Journal of Medicinal Plants and By-products (2012) 1: 55-59

Original Article

Effect of Thermal Period on Seed Dormancy of Damask Rose (*Rosa damascena* Mill.)

Zahra Abravesh*, Mohammad Hassan Assareh, Séyed Reza Tabaei-Aghdaei

Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran

Article history: Received: 25 July 2012 /Accepted in revised form: 01August 2012

Abstract

Damask rose (*Rosa damascena* Mill.) has high economic importance and commercial value. The plant essential oil is used for perfumery, food and medicinal industries. It is necessary to know much about seed germination for study of genetic variation and breeding improved varieties of *R. damascena*. In this study seed germination of *R. damascena* were evaluated, using thermal period treatments. The data were analyzed based on the Randomized Completely Design with 3 replications. Seeds were sterilized for elimination with NaOCl. Seeds of *R. damascena* were subjected to five different temperature regimes. For control, the seeds were placed at 4°C continuously. The seeds of all other treatments initially were placed at 25°C for the first warm stratification. Then they were placed at 4°C for 2, 4, 6 and 8 weeks for cold stratification as 2w/2w, 2w/4w, 2w/6w and 2w/8w, respectively. For the second cycle, seeds were placed at 20 °C for warm stratification followed by 2, 4, 6 and 8 weeks for cold stratification. The treatments were repeated for 3 cycles. Then the all of treatments were applied 30 weeks of continuously cold stratification at 4°C. The results of this study showed that the thermal period treatments had significantly promoted the germination percentage. The highest germination percentage and speed of germination were obtained for the 6w/2w treatment as (92%) and (1.17 seeds per day), respectively. The lowest germination percentage was found in the control and 8w/2w treatments (50%). In conclusion, it was observed that with application of thermal period (particularly 6w/2w) and 30 weeks of cold stratification to Damask rose seeds, dormancy was broken and germination was also highly improved.

Key words*: Rosa damascena* Mill, Damask rose, Seed Dormancy, Germination, Thermal period

Introduction

The genus Rosa includes 200 species and more than 18000 cultivars [1]. Damask rose (*Rosa damascena* Mill.) is widely cultivated for it's essential oil, medicinal properties, and ornamental aspects in many areas of the world such as Bulgaria, Turkey, India, Morocco, Egypt, France, China and Iran [2- 4]. These countries are the main producers of oil-bearing rose in the world [4]. Flowers are the main part of the Damask rose, thus, flowers yield is the most important trait for this crop. Considerable variation had been reported for many traits such as flower yield, oil content in Iranian Damask rose populations [3- 6] and molecular markers [2, 7, 8].

In many plant species delay occurs between period of maturation and germination time. The phenomenon is called seed dormancy. True dormancy or innate dormancy is caused by conditions within the seed that prevents germination under normally ideal conditions. Rose seeds show both endogenous (morphological and/or physiological) and exogenous (physical and/or mechanical) dormancy [9-12]. Rose seeds are surrounded by a hard-coated pericarp prevents water absorption and air diffusion of the seed and at the same time is a physical barrier to embryo expansion [10, 13, 14]. In addition, it was stated that high level of abscisic acid (ABA) in the pericarp and testa of rose seeds was a major germination inhibitor in roses [15-18]. It was reported that the amount o ABA in a rose seed was 10 to 1000-fold higher than those in other plants [10]. Due to the above-mentioned reasons, germination of rose seed is generally difficult. Prolonged dormancy delays germination and reduces germination percentage. This is a serious problem particularly in rose breeding and plant propagation [11-13, 17, 19, 20]. Primary seed dormancy often occurs in seeds on the mother plant in order to prevent germination during development and maturation and often also occurs for some time after seed harvesting [21]. Primary dormancy of many rose species can be broken by a cold stratification period at low temperature and with high moisture content [22].

*Corresponding Author: Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran
Email Address: z.abravesh@gmail.com

Seed dormancy of many rose species can be released at 4°C, but required stratification period differs among them. Germination of many rose species can occur at 4°C [23]. Since, dormancy can only be measured by germination; it is often difficult to distinguish between the processes of dormancy release and the processes of germination [22]. Secondary seed dormancy can occur after harvest in seeds which are non-dormant or have emerged partly or fully from primary dormancy [21]. Secondary dormancy may be induced by different environmental conditions which are unfavorable for germination, for instance high temperature [24].

