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Effects of after-ripening, stratification and GA_3 on dormancy release and on germination of wild asparagus (*Asparagus acutifolius* L.) seeds

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ABSTRACT

Seeds of wild asparagus (*Asparagus acutifolius* L.) were treated and compared in this research to investigate seed dormancy class and level involved in this species. Four seed lots were compared: (i) freshly harvested seeds in 2007 (07Fr); (ii) freshly harvested seeds in 2008 (08Fr); (iii) after-ripened (AR) 2007 seeds dry stored in glass jars (AR*g*); (iv) AR 2007 seeds dry stored in paper bags (AR*p*). The 07Fr seeds were exposed to (1) chemical scarification combined with gibberellic acid (GA_3) levels (0, 200, 400, and 600 mg L^{-1}) and to (2) 28-day moist stratification at 5 and 23 °C, and two sequences of 5/23 °C combined with 0 and 400 GA_3 mg L^{-1} levels, and (3) together to the 08Fr and AR seeds were exposed to 56-day moist stratification at 5, 23, or 5/23 °C. With the 08Fr and AR seed lots this last stratification treatment was combined with 0 or 800 GA_3 mg L^{-1} levels. The dormancy depth of 08Fr (32% germination) was less than 07Fr seeds (2%). The latter after-ripened during dry storage and when stored in glass germinated more (47.5%) than in paper (12%). Stratification for 4 weeks was ineffective in improving germination of 07Fr seeds; when chemically scarified they did not germinate at all. The highest (nearly 70%) and the most rapid and uniform germination were observed for all the lots when they were warm stratified for 56 days. Warm stratification improved germination more than alternate temperature stratification, while cold stratification inhibited germination especially for the 08Fr and ARg lots, thus seeds seem not to have a morphological component to their dormancy. GA_3 only improved germination of 07Fr seeds, at a low rate. *A. acutifolius* seeds fit the characteristics of a non-deep physiological dormancy.

1. Introduction

Wild asparagus (*Asparagus acutifolius* L.), formerly classified in the *Liliaceae* family, has been recently included in the *Asparagaceae* family (Angiosperm Phylogeny Group II, 2003). The centre of origin and its geographic distribution is South Europe as far east as southeast Bulgaria (Štajner et al., 2002). Currently, wild asparagus is a common species in all Mediterranean areas (Sica et al., 2005). In Italy, this evergreen species is found mainly in its southern regions, where it grows spontaneously in uncultivated areas, on dry stonewalls, fences and especially in the Mediterranean macchia ecosystem. The spears are highly valued and consumed in a vast number of regional dishes (Ghirardini et al., 2007). Wild asparagus could become a new crop with high income potential, especially for marginal areas where its cultivation will fit perfectly within a sustainable agriculture framework of both biodiversity and environmental conservation. Currently, some limitations exist in the cultivation of this vegetable, the most important is related to its low and erratic seed germination that limits seedling or crown production (Rosati, 2001). Rosati and Falavigna (2000) found that dry storage of *A. acutifolius* seeds for more than one year did not completely break dormancy, obtaining a satisfactory germination (~90%) of freshly harvested seeds only after moist stratification in sand for at least 8 months. They also observed that irrespective of dry storage or stratification treatments, germination responses were strongly influenced by ecotype. Other authors, working with different ecotypes, reported 60–70% germination with seeds maintained in open field conditions of a Mediterranean environment under 70% shading for almost 5 months (January–May: winter–spring period), independently of whether stratified in sand or directly sown in peat (Fiori et al., 2001).

A more recent study with an *A. acutifolius* ecotype from an hilly area (Conversa and Elia, 2009) has reported that a period of 13 months of dry storage in paper bags at room temperature (21 ± 2 °C; R.H. 50 ± 10%) is effective in after-ripening seeds by enhancing seed sensitivity to subsequent seed treatments (soaking in warm water at 35 °C and moist stratification): the highest germination (76%) was obtained when after-ripened seeds were stratified and soaked,

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without any significant difference between cold or warm stratification. When singularly applied, soaking or warm stratification were found to be less effective (47% germination) while cold stratification did not improve germination. Seeds appeared fully imbibed at the end of the pre-germination, which points to the absence of physical dormancy, as already noted by other authors (Rosati and Falavigna, 2000). Following these findings a non-deep physiological dormancy (according to seed dormancy classification proposed by Baskin and Baskin, 2004) has been hypothesized for the seeds of this species, nevertheless more information is necessary to confirm this and to exclude a morphological component.

Scarification is reported to be effective to break non-deep physiological dormancy, by weakening the embryo cover layer, as well as gibberellic acid application. Moreover, to overcome most morpho-physiological dormancy types, a sequence of warm and cold moist stratification is required (Baskin and Baskin, 2004).

To investigate seed dormancy class and level involved for this species, in this paper we report the results of chemical scarification, gibberellic acid levels, temperature regime and sequence during the 28- and 56-day periods of moist stratification applied to non-after-ripened and after-ripened seeds of *A. acutifolius* collected in Southern Italy.

