Breaking seed dormancy in oil rose (*Rosa damascena* Mill.) by microbial inoculation

Soner Kazaz	extsuperscript{1*}, Sabri Erbaş	extsuperscript{2} and Hasan Baydar	extsuperscript{2}

*Department of Horticulture, Faculty of Agriculture, Suleyman Demirel University, Isparta 32260, Turkey.  
\textsuperscript{2}Department of Field Crops, Faculty of Agriculture, Suleyman Demirel University, Isparta 32260, Turkey.

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This study was carried out to determine the effects of microbial inoculation in breaking seed dormancy and on the germination of *Rosa damascena* Mill. Seeds of *R. damascena* Mill. are the most used scented rose species in rose oil production. The most important production centers around the world are Turkey and Bulgaria. The seeds were subjected to 4 weeks of warm stratification at 25°C, followed by 150 days of cold stratification at 4 ± 1°C. Before stratification, 4 different microbial fertilizers, EM•1®®, B:speel™, Bioplin™ and Phosfert™ were inoculated to the seeds. In the study, the microbial inoculation treatments significantly (p < 0.01) promoted the premature germination percentage during cold stratification. During the stratification, the highest premature germination percentage was obtained from the EM•1® (69.3%). The highest germination percentage in terms of cumulative germination percentage was determined in EM•1® (100.0%), followed by Phosfert™ (84.0%) and B:speel™ (84.0%), whereas the lowest germination percentage was found in the control treatment (69.3%). The EM•1® shortened the mean germination time by 1.7 days in comparison to the control. In conclusion, it was observed that with microbial inoculation (particularly EM•1®) to oil rose seeds and a stratification time of 150 days, dormancy was broken and germination highly improved.

**Key words:** *Rosa damascena* Mill, dormancy, germination, microbial inoculation, stratification.

**INTRODUCTION**

The genus *Rosa* has over 130 species (Cairns, 2001) that are native to the Northern Hemisphere (Krüssmann, 1981), and of these species, 25 are distributed in Turkey (Kutbay and Kilinc, 1996; Ercisli, 2004). The primary species used in rose oil production among rose species are *Rosa damascena* Mill, *Rosa gallica* Linn., *Rosa centifolia* Linn. and *Rosa moschata* Herm. (Tucker and Maciarell, 1988). Among these species, *R. damascena* is commonly used in oil production (Douglas, 1993). Although oil rose is cultivated in many countries such as Turkey, Bulgaria, India, Iran, Egypt, Morocco and Syria (Büttner, 2001), the most important production centers in the world are Turkey and Bulgaria. *R. damascena* Mill., a perennial shrub, produces pink flowers in May-June. Oil rose is a temperate zone plant and has well adapted to climate zones, which receive abundant light and adequate rain, and do not experience negative climatic factors such as drought, excessive rainfall and freezing during the flowering period but in which dew occurs during the early morning hours. The primary products that are obtained from oil rose and that are greatly demanded in cosmetics industries include rose oil, rose water, rose concrete and rose absolute (Kaur et al., 2007; Kazaz et al., 2009). Fruits, fruit flesh and seeds of *R. damascena* contain ascorbic acid 332.0, 546.0 and 145.0 mg/100 g, respectively. Also *R. damascena* fruits can be used as food and food additive similarly as with dog rose fruits (*Rosa canina*) (Kazaz et al., 2009).

Rose seeds show both endogenous (morphological and/or physiological) and exogenous (physical and/or...
mechanical) dormancy (Gudin et al., 1990; Ueda, 2003). Rose seeds are surrounded by a hard-coated pericarp, and the pericarp prevents water absorption and air diffusion of the seed and at the same time is a physical barrier to embryo expansion (Ueda, 2003; Zlesak, 2007; Meyer, 2008). In addition, it was stated that high concentrations of abscisic acid (ABA) in the pericarp and testa of rose seeds was a major germination inhibitor in concentrations of abscisic acid (ABA) in the pericarp and testa of rose seeds was a major germination inhibitor in roses (Jackson, 1968; Cornforth et al., 1966; Bo et al., 1995; Hartmann et al., 2002). It was reported that the amount of ABA in a rose seed was 10- to 1000-fold higher than those in other plants (Ueda, 2003). Due to these characteristics, Isparta features a semi-arid climatic characteristic (Ucar et al., 2009).

Experimental site

The research was conducted in a plastic covered greenhouse located at the Agricultural Research and Application Center of Agricultural Faculty at Süleyman Demirel University (latitude 37°50’ N, longitude 30°32’ E, altitude 1019 m).

