The major conservation issue of endemic Iranian *Salvia* species is seed germination

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**Abstract**

*Salvia* species produces important medical materials and have attracted attention for their source of essential oils. In Iran, there is a high diversity of *Salvia* species and accessions which includes 70 species that 40% of them are endemic. The objective of this investigation was to find a practical treatment for germination of *Salvia* species, particularly, endemics and to find conservation issues and appropriate approaches. We observed that there was a huge diversity in color (RGB channels), seed area and 1000-seed weights among the population in this study, including 60 accessions (23 species) that thirteen of them (five species) are endemic of Iran. These accessions were soaked in four gibberllic acid (GA3) levels (0, 100, 150 and 200 mg/l). The germination rate and percentage of 62% of accessions, including *S. lachnocalyx* that is endangered, were, extremely, increased in response to the GA3 treatment; nonetheless, some accessions did not germinate at all which indicates there are demands for more efforts to conserve these accessions. Germination percentage of endemic species was significantly lower than non-endemic ones, indicating a serious concern for their conservation. A significant correlation between the 1000-seed weights and area under germination percentage curve (AUGPC) was found that indicates seeds were evolved to have more storage to survive for a long time until germination. Without the practical conservation programs for *S. lachnocalyx* and S*. mirzayanii*, there will be a big extinction risk in near future.

*Keywords*: Conservation; Endangered; Endemic; Iran *Salvia* diversity; Germination; gibberllic acid (GA3)

Abbreviations: ANOVA, analysis of variance; p, error probability level; \*\* P < 0.01, r, coefficient of correlation (Pearson-r) n, number.

The name of accessions in this study include: scientific name + collection origin.

**Introduction**

*Salvia* is an important genus of the Lamiaceae family that includes more than 700 species which are spread throughout the world (Ewans, 1996).

*Salvia* species has been famous for its medicinal properties since ancient times (Rivera et al., 1994). Most of *Salvia* species are, commonly, utilized for their essential oils in the foods, medicines and perfumery industries (Goren et al., 2006; Ozcan et al., 2003; Ulubelen and Topcu, 1998).

With total of 70 species and 40% endemism, *Salvia* has a consequential center of diversity in Iran. It exhibits an interesting range of morphological variation which is as great as, if not more than, anywhere in the Old World (Rechinger, 1982). Despite this huge diversity in Iran and considering very rare species, natural or even anthropogenic processes are not included within the current conservation management.

Some endemic species in this study grow in limited areas *(S. lachnocalyx* is restricted to one region) (Rechinger, 1982). A major priority in efforts to conserve these rare species is to maintain their evolutionary potential by improving their germination.

Due to some problems such as destroying pastures and their replacement with farm, uncontrolled use, lack of conservation, extension of urban areas and their narrowness of geographical stretch, some exclusive species of Iran are in threat of extinction and their gene pool is experiencing genetic drift. Therefore, there is, permanently, the risk of extinction of threatened endemic species that grow only in an exceptional situation or locations (Fay and Muir, 1990; Fay, 1992).

The primary global problem of *Salvia* species has been germination and up to now an integral solution has not been reported for it. Considering the expanding usage of these species at multiplicity parts of the world, finding a solution for triggering their germination is so prominent. In this experiment the germination and response of Iranian accession to GA3 was studied. Gibberellins treatments were chosen because previous works have revealed that this factor improves seed germination in *Salvia* and other plant species (Chan and Lambers, 1970; Finch-Savage et al., 1991; Kosikova, 1960; Thompson, 1969).

The present work focused on ex situ need of seed germination of *Salvia* species. Other aims were: (1) investigation of diversity for seed germination and GA3 response among species and comprising endemic and non-endemic species; (2) determination of species and accessions that their germination and, consequently, conservation is not acceptable and (3) study of genetic diversity in some *Salvia* species and their accessions.

