text{C}$ for eight weeks (dark). The seeds belonging to the second, third and fourth levels were alternate cold treated in dark at 4°C for 6, 4 and 2 weeks followed by warm treatments for 2, 4 and 6, weeks (16 h light photoperiod) respectively, as shown in Table 1. The seeds in the 5th treatment were cultured at $24 \pm 1^{\circ}\text{C}$ for eight weeks (16 h light photoperiod).

Scarification under light and dark conditions on MS medium. In the third experiment, H_2SO_4 scarified seeds for 10 min were placed on MS medium supplemented with 20 g L^{-1} sucrose at $24 \pm 1^{\circ}\text{C}$ under 16 h light photoperiod using $35 \mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity or darkness at 4°C .

The control treatment consisted of non scarified seeds. Similarly, these were also cultured on MS medium and incubated under $35 \text{ mol m}^{-2}\text{s}^{-1}$ light intensity at $24 \pm 1^{\circ}\text{C}$ under 16 h light photoperiod using $35 \mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity or darkness at $24 \pm 1^{\circ}\text{C}$.

Every treatment contained 60 seeds, that were equally divided into six replications.

Acclimatization. Before transplanting the plants, they were taken out of agar containing medium,

washed with water and placed in water for 30–40 min in agreement with Aasim et al. [2010] to avoid shock to the germinated plantlets.

The germinated iris plantlets were transferred to 20-cell seedling trays covered with 17.78 cm long and 15.24 cm wide transparent adjustable rectangular boxes provided with two controlled vents that could be opened and closed any time to allow air circulation and maintain/adjust moisture level. The seedling trays contained peat moss. These (seedling trays covered with vented plastic domes) were kept up in the growth room for hardening for 15 days. Thereafter, the plants were transferred to the greenhouse under controlled conditions of temperature and humidity. They were transferred to the fields during April 2017 under ambient conditions of temperature (16–20°C during days and 8–12°C during night) and 55–65% humidity. The soil in each tray was spray treated with aluminum ethyl-14C phosphite after three days of culture to avoid development of any fungal disease [Soyler et al. 2013]. The plantlets were regularly monitored and compared with original plants (control) for the seeds, fruits, flowers (spathe, perigonium, androecium, gynoecium), inflorescence, and leaves to check morphological aberrations.

Statistical analysis. Sixty seeds, equally divided into 6 replications of 10 seeds (6 replications × 10 seeds), were used for each treatment. Statistical analysis of data in all experiments were performed by IBM SPSS 24. The data presented in percentages were subjected to arcsine transformation [Gomez and Gomez 1984]. All values were reported as means ± standard error.

RESULTS

Tetrazolium test. All viable seeds showed red staining with formation of formazon after reduction of the tetrazolium solution during seed testing. Non-viable seeds and embryos that were shrivelled and smaller in size could be distinguished by a white color or non-formazan staining. No intermediate shading or mosaics were noted on them. Therefore, it was conceived that tetrazolium test could be used as a reliable viability test for *I. suaveolens* with viability of 64.5%.

Plant-growth regulators treatments on MS medium. Plant growth regulators (BAP + NAA) treatment on seeds cultured on MS medium containing 0, 0.3, 0.9, 1.5, 2 mg L⁻¹ BAP + 0.6 mg L⁻¹ NAA supplemented with 20 g L⁻¹ sucrose and MS medium used singly

Seed germination (%)

