Scientia Horticulturae 119 (2009) 434–441

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Dormancy and germination in *Rosa multibracteata* Hemsl. & E. H. Wilson

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ARTICLE INFO

Article history:
Received 13 April 2008
Received in revised form 18 August 2008
Accepted 19 August 2008

Keywords:
Scarification
Warm plus cold stratification
GA_3
Intermediate physiological dormancy
Rosa multibracteata

ABSTRACT

Most of the achenes produced by *Rosa multibracteata* Hemsl. & E. H Wilson are dormant on maturity and require pretreatment to stimulate germination. To investigate the mechanism of dormancy and to develop effective methods of improving germination, roles of the pericarp, testa, and embryo of *R. multibracteata* in regulating dormancy were studied by investigating the effect of different pretreatments on germination. The effects of temperature and water stress were also tested with achenes treated by warm plus cold stratification. In freshly harvested achenes, pericarps are permeable and the embryo fully developed, which eliminates the possibility of physical, morphological, or morphophysiological dormancy. Germination percentage remained low (<5%) despite softening the pericarp or even removing it fully. However, fully removing the testa improved germination significantly (39%), indicating the possible presence of germination inhibitors in the testa. Dry storage, scarification with sulphuric acid (H_2SO_4), and warm stratification proved ineffective by themselves but when combined with cold stratification, improved germination and shortened the cold stratification period needed to break dormancy. Dry storage for 68 weeks followed by cold stratification for 16 or 24 weeks resulted in maximum germination (72–79%) among all the treatments. In achenes scarified with H_2SO_4, germination increased with an increase in the duration of cold stratification. Neither gibberellic acid (GA_3) nor 'smoke water' (water through which smoke had been bubbled for 2 h) had any positive effect on germination even on seeds that had been mechanically scarified or stratified. Both high temperature and water stress lowered germination in achenes treated with warm plus cold stratification. Our results suggest that *R. multibracteata* achenes have an intermediate physiological dormancy, and that dry storage for 68 weeks followed by cold stratification for 16 or 24 weeks is the best method for propagating *R. multibracteata* from seed.

1. Introduction

Rose is one of the most important commercial crops worldwide because of its value in landscape gardening, as an ornamental plant, as a medicinal plant, and as food and also because the plant is adapted to a wide range of habitats (Hornero-Méndez and Minguez-Mosquera, 2000; Uggla, 2004; Winther et al., 2005). Conventionally, rose is propagated mainly by such vegetative methods as stem cutting, layering, budding, grafting, and tissue culture (Pati et al., 2006). All these methods are associated with various problems such as shortage of rootstocks and longer production time. Seed propagation of roses is used for breeding new cultivars, restoring native plants, selecting rootstocks, and, in some varieties, for producing the hips; however, seed propagation is difficult because of the low germination percentage, a result of prolonged seed dormancy (Tincker and Wisley, 1935; Xu et al., 1993; Bo et al., 1995; Hoşafçı et al., 2005).

The dormancy in rose achenes and delayed germination may be due to the hard pericarp, inhibitors in pericarp and testa, and physiological barriers in the embryo (Jackson and Blundell, 1963; Densmore and Zasada, 1977; Bo et al., 1995). The pericarp is permeable, although it sometimes prevents full imbibition (Svejda, 1972; Xu et al., 1993; Bo et al., 1995). The barrier in the form of a hard pericarp contributes to dormancy in some rose achenes (Bhanuprakash et al., 2004) but is not its sole cause, since cracking the pericarp fails to break the dormancy in some other achenes (Tincker and Wisley, 1935; Svejda, 1968). Moreover, Bo et al. (1995) report that high concentrations of abscisic acid (ABA) in the pericarp and testa of rose achenes may inhibit germination. It has been confirmed that embryos in the achenes are fully developed and have no morphological dormancy (Bo et al., 1995; Jackson and Blundell, 1963). Further, physiological barriers to germination in embryos have been overcome by cold stratification in a number of rose species (Tincker and Wisley, 1935; Svejda, 1968; Densmore

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doi:10.1016/j.scienta.2008.08.017

and Zasada, 1977). The mechanism of dormancy in rose achenes is thus a complex phenomenon and the few studies that have been conducted have focused on only a few species. Therefore, a better understanding of dormancy in rose achenes would contribute to successful propagation of roses from seeds.

A higher percentage of germination in rose seeds is possible only when the dormancy is overcome. Current attempts to release the dormancy have centred on two approaches, namely (a) eliminating the mechanical barrier in the form of the pericarp, which restricts the growth of embryo and its access to water and air and (b) reducing the period of after-ripening required by the embryo (Stewart and Semeniuk, 1965). Treatments involving soaking achenes in concentrated H_2SO_4, exposing them to an oxygen-rich environment (100% oxygen) (Tincker and Wisley, 1935; Svejda, 1968; Zlesak, 2005) or to various chemicals (such as GA_3), and dry storage or cold stratification alone (Stewart and Semeniuk, 1965; Semeniuk and Stewart, 1966) have had little success. However, a combination of different treatments – for example, H_2SO_4 scarification combined with cold stratification (Svejda, 1968; Densmore and Zasada, 1977; Bhanuprakash et al., 2004) or a combination of warm and cold stratification (Semeniuk and Stewart, 1966; Svejda, 1968; Densmore and Zasada, 1977) – may greatly improve germination. However, the efficacy of different pretreatments in stimulating germination varies with the species (Semeniuk and Stewart, 1966; Bhanuprakash et al., 2004).