One of the most commonly methods to break dormancy and stimulate germination in rose seeds is stratification [11-13]. Other methods, such as gibberellic acid treatment [11, 12, 20], hot water treatment [25], scarification with sulphuric acid [11, 12, 26], scarification with sand paper and chemical treatments (e.g. potassium nitrate, calcium nitrate, gibberellic acid, citric acid, 6-benzylamino purine) [11, 12] and macerating enzymes [19], have also been breaking seed dormancy. The aim of this research was use of thermal period treatment to overcome seed dormancy of *Rosa damascena* Mill.

Materials and Methods

The mature hips of the species *R. damascena* Mill. (Fars1) were collected from the oil rose germplasm in Research institute forests rangelands Tehran Province (Tehran, Iran, 35° 41' N latitude, 51° 19' E longitude and 1190.8 m altitude) in October 2005. Rose hips contained 8 seeds per hip on average. The annual mean temperature, relative humidity, total annual precipitation, wind speed in the area are 18.4 °C, 37%, 325.6 mm, 3 m s^{-1}, respectively [27]. The research was conducted in pots and Petri-dish which placed in cold room.

After that seeds had been manually extracted from hips, they were cleaned in water and the unwanted materials were removed. Later seeds were soaked in water for 24h, floating seeds were discarded and seeds which sunk in water were used in the treatment as they were assumed to be mature and viable [11]. After drying seeds in open air for 5 days, they were kept in white paper at laboratory temperature (20–24 °C) until the beginning of the treatments. Seed weight was determined based on 8 replications of 100 seeds (8 × 100 seeds) [28].

The sterilization step involved treatment of seeds with 0.3% (v/v) NaOCl for 30 min. A few drops of detergent were added to the treatment solutions. Then they were rinse off with distilled water. The scarification step involved treatment of seeds with sand paper for 1 min.

Seeds of *R. damascena* were germinated at five different temperature regimes: 4°C continuously (control), first for 2 weeks of warm stratification at 25 °C, then 2, 4, 6 and 8 weeks of cold stratification at 4 °C, then two-weeks of warm stratification at 20 °C in 3 cycles and then total treatments was applied 30 weeks cold stratification at 4 °C.

Plant seeds were cultivated at two different containers: Petri-dish with filter paper and pot containing: pith: vermiculite: perlite in the ratio of (1:1:1 v/v). A completely randomized design with 3 replications was used, and each replication consisted of 25 seeds. Germination percentage and speed of germination of seeds under different duration of cold stratification were analyzed using SAS9 (2004) [29]. Means values were compared by Duncan's multiple range test at the 0.05 probability level. The data were transformed to Arcsine before analysis.

Results

In this study, the average value of 1000 seeds weight was 43.03 gr. The results showed that there were no germinated seeds in Petri-dish, therefore, only germination of pot samples studied and analyzed.

Thermal period treatments significantly ($P< 0.05$) stimulated germination percentage during warm and cold stratification (Table 1). At the end of this period, germination was observed in all treatments. The highest germination percentage values was obtained in the 6 weeks of cold stratification at 4°C and then two-weeks of warm stratification at 20°C in 3 cycles (2w/6w) (92%), followed by 2w/2w (83.33%), 2w/4w (54.17%) and 2w/8w (50%). However, the lower germination value was 50% of the control (Table 1).

Table 1 Effects of thermal period treatments on germination percentage (%) and germination rapidity of *Rosa damascena* Mill. seeds

Treatment	Germination percentage (%)	Speed of germination
Control	50.00b	0.47a
2w/2w	83.33ab	0.78a
4w/2w	54.17b	0.55a
6w/2w	92.00a	1.17a
8w/2w	50.00b	0.54a
F value	718.34*	0.34^{ns}

* ns= significant at $P\leq 0.05$ and non significant, respectively.

*Means of treatments in each column followed by the same letters are not significantly different at 5% level of probability.

Speed of Germination

No Statistically significant differences (P>0.05) were found between the speed of germination for the thermal period treatments and control (Table 1). Nevertheless, the higher values (1.17 seeds per day was obtained for the 6 weeks of cold stratification at 4°C and then two-weeks of warm stratification at 20°C in 3 cycles (6w/2w) than that of the control with values of 0.47 seeds per day (Table 1). Therefore, 2w/6w treatment suggested as the best treatment for rose seeds germination.