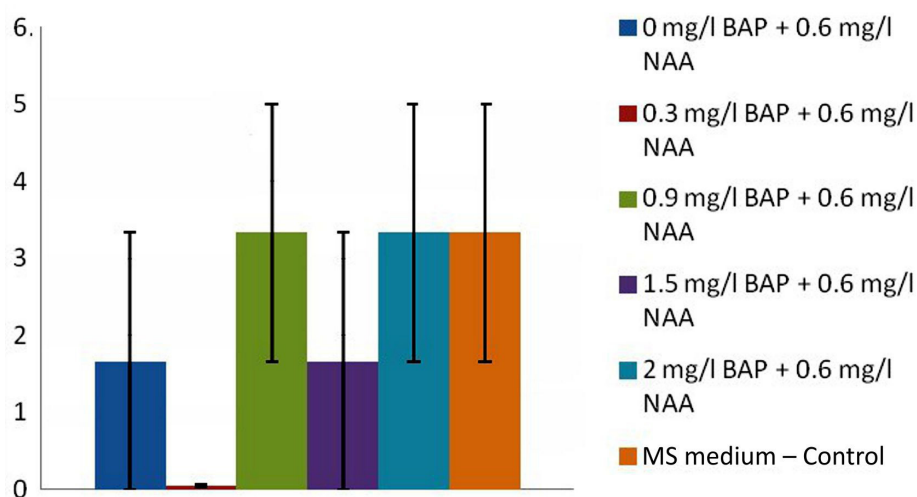


Fig. 1. Effects of varying BAP + NAA plant growth regulators' treatments on MS medium to germinate *I. suaveolens* seeds

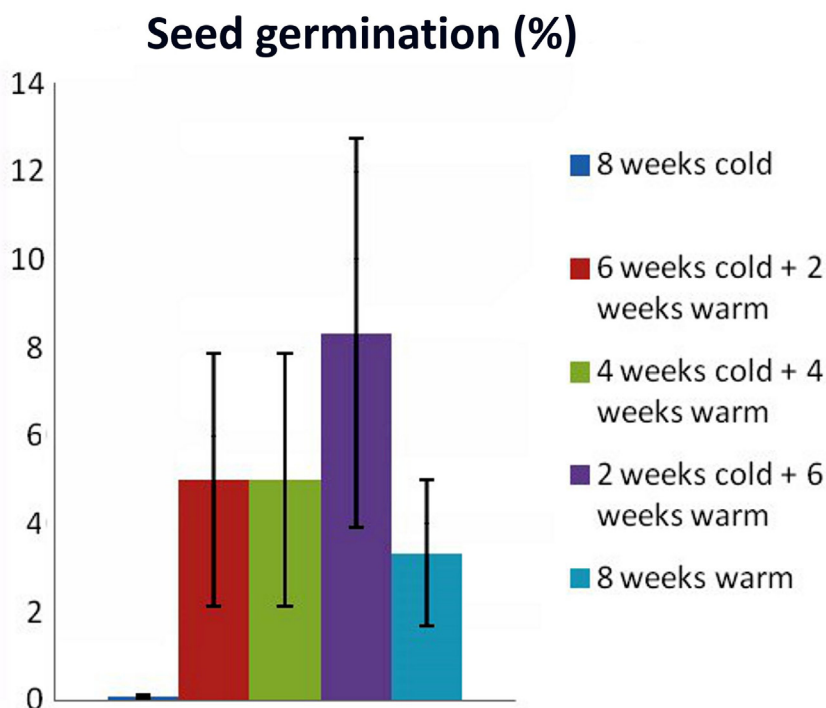


Fig. 2. Effects of alternate cold-warm treatments on MS medium on germination percentage of *I. suaveolens*

as control under *in vitro* conditions induced very low (0–3.33%) or negligible seed germination after eight weeks of culture (Fig. 1).

Combined alternate cold-warm stratification treatment on MS medium. It was assumed that culturing *I. suaveolens* seeds on MS medium ensured by combined alternate cold-warm stratification treatments could effectively break their dormancy.-

However, (i) cold, (ii) warm and (iii) cold + warm stratification treatments were generally not effective. Seeds exposed to cold treatment for 8 weeks did not germinate (Fig. 2).

Only 5% seed germination was noted on 6 weeks cold + 2 weeks warm and 4 weeks cold + 4 weeks warm stratified seeds. The seeds showed 8.33% and 3.33% germination on (i) 2 weeks cold + 6 weeks warm and (ii) 8 weeks warm stratification, respectively. All cold and warm stratified seeds showed abnormal growth (Fig. 3a) with albino, weak, feeble and undersized seedlings. These seeds never turned to seedlings.