It is important to know the kind of seed dormancy for successful propagation of horticultural plants, but presently most publications on seed dormancy do not indicate the kind of dormancy that was investigated (Baskin and Baskin, 1998). Lack of an internationally acceptable system of specifying dormancy may have discouraged researchers from investigating the different kinds of seed dormancy. Baskin and Baskin (2004) propose a new classification system for seed dormancy that consists of five types of dormancy: physiological, morphological, morphophysiological, physical, and combinatorial. Definition of these various classes of dormancy is based on a number of attributes such as permeability of the seed coat (or the fruit) to water (impermeable or permeable), morphology of the embryo (underdeveloped or fully developed), and physiological responses of whole seeds to temperature or to a sequence of temperatures. The new system of classifying seed dormancy makes it possible to determine the type of dormancy by investigating the effect of various pretreatments on germination.

Rosa multibracteata Hemsl. & E. H. Wilson is a perennial shrub found in Sichuan and Yunnan provinces of China (Yu, 1985). The plant produces its characteristically pink flowers from May to July and the red fruits in August and September. The leaves, pollen, and fruits are of great economic value as a source of vitamins and as constituents of medicines (He et al., 1994; Chen et al., 2000). *R. multibracteata* is abundant in arid and semi-arid habitats where it checks soil erosion and provides food and shelter to animals. The plant may be a good candidate for restoring vegetation to these dry areas. However, paucity of information on seed dormancy and germination greatly limits the utility of the species.

The objectives of this study were to investigate the mechanism of dormancy and to develop effective methods of improving germination in *R. multibracteata*. In particular, we wanted to (1) determine kind of dormancy in this species according to the system developed by Baskin and Baskin (2004); (2) understand the role of the pericarp, testa, and embryo in the dormancy; and (3) determine the effects of temperature and water stress during incubation on the germination of *R. multibracteata* achenes subjected to warm plus cold stratification. The results will be helpful in large-scale propagation of the species and facilitate its economic exploitation and ecological restoration.

2. Material and methods

2.1. Collection of achenes and measuring their physical attributes

Hips (the fleshy hypanthium) of *R. multibracteata* were collected from at least 30 plants on 1 October 2005 from the dry Minjiang River valley (32°02′N, 103°40′E, 2370 m a.s.l.) in Maoxian county, Sichuan, China. The area is semi-arid with a typical dry climate (Zhang, 1992) characterized by low and unpredictable rainfall, rapid and intense evaporation, and infertile soil (Bao et al., 1999). The mean annual temperature is 11 °C, mean annual rainfall is 494 mm, and mean annual evapo-transpiration is 1019 mm (Liu et al., 1996). Immediately after collection, the achenes were extracted manually from the hips and mixed thoroughly. Only those achenes that sunk in water, and therefore assumed to be mature and viable, were used in the experiments. After drying the achenes for 3 days in the open, the achenes were stored at room temperature (10–25 °C) until needed (a period that amounted to less than 2 weeks). Table 1 presents data on such physical attributes as length, mass, and moisture content of the achenes.

2.2. Experiment 1: imbibition by achenes

To study imbibition and physical dormancy, imbibition was monitored in mechanically scarified and non-treated (control) achenes. A small portion of the pericarp on the side opposite to the radicle was carefully removed with a scalpel to obtain mechanically scarified achenes. As replications, three lots, each comprising 100 scarified achenes and 100 non-scarified achenes, were placed on a double layer of filter paper moistened with 10 ml distilled water in individual Petri dishes 9 cm in diameter, which served as growth chambers, and incubated in the dark at 25 °C for different durations: 0, 1, 3, 9, 24, 48, 72, 96, and 120 h. After incubation, the achenes were blotted dry, weighed to the nearest milligram, and returned to the growth chambers. The percentage increase in achene mass was determined as described by Baskin et al. (2004).

2.3. Experiment 2: removal of the pericarp and testa

To examine the role of the hard pericarp and testa in seed dormancy, the achenes were scarified by any of the four methods: mechanically with a scalpel as described above, using H_2SO_4, by fully removing the pericarp, and by fully removing the testa. Each replication consisted of 25 achenes. For scarification with H_2SO_4, achenes were soaked in concentrated H_2SO_4 for 2, 4, or 6 h and then washed thoroughly with tap water. For removing the pericarp, a scalpel was used to carefully excise the seeds, whereas the testa was removed with tweezers to obtain excised embryos. The treated achenes were sterilized in 5% (v/v) sodium hypochlorite solution for 10 min and washed three times with sterile

Table 1
Achene traits of *R. mulbraceata* (mean ± S.E.)

Achene length (mm)	5.15 ± 0.11
Achene width (mm)	2.89 ± 0.06
Pericarp thickness (mm)	0.64 ± 0.04
Achene mass (mg)	20.01 ± 0.37
Seed:achene ratio (%)	22.95 ± 0.44
Pericarp:achene ratio (%)	77.05 ± 0.44
Achene water content (%)	8.22 ± 0.08
Percentage of sunken achenes (%)	72.50 ± 1.20
Viability of sunken achenes (%)	54.19 ± 0.90

20 achenes were measured for achene length, achene width and pericarp thickness, 6 replications of 100 achenes each for achene mass, achene water content and percentage of sunken achenes and 4 replications of 20 achenes each for seed:achene ratio, pericarp:achene ratio and viability of sunken achenes.