The results of speed of germination showed that thermal period treatments stimulated seed germination speed during cold stratification over one year (Fig. 1). The trend of germination speed indicated that seed germination was started from fifth month. Germination speed of in the (6w/2w) treatment (1.17 seeds per day) had increased rapidity than other treatments over times (Fig. 1). The results showed that thermal period treatments affected on the germination rapidity of seeds. This was indeed the case in this study, the seeds may have contained populations with physiological dormant.

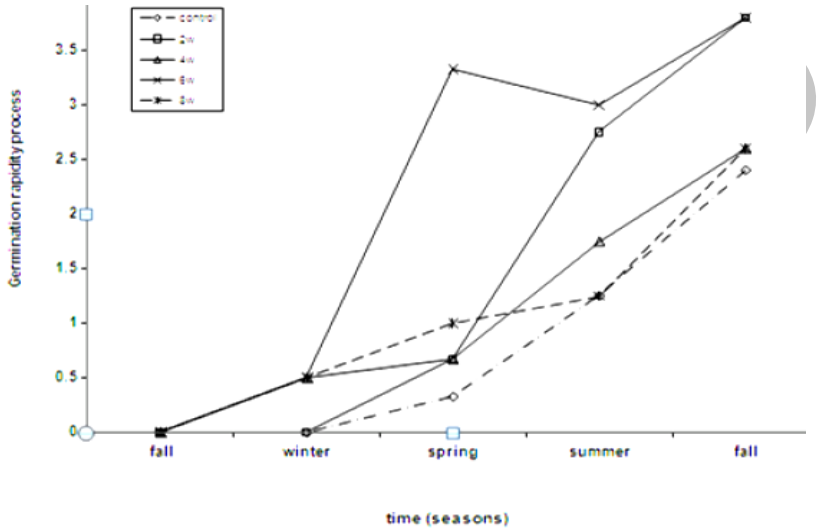

Fig 1. Effects of thermal period treatments on speed of germination by seedling growth in different seasons of year

Fig 2. Seed germination in cold room (4 °C) (thermal period) and after transferring to germinator (20 °C)

Discussion

This study showed that the germination percentage of *Rosa damascena* seeds was significantly affected by thermal period treatments.

Results showed that 2 weeks of warm stratification at 25 °C, followed by 2, 4, 6, 8 weeks of cold stratification at 4°C, then two-weeks of warm stratification at 20 °C in 3 cycles, seeds germination were observed in all thermal period treatments and control. In all treatments, seeds germination started after ending thermal period and application of cold stratification at 4 °C (Fig 1), except for the 8w/2w treatment, which seeds germination started before finishing cycle 3 of the thermal period, as well as seeds germination started from fifth month (140 days) in 4w/2w, 6w/2w and 8w/2w treatments. Seeds germination started from eighteenth month in control and 2w/2w treatments (Fig 1). This indicates that the alternate warm and cold stratification duration of thermal period might be adequate to break dormancy of the genotype seeds *R. damascena* Mill. The most common treatment to break rose seeds dormancy is cold stratification [11, -13, 30], and time required for breaking dormancy varies by species and duration of stratification [31]. For instance, the species *R.*

multiflora and *R. setigera* need 30 days of cold stratification; the species *R. wichuraiana* needs 45 days of cold stratification; and *R. setigera* 'Serena' and *Rosa x* reverse need 90 days of cold stratification to obtain maximum germination percentages [31]. Moreover, Hajian and Khosh-Khui were reported that a stratification duration longer than 150 days was needed to remove embryo dormancy of Damask rose seeds and that the germination percentage was over 80% through soaking seeds in 70-80% sulphuric acid for 10 min followed by 150 to 180 days of stratification [32]. Also Kazaz *et al* were reported that the highest premature germination percentage of *R. damascena* Mill. Seeds was determined in the EM•1® (69.3%), followed by B:seepel™ (52.0%) and Phosfert™ (44.0%). However, premature germination was 13.3% in the seeds treated only with warm plus cold stratification (control) [4].

In this study the highest germination percentage was obtained in 6w/2w treatment (92%). The premature germination percentage of *R. damascena* Mill. seeds in all thermal period treatments during stratification might be due to a temperature change . The temperature change raised the proportion of the growth regulators to inhibitors in the seed embryo during stratification that caused to stimulate the germination. The low rate of the germination might be due to negative effect of low temperature on enzyme activity, necessary for metabolism for seed germination, growth and development plantlets. A similar case was reported by Nishimoto and McCarty (1997) [33].