A comparison of results showed that only cold stratification treatment was not enough to break seed dormancy at sufficient level.

Combined scarification and stratification under dark and light conditions on MS medium. The impact of mechanically scarified seeds cultured on MS medium in 16 h light showed significant improvement with 60% germination compared to all other treatments (Fig. 4). Scarified seeds, incubated on MS medium in darkness, germinated in 8.33%.

Only 3.33% seed germination was noted on non-scarified seeds cultured under light and no germination was noted on non-scarified seeds under dark conditions.

Non-scarified seeds on control treatments under 16 h light photoperiod germinated abnormal, albino, poorly physiqued, weak, and undersized plantlets.

Healthy seedling growth was noted on scarified and stratified seeds (Fig. 3b, c) irrespective of photoperiod treatment and rate of germination. They had rapid

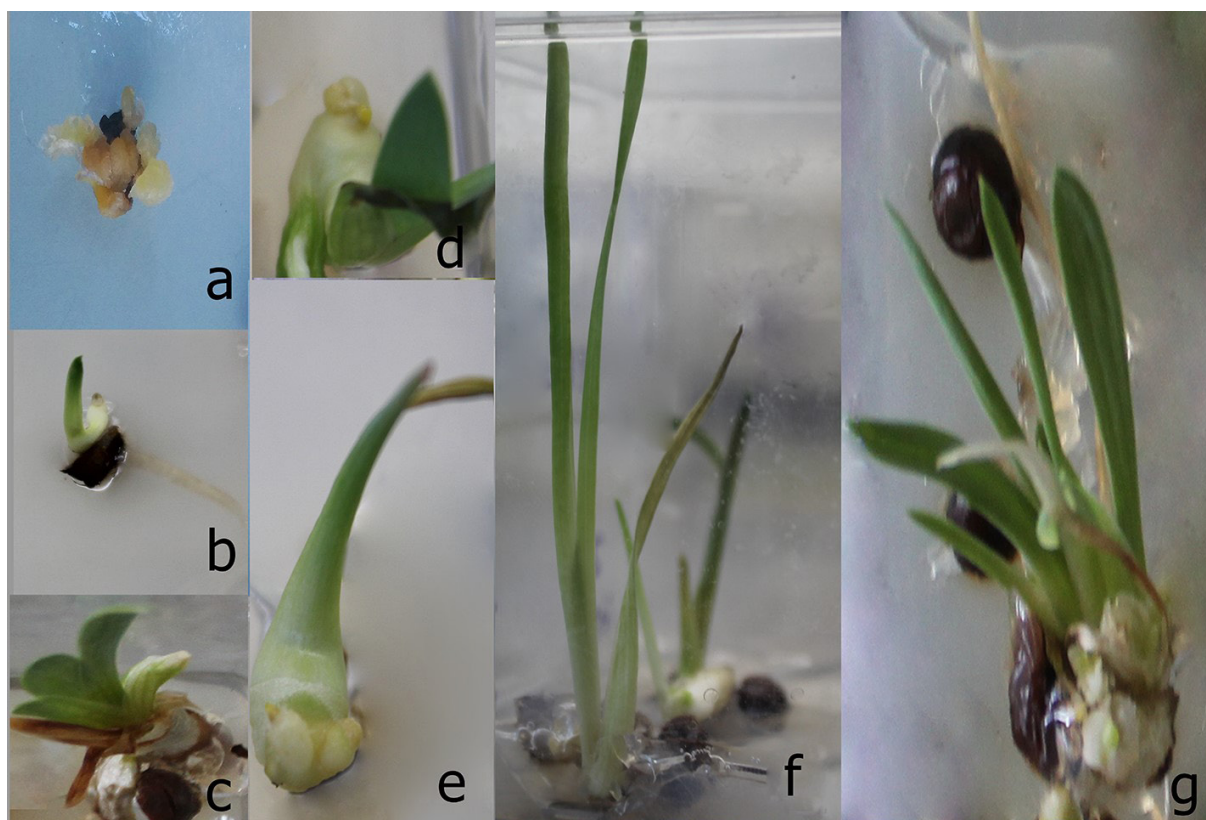


Fig. 3. Breaking seed dormancy *Iris suaveolens* (a); abnormal seed germination on cold + warm stratified seeds (b, c); seedling growth on acid scarified seeds (d, e, f, g); the seedlings showing normal, healthy and rapid growth

