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Full Length Research Paper

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

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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: seepel™ (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 Maciarello, 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

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Abbreviations: ABA, Abscisic acid; **PSB,** phosphate solubilizing bacteria; **PGPR,** plant growth promoting rhizobacteria; **GP,** germination percentage; **MGT,** mean germination time.

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 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 the above-mentioned reasons, the germination of rose seeds is generally difficult. Prolonged dormancy delays germination and reduces germination percentage. This is a serious problem particularly in rose breeding and seed propagation (Yambe and Takeno, 1992; Bo et al., 1995; Hosafci et al., 2005; Zlesak, 2007).

The degree of dormancy varies by the time and temperature required to overcome dormancy as well as by germplasm, maturity at hip collection, time of seed extraction, temperatures during seed development and temperature and duration of stratification (Semeniuk and Stewart, 1962; Gudin et al., 1990). One of the most commonly used methods to break dormancy and stimulate germination in rose seeds is stratification (Zlesak, 2007). Various methods, such as gibberellic acid (Hosafci et al., 2005), hot water treatment (Younis et al., 2007), scarification with sulphuric acid (Bhanuprakash et al., 2004) and macerating enzymes (Yambe and Takeno, 1992), have also been tried besides stratification. Even though these methods are used alone or as a combination, it has been reported that the germination percentages in some rose species are still low. It was reported that the germination percentages ranged from 0 to 10% in the 1st year and from 24.7 to 73.7% in the 2nd year (Hosafci et al., 2005). Belletti et al. (2003) reported that they ranged from 0.5 to 50.3% and that this percentage was 18.8% in *R. canina* L. according to Alp et al. (2009), while the germination percentages were 13.8 and 13.5% in *Rosa pulverulenta* Bieb. and *Rosa dumalis* Bechst., respectively (Alp et al., 2009). In *Rosa bracteata* Wendl, they ranged from 1.8 to 41.5% according to Anderson and Byrne (2007).

One of the methods used to break dormancy in seeds and promote germination percentage is microbial inoculation to seeds or germination medium. It was reported that microorganisms macerated the hard-coated seed pericarp and facilitated germination (Morpeth and Hall, 2000). The objective of this study is to determine the effects of microbial inoculation in breaking seed dormancy and on the germination of *R. damascena* Mill. seeds.

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).

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 Kinagro Agriculture Inc, Turkey), B: speel™ (Bioglobal Inc. Turkey), Bioplin™ (Bioglobal Inc, Turkey) and Phosfert™ (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:seepel™ 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). 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:seepel™ treatment, B:speel™ (20 g/kg seed) was sprinkled over the seeds, and the seeds were covered completely with B:speel™. 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

Table 1. Effects of microbial inoculations on seed germination percentage (%) and mean germination time (day).

Treatment	Premature germination[1] (%)	Greenhouse germination[2] (%)	Cumulative germination[3] (%)	Mean germination time (days)
Phosfert™	44.0 b	71.5 b	84.0 ab	7.8
B:seepel™	52.0 b	66.7 b	84.0 ab	7.3
Bioplin™	0.0 d	68.0 b	68.0 b	7.8
EM•1®	69.3 a	100.0 a	100.0 a	7.2
Phosfert™ + Bioplin™	14.7 c	63.9 b	69.3 b	9.3
Control	13.3 c	61.0 b	66.7 b	8.9
F value	129.40**	8.59**	14.34**	2.58 ns

[1]Germination during stratification; [2]germination in greenhouse (seeds without premature germination); [3]premature germination plus greenhouse germination.
**Mean values in the same column followed by the same letter are not significantly different at the 0.01 level according to the Duncan's test. ns: not significant at $p < 0.05$.

refrigerator at 4 ± 1°C in polyethylene bags. In order to keep sphagnum moss moist in the stratification medium and for aeration, the polyethylene bags were opened once a week during the stratification period, and water was added as needed.

Germination experiment

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 in the greenhouse, and only those seeds that did not germinate at the end of duration of stratification were sown. The seeds treated with warm plus cold stratification were sown in peat-containing vials in the plastic covered greenhouse on May 28, 2009. The misting irrigation system was used with adequate moisture both in the greenhouse and 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)

$$MGT = \sum nd/N \quad (1)$$

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.

Experimental design and data analysis

A completely randomized plot design of 3 repetitions was used, and each replication consisted of 25 seeds. The percentage of prematurely germinated seeds during cold stratification in the experiment, the germination percentage of those seeds that were not germinated at the end of the duration of cold stratification and sown in the greenhouse immediately afterwards, the cumulative germination percentage of both prematurely germinated seeds and the greenhouse-germinated seeds, and the MGT were analyzed using SAS (1998) statistical analysis program. The germination percentages were transformed into arcsine before analysis. After evaluation, data were back transformed and original data presented. The mean values were compared by Duncan's multiple range test at the 0.01 probability level.