**Materials and methods**

*Applied population*

Seeds of 23 *Salvia* species (60 accessions) comprising 5 endemic ones were used in this experiment during the spring to autumn of 2010. The five endemic species of Iran were *Salvia. reuterana, S. lachnocalyx, S. sharifii, S. mirzayanii* and *S. eremophila*. These seeds have been collected from different regions of Iran and were matured underwent a period of storage in darkness at -4°C because in general, fresh seeds are naturally dormant, requiring cold treatment and a period for ripening and maturity to germinate (Budvytyte, 2001; Hashemi and Estilai, 1994). For each accession 400 seeds in four replications were used to determine the viability of seeds by a tetrazolium test. *S. lachnocalyx* has been classified as nationally endangered because of its endemism, extremely localized allocation and exposure to disastrous events that could commence swift extinction (Jalili and Jamzad, 1999).

*Germination test*

Seeds were surface sterilized in 4% sodium hypochlorite solution containing a few drops of the surfactant Tween for 12 minutes and then rinsed three times with sterile distilled water; then placed on Whatman No. 3 filter paper discs in Petri dishes containing 0 (control), 100, 150 and 200 mg/l of GA3 (filter-sterilized and added to the growth media after autoclaving).

For each accession, seeds were allocated to four replicate Petri dishes, each containing 100 seeds in a completely randomized design. Seeds were incubated for 12 days in a germination chamber in the following environmental regime: 14/10 h light/dark cycle at 22±1°C. All Petri dishes were sealed to prevent from desiccating with parafilm and to ensure no systematic influences due to position within the chamber re-randomizing of Petri dishes was done every other day (Yang et al., 1999). Seeds with at least two millimeters radicle emergence were recorded daily as ‘germinated’.

*Seed morphological traits*

A total of sixty samples of *Salvia* seed were separated randomly from each accession and were situated in the image. The images of seeds were taken by a camera (SONY SyberShot DSC-H9 color digital camera with 8.1 Mega Pixels of resolution) installed on binoculars. The magnification coefficient was 1.5 and 15 for binoculars and camera, respectively. Two kinds of information were used to classify accessions, their seed area and RGB channels.

For calculating the 1000-seed weight, for each accession four replications and in each one 100 seeds were used.

*Data analysis*

Germination percentages of accessions were arcsine transformed before statistical analyses. Orthogonal comparisons to show germination differences of endemic and non-endemic in all treatments (α=%1) were performed. Germination rates in different clusters and treatments were analyzed. These Statistical analyses were performed with SAS software version 9.1 (SAS Institute, Cary, North Carolina).

A Dendrogram on germination percentages in different treatments was constructed based on WARD linkage method and Euclidean distance analysis and the Pearson correlation between 1000-seed weight and area under percentage germination curve (AUGC) were calculated by Minitab (Minitab, 2003).

The *R*, *G* and *B* channels and seed area values were calculated by digital images of seeds using MATLAB 7.1.4. In this study reported diversity with the Intensity component is given by this equation (Gonzalez et al., 2004):

*I* = (*R* + *G* + *B*)/3 (1)

Where *I* = Intensity component, *R* = the average of red channel, *G* = the average of green channel and *B* = the average of blue channel obtained by Image Processing.

***Results***

*Diversity in seed morphological traits*

Image Processing technique demonstrated that diversity in Intensity component (Fig. 1) and seed areas (Fig. 2) of *Salvia* species were very high, likewise there was a huge diversity in 1000-seed weights among species (varying from 0.625g to 12.725g with difference magnitude of more than 20 times).

*Germination responses to GA3*

The majority of accession samples were confirmed that their viability were more than 90% in tetrazolium test. Therefore, only samples with upper than 90% viability were separated and used in this experiment.

Exogenous application of GA3 resulted in a wide spectrum of changes in the percent and rate of germination. A wonderful variation of germination responses to GA3 was found. Different accessions were grouped in four clusters based on seed germination response to four levels of GA3 (Fig. 3). Clusters, obviously, separated accessions by their similarities so that it is easier to discuss about the differences based on these clusters instead of individual accessions (Fig. 4).