growth with induction of rhizomes (Fig. 3d, e, f, g) and roots (Fig. 5a, b, c) on MS medium after 18 weeks of culture. Germinated seedlings were transferred to pots for development into plantlets.

Acclimatization. The germinated iris plantlets (48 in number) had no difficulty to grow in seedling trays enclosed with transparent, vented domes, during hardening for 15 days in the growth room under 16 hour light $35 \text{ mol m}^{-2}\text{s}^{-1}$ light 8 hours dark photoperiod at $24 \pm 1^\circ\text{C}$. Thereafter, when the plants showed good growth under greenhouse without any stress, vented domes were completely removed. These plants did not face any problem under field conditions and showed 100% acclimatization. Growth and developmental behavior of the tissue cultured 13–14 cm long plants under field conditions guaranteed effective adjustment of tissue cultured plants compared to the 11–12 cm long plants growing at the

indigenous habitat. These plantlets induced flowers after about 12 months growth in the field and set flowers during late March–late April.

DISCUSSION

In spite of impressive advance over past decades [Graeber et al. 2012, Rajjou et al. 2012], seed dormancy stays “one of the minimum studied wonders in seed science” [Finkelstein et al. 2008]. Seed dormancy is an important trait to plant survival and guarantees that seeds grow just when natural conditions are ideal [Amen 1963]. This is a versatile attribute in various seed-plant species, empowering wild plants to make due under distressing conditions in nature [Finkelstein et al. 2008, Rajjou et al. 2012].

Previous literature portrays cold [Blumenthal et al. 1986] and warm treatment [Morgan 1990], synthetic

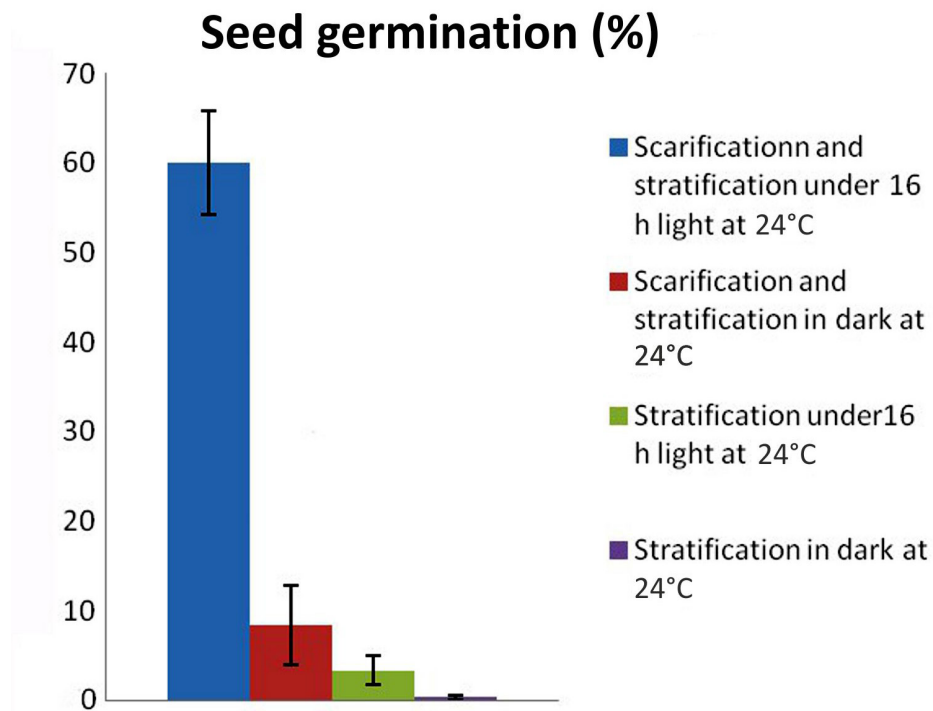


Fig. 4. Effects of stratification and scarification with warm or cold, treatments under light and dark conditions on MS medium