Seed preparation and determination of moisture content and 1000 seeds weight

After the seeds had been manually extracted from hips, they were cleaned in water and the unwanted materials were removed. Later, the seeds were soaked in water for 24 h and then the floating seeds were discarded and the seeds that sunk in water were used in the treatment as they were assumed to be mature and viable (Zhou et al., 2009). After the seeds had been dried in the open air for 3 days, they were kept in polyethylene bags at room temperature (20 - 24°C) until the beginning of the treatments. Seed moisture content (four replicates of 100 seeds) was determined at 103°C for 17 h and 1000 seeds weight was determined based on 8 replications of 100 seeds (8 x 100 seeds) (ISTA, 1993).

Microbial treatments and warm plus cold stratification

Some 4 different microbial fertilizers (EM•1® EM Agriton and Kina-gro Agriculture Inc, Turkey), B: speel™ (Bioglobal Inc. Turkey), Bioplin™ (Bioglobal Inc, Turkey) and Phostert™ (Bioglobal Inc, Turkey) were used in the study. EM•1® primarily contains 3 types of microorganisms, namely phototrophic bacteria (Rhodopseudomonas palustris), lactic acid bacteria (Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum and Lactobacillus delbrueckii) and yeasts (Saccharomyces cerevisiae). B: speel™ is a bioorganic seed dresser and contains a mixture of microorganisms (1x10^7 cfu/g) fixing nitrogen in dormant form, a mixture of phosphate solubilizing bacteria (PSB) (1x10^7 cfu/g), plant growth promoting rhizobacteria (PGPR) and metabolic extracts of different microbes. Bioplin™ contains efficient rhizosphere inhabiting, nitrogen fixing and plant growth promoter producing strains of Azotobacter (Azotobacter chroococcum and Azotobacter vinelandii 1 x 10^7 cfu/g). Phostert™ contains plurality of strains of Azotobacter (A. chroococcum, A. vinelandii, Bacillus polymyxa 1 x 10^7 cfu/g). Phosfert™ contains plurality of strains of Azotobacter (A. chroococcum, A. vinelandii, Bacillus polymyxa 1 x 10^7 cfu/g).

Firstly, the seeds were left in water for 24 h and then they were left in Bioplin™ (15 ml/l), Phosfert™ (15 ml/l) and Phosfert™+Bioplin™ (1:1, v/v) solution for 15 min and in EM•1® solution (300 ml/l) for 20 min. In the B: spool™ treatment, B: speel™ (20 g/kg seed) was sprinkled over the seeds, and the seeds were covered completely with B: spool™. On the other hand, no microbial fertilizer treatments were performed on the seeds in the control group.

Stratification was applied to the seeds treated with microbial fertilizer and to the seeds of the control group. Sphagnum moss was used as the stratification medium. Those seeds that were mixed with moistened sphagnum moss (1 part of seed and 4 parts of sphagnum moss, v/v) were subjected first to 4 weeks of warm stratification at 25°C and then to 150 days of cold stratification in

MATERIALS AND METHODS

Seed origin and seed collection

The mature hips of the species R. damascena Mill. were collected from the oil rose plantations in Isparta Province (Isparta, Turkey, 37°45’ N latitude, 30°33’ E longitude and 997 m altitude) in October 2008. Rose hips contain 2.35 seeds per hip on average. The annual mean temperature, relative humidity, total annual precipitation, wind speed and sunshine duration per day in the area are 12.4°C, 55%, 524.4 mm, 2.4 m s^-1 and 7.6 h, respectively (Anonymous, 2003). With these climate characteristics, Isparta features a semi-arid climatic characteristic (Ucar et al., 2009).
At the end of stratification, premature germination took place in all treatments, except for Bioplin™. The number of prematurely germinated seeds in each treatment was recorded, and the germination percentages of these seeds were further analyzed in order to determine the difference between the treatments. The prematurely germinated seeds were not sown in the germination medium after the sowing of seeds. Germination tests were carried out in greenhouse at 25°C day/15°C night temperature and a relative humidity of 70%. A seed was considered to have germinated when the cotyledons had emerged above the soil surface, and it was recorded for up to 30 days. Germinated seeds were counted and removed every 24 h for 30 days. Final germination percentage was calculated when no further germination took place for several days. The germination percentage (GP) was calculated for each experimental unit. Mean germination time (MGT) was calculated using Equation (1) (Chuanren et al., 2004)

$$\text{MGT} = \frac{\sum nd/N}{d}$$

Where, n is the number of seeds that germinated between scoring intervals; d the incubation period in days at that point in time and N the total number of seeds that germinated in the treatment.

