



Optimization of seed germination in an Iranian serpentine endemic, *Fortuynia garcinii*



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ARTICLE INFO

Article history:

Received 8 January 2017

Received in revised form 24 March 2017

Accepted 8 April 2017

Edited by Hermann Heilmeyer

Available online 12 April 2017

Keywords:

Gibberellic acid

Moist chilling

Nickel

Pericarp

Seed dormancy

ABSTRACT

Fortuynia garcinii (Brassicaceae) is endemic to serpentine soils in central Iran. It has indehiscent silicles. The effects of its fruit pericarp on seed imbibition and germination were determined. The effects of moist chilling (15 days) and gibberellic acid (GA₃, four levels), both alone and combined, were also investigated. In addition, the effects of nickel (Ni) on germination and early seedling growth were evaluated. When inside the indehiscent silicles, imbibition of seeds was hampered and germination completely inhibited. Immediately after removing the pericarp, the seeds were able to imbibe as good as isolated seeds, but their germination rates were significantly lower when the removed pericarps were included in the Petri dishes. All the GA₃ concentrations, moist chilling, both alone and combined, significantly shortened mean germination time (MGT), and increased the germination index (GI) and the germination percentage (GP). Excess Ni did not affect GP, but inhibited seedling growth. In conclusion, the pericarp inhibits seed germination, both chemically and mechanically, through impeding imbibition, in *F. garcinii*. Moist chilling or GA₃ improves the speed and final percentage of germination. Seedling growth is much more sensitive to Ni excess than seed germination.

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1. Introduction

Serpentine soils are characterized by physically and biologically stressful edaphic properties such as steep slopes, unstable substrates, coarse rocky textures and low water-holding capacities. They also have low Ca/Mg ratios, low contents of essential nutrient elements such as nitrogen, potassium and phosphorus, and relatively high, often toxic, concentrations of nickel (Ni), or occasionally Cr and Co (Ghaderian and Baker, 2007). Serpentine soils have a specific flora, often with considerable numbers of endemic species or specialized ecotypes. Most serpentinophytes are hypertolerant to Ni (Clemens, 2001; Asemaneh et al., 2006; Ghaderian and Baker, 2007). Most metal-hypertolerant species or ecotypes are 'excluders', which restrict the uptake and/or root-to-shoot transport of heavy metals (Baker, 1981), although at least part of their hypertolerance usually relies also on a superior capacity to sequester the metals inside their body, through chelation and subcellular compartmentalization, usually mainly in root cell vacuoles (Hall, 2002). A minority of metal-hypertolerant species are so-called 'hyper-accumulators', most of which hyperaccumulate Ni. These species

accumulate metals at extremely high concentrations, usually 2–3 orders of magnitude higher than 'normal' plants, in their leaves (Baker and Brooks, 1989).

Fortuynia garcinii (Burm.f.) Shuttlew, also known as *Fortuynia bungei* Boiss. (Brassicaceae), is a non-Ni-hyperaccumulating, woody based branched perennial, endemic to serpentine soils in central Iran (Ghaderian and Baker, 2007). It usually flowers in late spring, and produces a large number of fruits. The fruit is an indehiscent silicle, containing one or two seeds (Rechinger, 1968). The species is propagated through seeds only. Several reports indicated that in Brassicaceae with dry indehiscent fruits germination within the fruit is prevented by the rigid pericarp, which physically prevents the embryo to expand and protrude. However, the pericarp might also inhibit germination through hampering the diffusion of water or oxygen to the embryo (Mekenian and Willemsen, 1975; Lu et al., 2010; Ohadi et al., 2011; Lu et al., 2015). The fruits ripen during late May–early June, and are then dispersed by wind. Germination occurs early in spring of the next year, after decay of the pericarp. Thus, in this species fruit indehiscence may be considered to represent an adaptation to avoid exposure in the seedling stage to extreme heat and drought in summer, or extreme cold and frost in winter, which are characteristic for the local climate of the natural habitat of the species.

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In many species seeds do not germinate when placed under conditions normally regarded as favorable to germination and are said to be dormant. Five types of seed dormancy have been recognized: physiological, morphological, morpho-physiological, physical and combinational dormancy (Baskin and Baskin, 2004). Seed (or fruit) coat-dependent dormancy is due to restriction of water uptake, gas exchange, mechanical resistance, or the presence of inhibitors such as abscisic acid (ABA) (Bewley and Black, 2012; Afroze and O'Reilly, 2016). Seed dormancy is one of the most important adaptive properties of wild species, and is a mechanism for optimizing the distribution of germination in time and place (Bhatia et al., 2005; Cousins et al., 2014). Various seed treatments, such as disruption of the seed coat (scarification), a period of dry storage (dry after-ripening), moist chilling (stratification), exposure to light (Finkelstein et al., 2008; Rouhi et al., 2010) and gibberellin (GA) treatments have been applied (Rouhi et al., 2010; Barreto et al., 2016; Mattana et al., 2016) to increase the speed and the percentage of germination, or to synchronize the germination day. Gibberellins stimulate seed germination in a wide range of plant species. However, the effectiveness of gibberellins depends on the species (Rouhi et al., 2010). Breaking dormancy by incubating seeds under moist and cold conditions, to simulate overwintering, is known as stratification (Finkelstein et al., 2008). The effectiveness of GA₃ as a germination promoter is believed to increase with stratification treatment (Yamauchi et al., 2004), and stratification has been reported to induce an increase in the GA₃ concentration in seeds (Yamauchi et al., 2004; Rouhi et al., 2010).