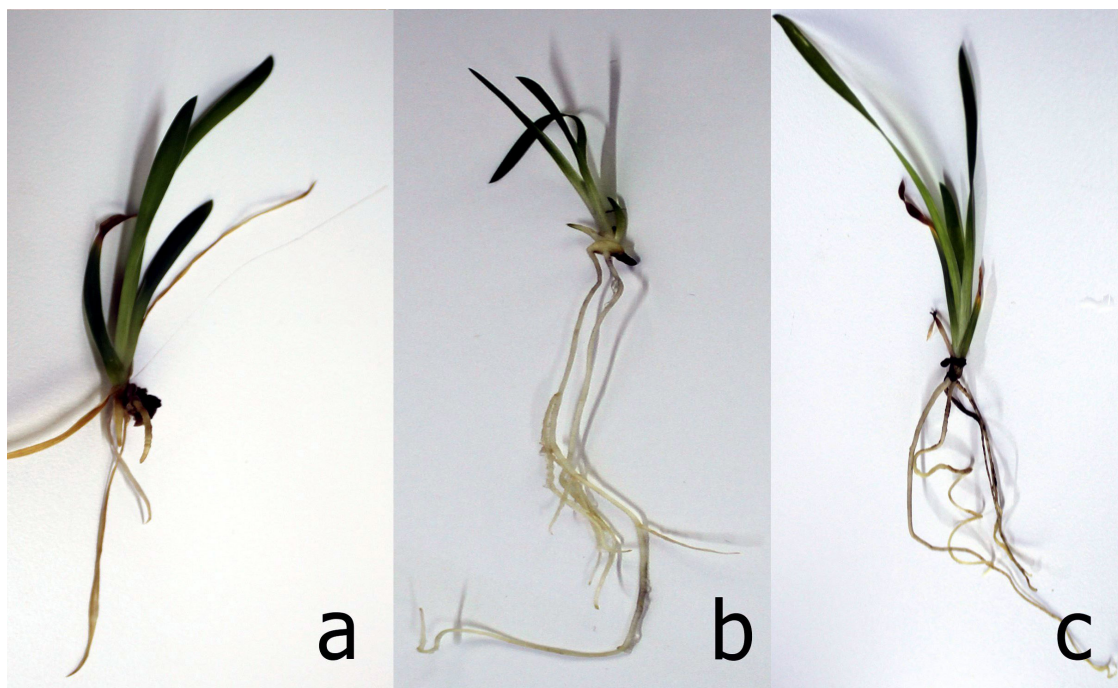


Fig. 5. Rooting and establishment of *Iris suaveolens* seedlings obtained after seed germination under *in vitro* conditions after 18 weeks of culture (a, b, c)

[Sun et al. 2006] and physical scarification of iris seed coats [Blumenthal et al. 1986] or techniques utilizing mixes of chilly, warm treatment [Jones 2012] and scarification to break iris seed dormancy. The results from this study confirm previous studies and approve the use of alternate cold and warm treatments along physical scarification for breaking seed dormancy of *I. suaveolens*. Many researchers have explored dormancy among *Iris* species [Blumenthal et al. 1986, Morgan 1990]. Germination of *I. suaveolens* seeds has not been examined, as the knowledge is limited and the net cause of dormancy is not known.

There are studies on morpho-physiological dormancy of different species of iris [Blumenthal et al. 1986, Sun et al. 2006], excluding *I. suaveolens*. These experiments were carried out to find the effects of BAP + NAA treatments, cold warm stratification and scarification + cold warm stratifications on breaking seed dormancy.