### RESULTS

#### Germination percentages

In this study, moisture content of seeds was 11.15%, and weight of 1000 seeds was 20.9 g. Microbial inoculation treatments significantly (p < 0.01) stimulated premature germination during cold stratification. At the end of this period, premature germination was observed in all treatments, except for the Bioplin™. The highest premature germination percentage 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) (Table 1). The germination percentages of seeds sown in the greenhouse after cold stratification are presented in Table 1. Statistically significant differences were determined between the germination percentages of the treatments (p < 0.01). Among the treatments, the highest germination percentage was obtained in the EM•1® (100.0%), whereas the other treatments were included in the same statistical group.

When the germination percentages of prematurely germinated seeds at the end of the duration of stratification and of greenhouse-germinated seeds were considered together (cumulative germination percentage), microbial inoculation treatments statistically significantly affected cumulative germination percentage. All seeds germinated with the EM•1®. Furthermore, Phosfert™ and B:seepel™, with their germination per-cent-age of 84%, were included in the same group with EM•1®. 66.7% germination occurred in the seeds (control) which were...
only stratified without any microbial inoculation treatments.

Mean germination time

No statistical difference in mean germination time was found between microbial inoculation treatments and the control treatment. Nevertheless, although no statistical difference was found between treatments, the mean germination time of the EM•1® (7.2 days) was 1.7 days shorter than that of the control (Table 1).

DISCUSSION

This study showed that the germination percentage of oil rose seeds was significantly affected by microbial inoculation. During 150 days of cold stratification following 4 weeks of warm stratification, premature germination was observed in seeds in all treatments, except for the Bioplin™. This indicates that the stratification duration of 150 days might be adequate to break dormancy of the seeds of the species *R. damascena*. The most common treatment to break dormancy of rose seeds is cold stratification (Ziesak, 2007; Zhou et al., 2009), and the degree of dormancy varies by species and duration of stratification (Stewart and Semeniuk, 1965). For instance, the species *Rosa multiflora* and *Rosa setigera* need 30 days of cold stratification; the species *Rosa 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 (Steward and Semeniuk, 1965). Moreover, it was reported that a stratification duration longer than 150 days was needed to remove embryo dormancy of oil rose seeds and that the germination percentage was over 80% through soaking seeds in 70 and 80% sulphuric acid for 10 min followed by 150 to 180 days of stratification (Hajian and Khosh-Khui, 2000). Higher germination percentages were obtained in this study. The higher premature germination percentage of oil rose seeds in all microbial inoculation treatments except for Bioplin™ during stratification than the control treatment might be due to an increase in the number of microorganisms in the seed pericarp during stratification and might be because these microorganisms macerated the hard and thick seed pericarp, thereby facilitating germination. A similar case was reported by Morpeth et al. (1997) and Morpeth and Hall (2000).

In this study, microbial inoculation treatments significantly increased germination percentage in comparison to the control. The results of the present study are also supported by the findings of Morpeth and Hall (2000) in *Rosa corymbifera* (95%) and of Belletti et al. (2003) in *R. canina* (50.25%) that microbial inoculation to the seeds increased germination percentage.

Among the treatments, the highest germination percentages were obtained from the EM•1®, followed by the Phosfert™ and B: speel™ (Figure 1). In both the prematurely germinated seeds during stratification and those seeds that did not germinate during stratification but germinated in the greenhouse immediately afterwards,
the lowest cumulative germination percentage was obtained from the control treatment (66.7%). Although there was no statistically significant difference between the Phosfert™, B: speel™ and the control (which might be because the EM•1® showed a very high germination percentage), both treatments showed a 20.6% higher germination percentage than that of the control treatment in terms of cumulative germination percentages. It might be stated that this percentage is quite high in commercial sense.

The effect of treatments on the mean germination time of oil rose seeds was statistically insignificant. However, despite the statistically insignificant difference among them, the mean germination times in EM•1® (7.2 days) and B: speel™ (7.3 days) were 1.7 and 1.6 days shorter than that of the control, respectively (Figure 2). Belletti et al. (2003) reported that different doses of compost activator treatments in <i>R. canina</i> further shortened the mean germination time by 8.48 to 9.64 days in comparison to the control.

**Conclusion**

This study suggested that microbial inoculations greatly increased the germination time and percentage of <i>R. damascena</i> seeds and that all seeds particularly germinated with the EM•1®. The observation of a high rate of premature germination (69.3%) of the <i>R. damascena</i> seeds during stratification with the EM•1® indicates that the time required for stratification in this species might be further reduced with the EM•1®. The inoculation of microorganisms to the seeds during preliminary treatment and the development of microorganisms immediately afterwards facilitated the germination of seeds. The study also showed that 150 days of cold stratification (4 ± 1°C) following 4 weeks of warm stratification (25°C) might be enough to break dormancy. How long it takes for dormancy of the species <i>R. damascena</i> to be broken will be clarified with further studies that we will be later conducted on EM•1® and other microbial fertilizers with different durations of stratification.

**REFERENCES**