The majority of species required a stimulus to trigger germination. Without GA3 treatment, the seed either failed to germinate (34 accessions) or germinated at a very low percentage (Fig. 5) and rate (Fig. 6). The Germination percentage of accessions which were treated by GA3 was significantly increased particularly for those in clusters 2 and 3 (Fig. 4), and smoothly increased for some accessions of cluster 4 (Fig. 4). However, in 40 accessions both germination rate and percentage increased considerably during 12 days.

Sixteen GA3 treated accessions failed to germinate (cluster 4) because seed dormancy was not broken or twelve days were not long enough for their germination. However, the germination percentages of more than 60% of the accessions (cluster 1 to 3) reached above 60% in 200 mg/l GA3 medium (cluster 1, 2 and 3). GA3 had a highly significant effect on germination percentage of many accessions (Fig. 5).

Germination of 44 accessions was significantly stimulated by GA3 but the effect was very diverse in different species and accessions. Accessions in cluster 2 responded very well and for 100, 150 and 200 mg/l of GA3, their responses were nearly the same (Fig. 4), but in cluster 1, the effect of GA3 treatment was intangible. Germination percentage of 23 accessions in cluster 3 positively responded to the increase of GA3 (100 to 200 mg/l), but comparing with other clusters, there were more differences among individuals of this cluster (Fig. 4). In all germinated accessions, the rate of germination, significantly, enhanced with GA3 treatment. The increasing rate of germination by different levels of GA3 was dissimilar in four clusters (Fig. 6).

*Comparison of endemic and non-endemic accessions*

The orthogonal comparison of endemic and non-endemic accessions showed that germination percentage of endemic accessions was, significantly, lower than non-endemic ones in all four treatments (α = 0.01) (Fig. 7).

*Correlation* *between seed weight and dormancy*

Accessions were categorized into 12 groups based on their 1000-seed weights (including 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 9-10, 10-11, 11-12, 12-13 gr.). A negative correlation was observed between 1000-seed weight and area under percentage germination curve (*r* = − 0.765\*\*, *n* = 12). Therefore, species with lighter seeds showed, considerably, lower dormancy than heavier ones.

**Discussion**

*Diversity*

Diversity observed in RGB channels, seed areas and 1000-seed weights proves accessions studied had enough diversity to investigate approximately all kinds of germination and responses to GA3 models. Accordingly, it seems that their physiological demands are completely different. The germination percentage of seeds in control had huge differences so that the germination mean for some accessions (like *S. sclarea* Kordestan) was 90% while in most of them, it was zero. Also, the response of accessions to GA3 was, highly, different. It confirms that genetic diversity among accessions is high and consequently their germplasm is valuable for conservation aims.

*Germination and response to GA3 models*

Propagation from seed is, efficiently, economical and common; but germination demands for native species are, in many cases, mysterious, particularly, for thinly distributed or endemic species of which material is additionally difficult to acquire. For *Salvia* species, propagation by seed is a preferred method for preserving their genetic diversity. However, in some cases another proper method must be chosen based on the demands of each species (Benson et al., 2000).

Hormonal treatments can effectively be used to increase the germination rate and percentage. Many *Salvia* species, similar to some other plant species, demand a stimulus to trigger germination and fail to germinate in the absence of gibberellins treatment (Hashemi and Estilai, 1994).

Four separate clusters respond, differently, to GA3 due to high diversity in germination of accessions. Previous studies have indicated that various responses to germination treatments of accessions in diverse inhabitants are possibly due to environmental differences, in which they have evolved (Maruta, 1994; Martin et al., 1995; Nishitani and Masuzawa, 1996). Results showed that in some species, germination behavior is, intensively, differing between regions (Fig. 5). For example, the species of *S. sclarea* from Kordestan have been located in cluster one while the accessions of this species gathered from Yazd, Dena and Shiraz grouped in cluster two, and the accessions of Lorestan and Khoram-Abad have entered into clusters three and four, respectively. It means that the location is very influential on this species and its accessions have acclimated to their growth areas. On the other hand, the grouping of all accessions of *S. mirzayanii*, *S. hydragea*, *S. sharifii* and *S. multicaulis* in cluster four and those of *S. limbata* and *S. macrosiphon* in clusters three and two, respectively, suggest that the environment in the evolution of these species had less effect.

