

Seed germination of *Paederotella pontica* (Rupr. ex Boiss.) Kem-Nath.- rare endemic species of the Caucasus.

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Abstract. *Paederotella pontica* is a Colchis tertiary relict species, regional narrow endemic to the Caucasus, representative of an oligotypic genus. The aim of this study was to determine dormancy-breaking requirements and develop seed germination protocol for *P. pontica*. Freshly matured seeds of *P. pontica* are morphophysiologicaly dormant (MPD). Mean length of seed is 820 μm , linear embryo is fully differentiated, on average 625 μm long. Penetration of tetrazolium salt indicates the permeability of seed coat and high percentage of vital seeds in capsules. Prior to root emergence, the E:S ratio increased from 0.76 to 0.9. Effects of warm and cold stratification and gibberellic acid (GA_3) on embryo growth and seed germination were studied under laboratory conditions. Since cold stratification is the only requirement for the loss of MPD, the longest embryo growth occurred during this treatment and GA_3 promoted MPD loss, we concluded that *P. pontica* seeds have intermediate complex MPD. Based on the treatment results a germination protocol is proposed: 1. Dry storage at 20°C, 2 months; 2. Cold-wet stratification at 3°C, 3 months; 3. Germination at 20/15°C day/night. Under developed conditions germination is fast, synchronous and yields to 80%.

1 Introduction

The oligotypic genus *Paederotella* (E. Wulff.) Kemul-Nath. from the family Plantaginaceae includes only three species endemic to the Caucasus: *P. daghestanica* (Trautv.) Kem.-Nath., *P. pontica* (Rupr. ex Boiss.) Kem.-Nath. and *P. teberdensis* Kem.-Nath.) [1].

P. pontica is northern Colchis tertiary relict plant with narrow distribution in Northern Caucasus – Transcaucasus, where it grows by rare and small populations in rock fissures, forest gorges mainly in the subalpine belt with an altitudinal range 300-2400 m a.s.l. In Georgia populations of *P. pontica* often consisting of a few numbers of individuals, are rare and categorized as vulnerable (B2ac(ii)) [2]. Potential threats caused by natural and anthropogenic factors promote the loss of species and increase its extinction risk. Several studies showed that the rare endemic species are in particular suffer from the changes of habitat quality in comparison with widespread taxa, due to limited distributional range [3]. Little is known about seed germination ecology of the *P. pontica*. Giving the risk of the

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species loss in the native habitats posed by natural and anthropogenic threats, the knowledge of factors promoting seed germination could greatly contribute to the plant propagation and support the ex situ conservation of *P. pontica*. Although the species is listed in regional conservation reports [4, 5] as rare endemic plant need to be protected, its reproduction requirements are still poorly known. In many angiosperms seeds are dormant at harvest, that means that they do undergo morphological or morphophysiological changes for dormancy breaking to meet specific germination requirements [6, 7]. As a rule, appropriate temperature and hormonal pretreatment can both release dormancy and promote germination [8, 9].

We have not been able to find data to show whether or not the seeds of *P. pontica* are dormant at harvest and if so, what type of dormancy is characteristic of the species. Therefore, the purpose of this study was to develop optimal seed germination protocol. We aimed to test temperature and hormone effect on the dormancy and identify requirements to overcome it. Another aim was to test the suitability of freezing microtomy technique to analyse growth dynamics of small-sized embryos in vital seeds, without using the fixatives.

2 Materials and methods

Capsules with mature seeds were collected from natural population of *P. pontica* growing in Western Georgia in the foothills of the Ajara-Imereti ridge (271743.14 E, 465178.45 N) on July 2016. Due to the limited number of the collected seeds and small seed sizes, viability, permeability of the seed coat and initial size measurements were made simultaneously on the same seed amount shortly after collection. To determine the initial length of seed (S) and embryo (E) seeds were sectioned in a freezing microtome. Median longitudinal 10 μm sections were measured under the light microscope Axio Lab. A1 (Carl Zeiss, Germany), equipped with the digital ruler and photcamera AxioCam 16 Erc 5s. Length and standard error were calculated for each sample of 10 seeds/embryos. The maximum embryo length for germination was determined by measuring embryos in seeds with the seed coat split open, indicating that root protrusion was started. All measurements were made on vital seeds without using the fixatives.

Seed viability was tested by staining the seeds in 1% solution of tetrazolium chloride [10].

To check the dormancy status of freshly harvested seeds, seeds were incubated at 20/15°C at day/night regime for 2 months.

To test the effect of dry storage on dormancy and germination, seeds were stored at 17°C for 2 month and then germinated at 20/15°C, or subjected to incubation in GA₃ or GA+cold stratification (CS) at 5°C.