The reported studies show that there is a conceivable significance of morpho-physiological dormancy in almost all species of iris with a very few species that do not need any treatment to break seed dormancy [Stoltz 1968, Arditti and Pray 1969, Tillich 2003, Jones 2012]. Most of these need a growth and development time to get mature embryos in seeds to break dormancy [Tillich 2003].

The zygotic embryo of *I. suaveolens* in a developing seed is rudimentary and around 1.5–2 mm in length (personal observation), which needs maturing with multiplication of cells to germinate the seeds by emergence of radicle. Scarification + warm treatment is important for germination proposing that inhibitors in the seed endosperm and embryo must be wiped out before radicle emergence [Amen 1963, Stoltz 1968, Arditti and Pray 1969]. Morpho-physiological dormancy is frequently overcome through a blend of scarification and either warm and chilly treatments or any of them used singly to break dormancy [Arditti and Pray 1969]. Results of the study confirm that *I. suaveolens* seeds coat mechanically confined germination like other *Iris* species [Stoltz 1968, Blumenthal et al. 1986]. Gibberellic acid treatment overcomes dormancy in the species recommending profound dormancy in its seeds [Jones 2012]. The results of this study also confirm that combined physical, morphological, and physiological barriers are also responsible for induc-

ing the seed dormancy of *I. suaveolens* and need very careful handling.

Results of this study underscore importance of blending scarification and warm treatments under light. Seed treatments with hormones were also ineffective in contradiction to Sumlu et al. [2010]. Scarification and cold treatments under dark failed to sprout seeds in contradiction to Morgan [1990], who germinated seeds of *I. virginiana* by applying cold moistened treatments after warm moistened treatments.

The highest germination of *I. suaveolens* was accomplished by culturing scarified seeds for eight weeks under warm and moistened conditions in light at 24°C. The same treatment induced 2% germination under cool dark conditions. The results confirm that the seeds had also thermal and photoperiod dormancy that can be alleviated by scarification treatments. Results (60% germination) are almost in parallel to those of tetrazolium test (64.5% viability). It is also assumed that inbreeding and small populations hinder reduced seed set along with high seed dormancy in remaining seeds of endemic and threatened plant species [Severns 2003].

Success in acclimatization of *in vitro* grown plants is a vital for commercialization [Mokhtarzadeh et al. 2013]. Breaking seed dormancy guarantees a higher yield and quick proliferation of sprouted plants, consequently survival of the plantlets following transfer to the fields determined the effectiveness of this protocol. Blossoms of *in vitro* germinated plants were likewise seen to be morphologically like the naturally grown plants. All sprouted seedlings showed normal growth and development under field conditions, as they were hard enough to acclimatize under indigenous conditions and guaranteed a superior survival with no morphological variations from the norm.

CONCLUSIONS

This study underlines the importance of combined treatments to break morpho-physiological seed dormancy of *I. suaveolens*. The study further points out the significance of combined scarification, phytohormonal treatments and alternate cold (dark) – warm (16 h light photoperiod) treatments on agar solidified MS medium under *in vitro* conditions for easy breaking of *I. suaveolens* seed dormancy.