Different levels of GA3 have different affects on different accessions. In cluster 2, the response to GA3 has been very high and there is no significant difference among treatment levels (Fig. 4) while in accessions of cluster 3, the GA3 effect was less pronounced. Therefore, at least, 200 mg/l GA3 is necessary for their acceptable germination. For accessions with extreme dormancy in cluster 4, it seems that they need other treatments.

*Conservation condition of endemic species*

Among endemic species, *S. lachnocalyx* whose territory has been restricted for many years at a small region near Eghlid in Fars province, although its germination percentage and rate were very low at control, but they increased, noticeably, by applying GA3.

For *S.* *reuterana* the germination percentage in one accession was zero and in other two accessions was less than 60% which does not seem acceptable and another treatment should be tested to improve its germination.

The *S. sharifii* and *S. mirzayanii* that evolved to grow in arid and semi-arid warm in south of Iran regions with too disperse precipitation, did not germinate at all. Other researchers suggested in extreme environment strength of dormancy increased (Beckstead et al., 1996; Meyer et al., 1997; Meyer and Allen, 1999) and germination with extreme ‘opportunistic’ or ‘cautious’ strategies may be expanded (Gutterman, 2000). Based on above conclusion it seems there is a sensible evolution in these species to survive against variable environment with delayed germination. However seeds that remain dormant are subjected to increasing mortality, growth reduction, and low or unsuccessful reproduction (Kevin and Andrew, 2001). Therefore it is seriously important to perform conservation management to rescue these species.

*S. eremophila* as a desert species has evolved to germinate rapidly during the rainy period, furthermore, germination rate and percentage of its accessions with application of GA3, significantly, increased.

*Comparison of endemic and non-endemic species*

Species that subsist in, extremely, specific territory often generate seeds with, exceedingly, specialized adaptations. Iranian endemic accessions had delay in germination. They were grouped in clusters 2 to 4 whose common characteristics were their lack of ability to germinate in absent of germination stimulators.

Most part of Iran has semi-arid climate with highly uneven distribution and dispersed precipitation, therefore, it seems that they delay to germinate because this strategy helps them to keep their soil seed bank.

In wild plants, delay in germination slows the establishment of plants by increasing the probability of site occupation by other plants resulting in reduced seedling growth. This leads to slighter adult mass and the decrease of reproductive productivity (Abul-Fatih and Bazzaz, 1979; Miller, 1987; Ross and Harper, 1972). Therefore, it is necessary to pay attention to their germination problem exclusively.

* 1. *Correlation of seed weight with dormancy*

Investigation on numerous species have proved that seed weight in most cases have significant effects on germination rate and percentage (Navarro and Guitián, 2003). Several studies have revealed that seed weight is a good predictor of different variables, including germination capability (Schaal, 1980; Stanton, 1985).

In general, accessions with lighter seeds showed considerably superior germination than heavier seeds. It seems that some *salvia* accessions have evolved in a way that do not easily enter the germination phase due to the semi-arid climate and disperse precipitations of Iran. Since seed is alive, it has evolved to have more material stored to survive for a long time until germination. Likewise, accessions with germination delay evolved to have thicker seed coat. The thicker coat acts as a protection against erosion and soil organisms.

* 1. *In conclusion*

This research showed that there is a high variation and genetic diversity among the *salvia* species and accessions, consequently, their germplasm is valuable for conservation purposes. For *Salvia* accessions, propagation by seed is the preferred method for preserving their genetic diversity. For the majority of accessions, exogenous application of GA3, significantly, increased the rate and percentage of germination. The application ofGA3 also increased germination rate and percentage of *S. lachnocalyx*. Dormancy of heavier seeds was higher because they were evolved to have more stored material and thicker coat to help them survive for a long period before germination. Endemic accessions had delayed germination in comparison with non-endemic ones, because they have more, specifically, evolved for Iran's climate.

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