2. Materials and methods

2.1. Seed collection

In November 2007 and 2008, plant stems with mature green-brown berries were collected from wild *A. acutifolius* L. plants selected in Manfredonia territory (41°37′36″84 N, 15°54′36″72 E, Foggia province, Apulia Region). The site is the wetland of Lake Salso Oasis in the Gargano National Park. It is about 1 km far from the coastline and 2 m above sea level and is dominated by a Mediterranean climate with mild winter and dry-and-warm summer (Macchia et al., 2000); mean minimum and maximum temperatures are 10.8 ± 1.7 and 19.9 ± 2.2 °C, respectively, and the mean temperature of the coldest (January) and hottest (August) months are 7.1 and 24.5, respectively. Mean annual rainfall is 537 mm (Caliandro et al., 2005). The stems were air-dried for 2 weeks under 90% shading before removing berries. Once the berries were picked off from stems, the pericarps were manually removed, thus releasing the seeds. In both years the weight of 1000 seeds and moisture content of seeds were on average 39 ± 1.5 and 6.8 ± 1.0 g 100 g^{-1} dw, respectively.

2.2. Preparation of seed lots

In total four seed lots were prepared. Seeds harvested in the first year (2007) were randomly divided into three lots: the first was used for the tests carried out 2 months after harvest, hereinafter, referred to as the 2007 fresh seed lot (07Fr), the other two were dry-stored for 14 months (after-ripening—AR) in glass jars (AR*g*) or in paper bags (AR*p*), at room temperature (21 ± 2 °C; R.H. 50 ± 10%). The last lot was represented by the seeds harvested in 2008 that were used in germination tests 2 months after harvest and hereinafter referred to as the 2008 fresh seed lot (08Fr).

At the end of the after-ripening period the AR*g*, and AR*p* seed lots had 7.1 ± 0.6 and 8.1 ± 0.4 g 100 g^{-1} dw of water content, respectively.

2.3. Seeds were sterilized according to the Reid et al. (2002) procedure

Soaking, which in a previous work (Conversa and Elia, 2009) proved effective in improving *A. acutifolius* germination, was always applied. Seed soaking was performed by putting seeds in 100 ml vials (50 seeds/vial—average weight 3 g) filled with 50 ml of distilled water. The vials were maintained in a thermostatic bath at 35 °C for 12 h in the dark.

2.4. Seed treatments

Seed treatments were arranged (Table 1) in order to evaluate the following effects.

2.4.1. Experiment 1—chemical scarification and gibberellic acid level on 2007 fresh seed germination

The test was aimed to evaluate the effect of chemical scarification combined with different level of GA_3 treatment on 2007 freshly harvested seeds (07Fr). The trial started in January 2008, seeds were submerged in a sulphuric acid solution (96%) for 2 min and then rinsed with running water. Both scarified (*SC*) and control seeds (no*SC*) were soaked, sterilized and then treated with GA_3 at 0, 200, 400, and 600 mg L^{-1} by maintaining seeds constantly for 24 h under submerged conditions (12 g/20 ml) in the GA_3 solution at room temperature.

2.4.2. Experiment 2—GA_3 and temperature regimes/sequences during 28 days moist stratification on 2007 fresh seed germination

In January 2008 a separate test was carried out to evaluate the effect of 28 days of moist stratification on 07Fr seeds, performed with different temperature regimes and sequences and combined with the GA_3 treatment.

Sterilized seeds were first treated with GA_3 at 0 and 400 mg L^{-1} ($GA_3$0 and $GA_3$400) (as above described), and then they were subjected to moist stratification for 28 days (*ST28*) with the following temperature regimes and sequence: 28 days at 23 °C ($ST28_{23}$); 28 days at 5 °C ($ST28_5$); 14 days at 5 °C followed by 14 days at 23 °C ($ST28_{5/23}$); a repetition of two cycles of 7 days at 5 °C plus 7 days at 23 °C ($ST28_{5/23/5/23}$) (these latter two to simulate seasonal fluctuations). After the stratification period the seeds were soaked. For each GA_3 level non-stratified controls ($GA_3$0-no*ST* and $GA_3$400-no*ST*) were also carried out; they were soaked, sterilized and finally treated with gibberellic acid.

Stratification was carried out in flat aluminium containers (6 cm × 10 cm × 20 cm) filled with a 5 cm layer of washed river-sand saturated with distilled water. The containers were placed in plastic bags to avoid dehydration of the substrate and were stored in the dark in a growth chamber.

2.4.3. Experiment 3—seed lot and temperature regimes/sequences during 56 days of moist stratification on seed germination

The seed lots used in this test were: 07Fr, 08Fr, AR*p* and AR*g*. The tests on 07Fr seeds were carried out in January 2008, while those on the other seed lots were carried out in January 2009.

The effect of a longer stratification period was investigated, performed with the same temperature regimes (23 and 5 °C), but with different time sequences, than those applied in the previous "Experiment 2". Sterilized seeds were subjected to moist stratification (in the conditions described above) for 56 days (*ST56*) with the following temperature regimes: 56 days at 23 °C ($ST56_{23}$), 56 days at 5 °C ($ST56_5$), 28 days at 5 °C followed by 28 days at 23 °C ($ST56_{5/23}$). After the stratification period, seeds were soaked before placing them in Petri dishes. For each seed lot, non-stratified (no*ST*) controls were carried out which were only soaked and sterilized before starting incubation in Petri dishes.

2.4.4. Experiment 4—GA_3 and temperature regimes during 56 days moist stratification on 2008 fresh and after-ripened seed germination

A separate test was performed in January 2009 to evaluate the effect of 800 mg L^{-1} GA_3 on the 08Fr, AR*g* and AR*p* seed lots, higher