Nickel (Ni) is considered an essential micronutrient for plants (Brown et al., 1987), but becomes toxic at excess concentrations in most plant species. Typical symptoms of Ni toxicity in plants are inhibition of growth, chlorosis, wilting, foliar necrosis and root browning or die-back (Gerendás et al., 1999; Gajewska et al., 2013). Serpentine soils are often toxically Ni-enriched (Proctor and Woodell, 1975), and serpentine plant communities are largely composed of species or ecotypes with extreme levels of Ni tolerance, as compared to related non-serpentinophytic species or con-specific ecotypes (Baker and Brooks, 1989). Since seed germination is the first physiological process to be potentially affected by high Ni concentrations in the soil, the ability of a seed to germinate in a medium containing high Ni might be indicative of its level of tolerance to this toxic element, although Ni toxicity seems to impact more on seedling growth than on germination (Di Salvatore et al., 2008; Visioli et al., 2014).

Human activities and climate change, such as global warming, or changes in precipitation patterns can easily lead to extinction of rare species in restricted habitats, such as serpentine areas. Strengthening of populations using plant specimens that are propagated *ex situ* is regarded as a possibility to reduce the risk of local extinction of endangered species (Kirmizi et al., 2011). The germination requirements of *F. garcinii* have not previously been studied. Data on the germination conditions of this species could be employed in *ex situ* conservation management.

To better understand the germination ecology of *F. garcinii* and the potential role of pericarp-imposed seed dormancy therein, we determined the effects of pericarp removal and cold stratification on germination in *F. garcinii*. To check for a potential role for Ni in seedling establishment, we also investigated the effects of different concentrations of Ni on seed germination and early seedling growth.

2. Materials and methods

2.1. Plant material

Approximately 2000 mature fruits of *Fortuynia garcinii* were harvested from at least 50 randomly selected individual plants

growing on serpentine soil at Anarak (33° 23' N, 53° 41' E and 33° 02' N, 53° 03' E), Iran, in June 2014. The fruits were mixed thoroughly, air-dried and stored at 4 °C for at least one month before starting the experiments. Seeds were isolated from the silicles and surface sterilized in 5% (v/v) sodium hypochlorite for 15 min and rinsed three times in distilled water.

2.2. Effect of pericarp (fruit parts) on germination

Experiments were performed with seeds in three states: within intact fruits, and separated from the silicles, either with or without the removed pericarps present in the Petri dishes. Seeds were placed about 1 cm apart from each other. The pericarp was removed and placed adjacent to its seed (at 0.5 cm from each other). The water permeability of seed coats and pericarps was determined by weighing 25 seeds before and after a 60-h imbibition period (maximum water absorption in isolated seeds), and the percentage weight increase (% Wr) was calculated as $Wr(\%) = [(Wi - Wd)/Wd] \times 100$, where Wi and Wd are mass of imbibed and air-dry dispersal units (seeds), respectively (Lu et al., 2015).

2.3. Effects of GA₃ and moist chilling on germination

Seeds without pericarp were used in the following experiments: germination tests in Petri dishes (9 cm diameter) with two layers of filter paper, moistened with 5 ml distilled water with different GA₃ concentrations (0, 200, 500 and 750 mg/L), and sealed with adhesive tape (Parafilm™) to avoid desiccation. To check the effects of cold stratification fully imbibed seeds were stratified at 4–5 °C in the dark for 15 days in the absence or presence of GA₃ at different concentrations (a pilot experiment showed that longer stratification periods did not further enhance germination, irrespective of the presence or absence of GA₃). Three replicates with 25 seeds each per GA₃ treatment level were then exposed to 25 ± 2 °C. Experiments were performed in the dark, because it appeared in pilot experiments that light did not affect germination. Seeds were considered germinated when the radicle protruded from the seed by one mm.

2.4. Effects of Ni concentration on germination and early seedling growth

Ni toxicity was evaluated by testing seed germination and hypocotyl and root elongation in a series of nickel sulfate (NiSO₄) concentrations in distilled water. The concentrations of Ni were: 0, 5, 10, 25, 50, 100, and 150 μM. To accelerate seed germination, based on the results obtained from preceding experiments, Petri dishes containing different concentrations of Ni were incubated at 4–5 °C for 15 days in darkness and then transferred to 25 ± 2 °C in dark. Root and hypocotyl length were measured after 8 days.

2.5. Germination characteristics

The germination percentage (GP) and the mean germination time (MGT) were calculated from the germination counts during 8 days (after 6 days, there was no further germination in pilot experiments).

The mean germination time (MGT) was calculated as:

$$MGT = \frac{\sum Dn}{\sum n}$$

in which n is the number of seeds germinated on day D, and D is the number of days after the start of the experiment (Dastanpoor et al., 2013).

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