To test the effect of CS on dormancy breaking, seeds were sown in Petri dishes on filter paper moistened with distilled water at 5°C for 1, 2 or 3 months. After the end of each stratification period seeds were transferred at 20/15°C for germination A seed with at least 2 mm long radicle was considered to be germinated.

To determine the effect of the gibberellic acid (GA₃) and CS on dormancy breaking, 1000 mg/L solution of GA₃ (Sigma-Aldrich, St. Louis, MO, USA) was applied for 1-3-month period of CS. Analysis of parameters related to embryo growth was conducted at the end of each month. A 90-seed lot was used to CS treatment, combination of GA₃ + CS condition and control, distributed into 15-seed per dish. For warm stratification and dry after-ripening study, 8 dishes of 10 seeds each were used. A total 180 seeds were analysed in the study.

The statistical analysis was made using the ANOVA procedure of SPSS 25.0. The difference between the means was compared using the Duncan's multiple test ($p < 0.05$). The significance of differences between the variants was evaluated by post-hoc Tukey test.

Treatments, in which none of the seeds germinated or the germination percentage was lower than 5.0 %, were not included in the statistical analysis.

3 Results and Discussion

There was a statistically significant difference between treatment groups as determined by one-way ANOVA ($F_{2,127}=514.79$, $p=.000$). A Tukey post hoc test revealed that the time to overcome dormancy during the cold stratification was statistically significantly reduced when GA_3 was applied during the cold stratification. There was no statistically significant difference between GA_3+CS and CS lots in final germination percentage ($p=.98$). After one month of CS >35% of seeds germinated; 54 and 75 % of these seeds germinated after 2 and 3 months, respectively. Application of GA_3 during the CS significantly shortened the dormancy breaking period. Thus, substantial difference between CS and GA_3+CS treatments could already be observed at the end of the first month of chilling: germination percentage of the seeds from GA_3+CS treatment was much higher to compare with CS (68 versus 35%). After 2 and 3 months of GA_3+CS treatment 76 and 79 % of the seeds germinated, respectively. However, it should be noted that the final germination percentage of CS and GA_3+CS seeds were more or less the same (75 and 79% respectively).

Results of our study showed that the seeds subjected to the 1 month of warm incubation and dry-stored seeds did not germinate at 20/15°C, indicating dormancy of both seed group. We also consider a possible nondormant status of small portion of mature seeds, as less than 5% of seeds germinated under these conditions. When seeds were subjected to WS for 2 months, it caused seed deterioration.

Our experience shows that the use of freezing microtome technique could be useful when working with seeds having an extremely small size. Permeability of seed coat was clearly confirmed by staining in tetrazolium chloride solution. Penetration of the dye and the intensive red colour of the tested embryos indicate the high number of viable seeds in the population, despite the small population size.

Mean length of the mature seed measured on the median longitudinal sections is 820 μm . Embryo is linear, mean initial length of fully differentiated embryo is 625 μm . During warm stratification no obvious change in the seed morphology and embryo size was observed. Thus, embryo to seed size ratio did not exceed 0.76. A sufficient embryo elongation was fixed in seeds subjected to CS or GA_3+CS treatment. Critical (maximum) embryo length was measured after seed transfer to the germination conditions at 20/15°. Prior to root emergence, the E:S ratio reached to 0.9.

Classification system of dormancy types includes 5 classes: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and combinational dormancy (PY + PD). A simple, intermediate and complex levels of MPD have been distinguished with respect to temperature and hormonal requirements for dormancy breaking. Embryos with simple level of MPD grows at temperatures suitable for WS (>15°C), while in case of complex level of MPD the embryo grows at low temperatures (0-10°C) suitable for CS [7]. Seeds with intermediate PD can after-ripen to some degree, which results in a decrease in the length of the cold stratification period required to break PD [11-13]. Also, in case of intermediate level of MPD only cold stratification requires to break MPD of the embryo and applying of GA_3 may substitute for CS in these seeds.

According to the results of our experiments, dry storage does not promoted after-ripening i.e. breaking of the PD. Morphometric data obtained in this study indicate that embryo growth of *P. pontica* does not initiated until seeds are transferred to temperature effective for CS. Exogenous application of 1000 mg/L solution GA_3 shortened the time necessary to overcome dormancy and promoted germination, only when combined with CS

at 5°C. However, it was moderately effective to achieve maximum germination percentage to compare with seeds subjected to CS only. Since CS is the only requirement for the loss of MPD, the longest embryo growth occurred during this treatment and GA₃ promoted MPD loss, we concluded that *P. pontica* seeds have intermediate complex MPD.

Based on the treatment results a germination protocol is proposed: 1. Dry storage at 20°C, 2 months; 2. Cold-wet stratification at 3°C, 3 months; 3. Germination at 20/15°C day/night. Under developed conditions germination is fast, synchronous and yields to 80%.

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