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REFERENCES

- Aasim, M., Khawar, K.M., Özcan, S. (2010). Efficient *in vitro* propagation from preconditioned embryonic axes of Turkish cowpea (*Vigna unguiculata* L.) cultivar Akkiz. Arch. Biol. Sci., 62(4), 1047–1052.
- Ali-Rachedi, S., Bouinot, D., Wagner, M.H., Bonnet, M., Sotta, B., Grappin, P., Jullien, M. (2004). Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds, studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. Planta, 219(1), 479–488.
- Amen, R.D. (1963). The concept of seed dormancy. Am. Sci., 51, 408–424.
- AOSA/SCST (2010). Tetrazolium testing handbook. Association of Official Seed Analysts and Society of Commercial Seed Technologists, Moline.
- Arditti, J., Pray, T.R. (1969). Dormancy factors in *Iris* (Iridaceae) Seeds. Am. J. Bot., 56(3), 254–259.
- Atak, A., Kaya, E., Erken, K. (2014). Determination of quantity and purity of some geophytes collected from the flora of Turkey. Asian J. Plant Sci., 13(3), 98–110.
- Bell, D.T., Rokich, D.P., McChesney, C.J., Plummer, J.A. (1995). Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. J. Veg. Sci., 6, 797–806.
- Bethke, P.C., Libourel, I.G., Aoyama, N., Chung, Y.Y., Still, D.W., Jones, R.L. (2007). The *Arabidopsis aleurone* layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiol., 143, 1173–1188. DOI: 10.1104/pp.106.093435
- Blumenthal, A., Lerner, H.R., Werker, E., Pojarkoff-Mayber, A. (1986). Germination preventing mechanisms in *Iris* seeds. Ann. Bot., 58, 551–561.
- Cone, J.W., Spruit, C.J.P. (1983). Imbibition conditions and seed dormancy of *Arabidopsis thaliana*. Physiol. Plant, 59, 416–420.
- Coops, H., Veld, G. van der (1995). Seed dispersal, germination and seedling growth of six helophyte species in relation to water level zonation. Freshw. Biol., 34, 13–20.
- Derkx, M.P.M., Karssen, C.M. (1993a). Changing sensitivity to light and nitrate but not to gibberellins regulates seasonal dormancy patterns in *Sisymbrium officinale* seeds. Plant Cell Environ., 16(1), 469–479.
- Derkx, M.P.M., Karssen, C.M. (1993b). Variability in light-gibberellin-and nitrate requirement of *Arabidopsis thaliana* seeds due to harvest time and conditions of dry storage. J. Plant Physiol., 141(1), 574–582.
- Finch-Savage, W.E., Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. New Phytol., 171(3), 501–23. <https://doi.org/10.1111/j.1469-8137.2006.01787.x>
- Finch-Savage, W.E., Cadman, C.S.C., Toorop, P.E., Lynn, J.R., Hilhorst, H.W.M. (2007). Seed dormancy release in *Arabidopsis Cvi* by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J., 51, 68–70. <https://doi.org/10.1111/j.1365-3113X.2007.03118.x>
- Finkelstein, R.R., Ariizumi, T., Steber, C. (2008). Molecular aspects of seed dormancy. Ann. Rev. Plant Biol., 59, 387–415.
- Gomez, K.A., Gomez, A.A. (1984). Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York.
- Graeber, K., Nakabayashi, K., Miatton, E., Leubner-Metzger, G., Soppe, W.J.J. (2012). Molecular mechanisms of seed dormancy. Plant Cell Environ., 35, 1769–1786.
- Greipsson, S. (2001). Effects of stratification and GA3 on seed germination of a sand stabilising grass *Leymus arenarius* used in reclamation. Seed Sci. Technol., 29, 1–10.
- Jones, K. (2012). Factors affecting establishment and germination of upland prairie species of conservation concern in the Willamette Valley, Oregon (Masters thesis). Corvallis, Oregon State University.
- Kaššák, P. (2012). Secondary metabolites of the chosen genus *Iris* species. Acta Univ. Agric. Silv. Mendel. Brun., 60(8), 269–280.
- Khawar, K.M., Cocu, S., Parmaksiz, I., Sarihan, E.O., Özcan, S. (2005). Mass proliferation of madonna lily (*Lilium candidum* L.) under *in vitro* conditions. Pak. J. Bot., 37(2), 243–248
- Liu, D.F., Chen, S.H., Chen, J.W., Aotegen, Z., Yang, S.M. (1998). Reproductive characteristics, ecological and geographical distribution of *Iris lactea* var. *chinensis* (in Chinese). J. Inner Mongolia Inst. Agr. Animal Husbandry, 19, 1–6.
- Manz, B., Müller, K., Kucera, B., Volke, F., Leubner-Metzger, G. (2005). Water uptake and distribution in germinating tobacco seeds investigated *in vivo* by nuclear magnetic resonance imaging. Plant Physiol., 138, 1538–1551.
- Mathew, B. (1984). *Iris* L. in flora of Turkey and the East Aegean Island, Davis, P.H. (ed.). Vol. 8. Edinburg Univ. Press, Edinburg, 382–410.
- Mokhtarzadeh, S., Hajyzadeh, M., Ahmad, H.A., Khawar, K.M. (2013). The problems in acclimatisation of *in vitro*