The control treatments indicated that germination occurred in continuous treatments of 4°C, and showed seed germination was increased by increasing cold duration (50%). It means that dormancy had been broken in these seeds during cold period stratification at 4°C. The 2w/2w, 2w/4w, 2w/6w and 2w/8w treatments (warm and cold stratification) and control indicated that in all treatments seed germination percentage was increased with increasing cold stratification period (with different percentages) and approximately had steady process. It means that seed dormancy was physiological (Fig 1). Also Fig 1 indicated that the 2w/4w, 2w/6w and 2w/8w treatments were advanced seeds germination percentage compared to the control and 2w/2w treatments. Therefore, the length of the thermal period (proportion cold to warm) affected in induce the germinated seeds. Low germinated seeds with get prolonged the cold stratification period in some treatments indicated that some seeds for germination either need to more cold or were unripe their embryos.

Conclusions

This study suggested that thermal period highly increased germination percentage and considerably increased the speed of germination of *R. damascena* seeds and 6w/2w treatment highly improved seeds germination percentage (92%). According to the observations, the highest germination rate occurred at of stratification with 6w/2w. The study also showed that one year of cold stratification (4 °C) followed by 2 weeks of warm stratification (20 ^{o}C) in 3 cycles thermal period 2w/6w might be enough to break dormancy. How long it takes for dormancy of the genotypes *R. damascena* to be broken will be clarified with further studies will be carried out on seed embryo in future.

References

1. Gudin S. Rose: Genetics and Breeding. Plant Breed. Rev. 2000;17:59-189.
2. Tabaei-Aghdaei S R, Hosseini Monfared H, Fahimi H, Ebrahimzadeh H, Jebeli M, Naghavi MR and Babaei A. Genetic Variation Analysis of Different Population of *Rosa damascena* Mill. in NW. Iran Using RAPD Markers. . Iranian J. Bot. 2006;12:121-127.
3. Yousefi B, Tabaei-Aghdaei S R, Assareh MH and Darvish F. Evaluation of Stability Parameters for Discrimination of Stable, Adaptable and High Flower Yielding Landraces of *Rosa damascena* Mill. J. Agr. Sci. Tech. 2011;13:99-110.
4. Kazaz S, Erba S, Baydar H. Breaking seed dormancy in oil rose (*Rosa damascena* Mill.) by microbial inoculation. Afr. J. Biotechnol. 2010;9:6503-6508.
5. Tabaei-Aghdaei SR, Rezaei M.B, and Jebeli M. Flower Yield and Morphological Characteristics in Some Genotypes of *Rosa damascena* Mill. Iranian J. Med. And Aro. *P*. 2004; 20(1): 111-122.
6. Tabaei-Aghdaei S R, Babaei A, Khosh-Khui M, Jaimand K, Rezaee MB, Assareh MH and Naghavi M R. Morphological and Oil Content Variations amongst Damask Rose (*Rosa damascena* Mill.) Landraces from Different Regions of Iran, Sci. Hortic. 2007;113:44-48.
7. Pirseyedi S M, Mardi M, Davazdahemami S, Kermani M, and Mohammadi S A. Analysis of the Genetic Diversity 12 Iranian Damask Rose (*Rosa damascena* Mill.) Genotypes Using Amplified Frament Length Polymorphism Markers. Iranian J. Biot. 2005;3:225-230.
8. Babaei A, Tabaei-Aghdaei S R, Khosh-Khui M, Omidbaigi R, Naghavi MR, Esselink GD and Smulders MJM. Microsatellite Analysis of Damask Rose (*Rosa damascena* Mill.) Accessions from Various Regions in Iran Reveals Multiple Genotypes. BMC Plant Biol. 2007;7:12 dio: 10. 1186/1471-2229-7-12.
9. Gudin S, Arene L, Chavagnat A, Bulard C. Influence of Endocarp Thickness on Rose Achene Germination: Genetic and Environmental Factors. Hort. Sci. 1990;25: 786-788.
10. Ueda Y. Seed maturation and germination, In: Roberts A, Debener T, Gudin S (eds). Encyclopedia of rose science. Elsevier, Oxford. 2003; pp. 623-626.
11. Assareh MH, Abravesh Z, Tabaei-Aghdaei S R, and Naraghi T S. Study of Different Methods of Seed Germination by Using Phisico-Chemical Treatments in *Rosa damascena* Mill. Final Repot of Research Plan Institute of Forests and Rangelands. 2008.
12. Assareh MH, Abravesh Z, Tabaei-Aghdaei S R, and Naraghi T S. Investigation Effect of Chemical and Mechanical in the Seed Dormancy Breakage and