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-centage of 84%, were included in the same group with EM•1®. 66.7% germination occurred in the seeds (control) which were

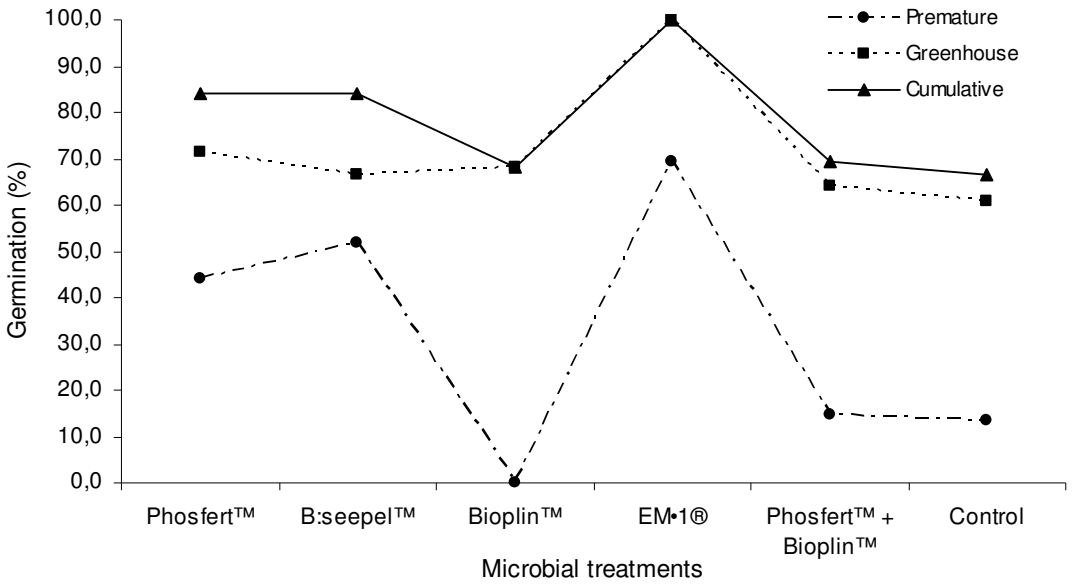

Figure 1. Effects of microbial inoculations on seed germination (%).

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 (Zlesak, 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 stratifi-cation (Hajian and Khosh-Khui, 2000). Higher germi-nation 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,

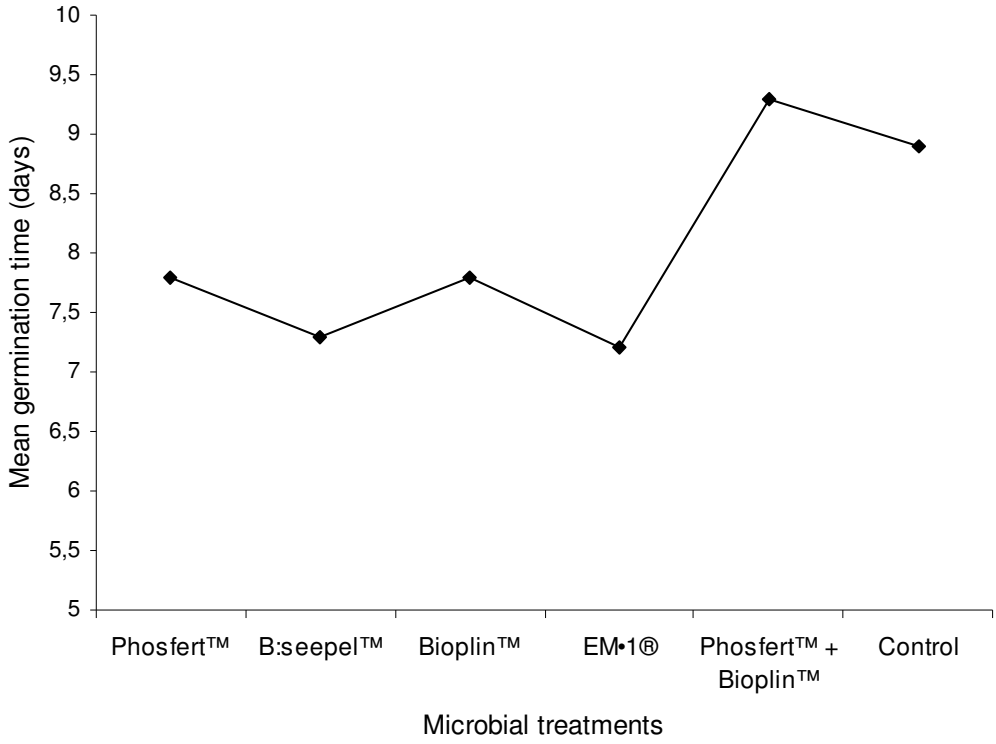

Figure 2. Effects of microbial inoculation on mean germination time (days).

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 *R. canina* 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 *R. damascena* seeds and that all seeds particularly germinated with the EM•1®. The observation of a high rate of premature germination (69.3%) of the *R. damascena* 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 *R. damascena* 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.

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