- multiplied plants of *Lavandula angustifolia* Miller under field conditions. *Acta Hort.*, 988, 71–76.
- Morgan, M.D. (1990). Seed germination characteristics of *Iris virginica*. *Am. Midl. Nat.*, 124, 209–213.
- Nadjafia, F., Bannayan, M., Tabrizi, L., Rastgoo, M. (2006). Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *J. Arid. Environ.*, 64, 542–547.
- Ozel, C.A., Khawar, K.M., Karaman, S., Ates, M.A., Arslan, O. (2008). Efficient *in vitro* multiplication in *Ornithogalum ulophyllum* Hand.-Mazz. from twin scale explants. *Sci. Hortic.*, 116(1), 109–112.
- Parmaksiz, I., Khawar, K.M. (2006). Plant regeneration by somatic embryogenesis from immature seeds of *Sternbergia candida* Mathew et T. Baytop, an endangered endemic plant of Turkey. *Propag. Orn. Plants*, 6(3), 128–133.
- Rajjou, M., Duval, K., Gallardo, J., Catusse, J., Bally, C., Job, D. (2012). Seed germination and vigor. *Annu. Rev. Plant Biol.*, 63, 507–533.
- Ritchie, S., Gilroy, S. (1998) Gibberellins, regulating genes and germination. *New Phytol.*, 140, 363–383.
- Severns, P. (2003). Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). *Biol. Conserv.*, 110, 221–229.
- Soyler, D., Canihoş, E., Temel, N., Hajyzadeh, M. (2013). Determination of chemical fungicide against soil borne fungal diseases of capers (*Capparis ovata* Desf. var. *Herbacea*) during early stages. *Pak. J. Agric. Sci.*, 49(3), 311–314.
- Soyler, D., Khawar, K.M. (2006). Effects of prechilling, scarification, incubation temperature, photoperiod, KNO₃ and GA₃ treatments on germination of caper (*Capparis ovata* Desf. var. *Palaestina* Zoh.) seeds. *Propag. Orn. Plants*, 6(4), 159–164.
- Soyler, D., Khawar, K.M. (2007). Seed germination of caper (*Capparis ovata* var. *Herbacea*) using α naphthalene acetic acid and gibberellic acid. *Int. J. Agric. Biol.*, 9(1), 35–37.
- Soyler, D., Canihoş, E., Temel, N., Hajyzadeh, M. (2012). Determination of chemical fungicide against soil borne fungal diseases of capers (*Capparis ovata* Desf. var. *Herbacea*) during early stages. *Pak. J. Agric. Sci.*, 49(3), 311–314.
- Stoltz, L.P. (1968). Iris seed dormancy. *Physiol. Plant*, 21, 1328–1331.
- Sumlu, S., Atar, H.H., Khawar, K.M. (2010). Breaking seed dormancy of water lily (*Nymphaea aiba* L.) under *in vitro* conditions. *Biotechnol. Equipment*, 24(1), 1582–1586.
- Sun, Y.C., Zhang, Y.J., Wang, K., Qiu, X.J. (2006). NaOH scarification and stratification improve germination of *Iris lactea* var. *chinensis* seed. *HortScience*, 41, 773–774.
- Tillich, H.J. (2003). Seedling morphology in Iridaceae, Indications for relationships within the family and to related families. *Flora*, 198, 220–242.
- Tubives (2017). www.tubives.com [date of access 11.11.2017].