Germination *Rosa damascena* Mill. Pagouhesh-va-Sazandegi in Natural Resources (Special Issue). 2009;21: 84-91.
13. Zlesak, D.C. Rose. In: Anderson N.O. (eds). Flower Breeding and Genetics. 2007; pp. 695-740.
14. Meyer SE. Rosa L. In Bonner and Karrfalt (eds). The Woody Plant Seed Manual. USDA For. Serv. Agric. Handbook. 2008;727:974- 980.
15. Jackson GAD. Hormonal Control of Fruit Development, Seed Development and Germination with Particular Reference to Rosa. Sci. Monogr. 1968;31:127-156.
16. Cornforth JW, Milborrow BV, Ryback G. Biochemistry, Identification and Estimation of (+)-Abscisin II ('Dormin') in Plant Extracts by Spectropolarimetry, Nature. 1966;210:627-628.
17. Bo J, Huiru D, Xiaohan Y. Shortening Hybridization Breeding Cycle of Rose a Study on Mechanisms Controlling Achene Dormancy. Acta. Hortic. 1995;404: 40-47.
18. Hartmann HT, Kester DE, Davies Jr FT, Geneve RL. Plant Propagation, Principles and Practices. Prentice Hall, Upper Saddle River, New Jersey. 2002.
19. Yambe Y, Takeno K. Improvement of rose achene germination by treatment with macerating enzymes. Hort. Sci. 1992;27:1018-1020.
20. Hosafci H, Arslan N, Sarihan EO. Propagation of Dog Roses (*Rosa canina* L.) by Seed. Acta Hortic. 2005;690: 159-164.
21. Karssen CM. Enviromental conditions and endosgenous mechanisms involved in secondary dormancy of seeds. Isr. J. Bot. 1980/81;29:45-64.
22. Hilhorst HWMA. Critical upDate on Seed Dormancy. L. Primary Dormancy Seed Sci. Res. 1995;5:61-73.
23. Jeansen m. Moisture Content Controls the Effectiveness of Dormancy Breakage in *Abies nordmanniana* (Steven) Spach sSeeds In: R.H.Ellis M, Black AJ, Murdoch and TD, Hong (eds), Basic and Applied Aspects of Seed Biology. KluwerAcademic Publishers, Dordrecht. 1997; pp. 181-190.
24. Corbineau F, Black M and Come D. Induction of Thermodormancy in *Avena sativa* Seeds. Seed Sci. Res. 1993;3:111-117.
25. Younis A, Riaz A, Ahmed R, Raza A. Effect of hot water, sulphuric acid and nitric acid on germination of rose seeds. Acta Hort. 2007;755:105-108.
26. Bhanuprakash K, Tejaswini Y, Yogeesha HS, Naik LB. Effect of scarification and gibberellic acid on breaking dormancy of rose seeds. Seed Res. 2004;32: 105-107.
27. Iran Meteorological Organization Calendar. 2005.
28. ISTA (International Seed Testing Association). International Rules for Seed Testing. Seed Sci. Technol. 1993;21: p. 259.
29. SAS Institute. INC SAS/STAT user's guide release 7.0, Cary, NC, USA. 1998.
30. Zhou ZQ, Wei-Kai B, Ning W. Dormancy and germination in Rosa multibracteata Hemsl and E.H. Wilson. Sci. Hortic. 2009;119:434- 441.
31. Stewart RN, Semeniuk P. The effect of the interaction of temperature with after-ripening requirements and compensating temperature on germination of seeds of 5 species of Rosa. Am. J. Bot. 1965;52:755-760.
32. Hajian S, Khosh-Khui M. Investigation on sexual and asexual propagation methods of Damask rose (Rosa damascena Mill.). Iranian Agric. Res. 2000;19:1-16.
33. Nishimoto RK and McCarty Lb. Fluctuating temperature and light influence seed germination of gosegrass (*Eleusine indica*). Weed Sci. 1997;45:426-429.