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Effect of Stratification on Seed Germination and Epicotyl Dormancy in *Arbutus andrachne* L.

Nana Shakarishvili[*], Lamara Asieshvili[**], Nino Eradze[**], Marina Siradze[**]

** Institute of Botany, Ilia State University, Tbilisi*
*** State Botanical Garden, Tbilisi*

(Presented by Academy Member Giorgi Nakhutsrishvili)

ABSTRACT. The stage of seed maturity at harvest, storage temperature and duration have profound interacting effects on seed viability, germination and dormancy. The topics were studied in seeds of *Arbutus andrachne* L. - a critically endangered species included in the Georgia Red Data List. The results of the present study have shown that the seeds from ripe fruits harvested in autumn are physiologically dormant and fail to germinate. However, dormancy stage of the seeds isolated from the unharvested fruits is fully released (mean germination time = 13 days) after winter low temperature effect on the mother tree. A secondary dormancy status is established in the winter harvested seeds that were subjected to warm and dry storage conditions. The induced dormancy breaks partly, when these seeds are exposed to chilling temperature +5° C. Thus, radicle protrusion begins after 1-3 months of cold stratification, whereas shoot apical meristem remains inactive, after cotyledon unfolding. The effect of cold stratification on shoot development is revealed only in those seeds that where subjected to the additional 4-7 wet chilling. Despite favorable germination conditions secondary after-ripening epicotyl dormancy breaks gradually, depending on the duration of dry storage. Conclusion: seeds of *A. andrachne* may demonstrate 3 different dormancy status: non-deep physiological dormancy (in ripe seeds harvested before winter frost), non dormancy (in seeds harvested after winter frost) and deep secondary epicotyl physiological dormancy (in dry storage seeds), depending upon the extent of previous dry-afterripening.

Key words: *after-ripening dormancy, stratification, Arbutus andrachne.*

Strawberry tree – *Arbutus andrachne* L. (Ericaceae) is a species native to the Mediterranean region, the Middle East and southwestern Asia. A few populations of *A. andrachne* naturally occur in coastal rocky limestones of Abkhazeti and Ajara. As a Tertiary relict species *A. andrachne* is included in the Georgia Red Data List and needs to be protected. Small number of trees and weak natural regeneration of these populations indicate that seed germination and seedling establishment are insufficient. Seeds of *A. andrachne* possess a physiological dormancy that prevents synchronized and rapid germination.

The purpose of the present study is to classify the seed dormancy type and examine the requirements for root protrusion and shoot emergence in *A. andrachne* L.

Materials and Methods.

Ripe fruits of *A. andrachne* were collected in the Georgian National Botanical Garden from the mature tree growing at the plot of Rare and Medicinal Plants of the Caucasus. Seed coat permeability was tested by staining the seeds in 1% methylene blue water solution for 24 h. For *in vitro* storage tests the seeds were dried at room temperature in the laboratory for 4-7 months. Seedling emergence was examined for fruits harvested in September 2011 and January 2012. The warm (+20 °C) stratification of dry seeds, stored at 17°C for 3 or 8 months, was performed for 1 month, seeds were then given 1-7 months of cold stratification at 5°C. Mean germination time was calculated according to [1]. All germination experiments were conducted on moistened filter paper in Petri dishes and light/dark conditions.

Results and Discussion.

Seeds from mature ripe fruits of *Arbutus andrachne* L. harvested in September 2011 had a water permeable yellowish seed coat. The embryo axis and two large cotyledons were about 2–3.5 mm in length. The plumule - the meristematic part of the embryo shoot axis and epicotyl were slightly detectable. Despite the well-developed embryo, with high embryo to seed size ratio, no radicle protrusion had occurred and seeds failed to germinate in 30 days – the time span determined for germination of nondormant seeds. A small dome-shaped shoot apical meristem was well-developed in winter harvested seeds. Mean germination time of these seeds was 13 days and four-leaf stage was achieved during the next 2 months (Fig. 1). Seeds sown at 20 °C after 4 months of dry storage did not germinate. However, when cold stratified at 5 °C, radicle protrusion was observed and at 30 day of cold treatment radicle emergence (2-4 mm long) was completed in 100% of seeds. Further additional

Fig.1. A four-leaf stage seedlings developed from the nondormant seeds.

warm stratification arrested seedling development at true leaf development stage. Seeds sowed at 20 °C after 7 months of storage at ambient temperature had rather longer dormancy period. Thus, radicle emergence occurred after 4 months of the subsequent cold stratification. No true leaf development was observed in both cases. An epicotyl afterripening dormancy was expressed in *A. andrachne* seeds by slow growth of the shoot apex relative to rapid growth of the radicle, when stratified seeds were then germinated at 20 °C.

The period of cold stratification required for germination of seeds harvested in September and for dry-stored seeds is much longer than 30 days - the upper limit of germination time for undormant seeds. Moreover, seed germination (radicle emergence) is impossible without cold stratification pretreatment. The data obtained make it possible to suggest that these seeds are fully dormant.

According to the modern hierarchical classification scheme [2, 3], there are 5 classes of seed dormancy, each of which contains several levels and types. In the case of intermediate physiological dormancy, seeds require 3–4 months of cold stratification for dormancy break. Our study shows that the dormancy in seeds harvested in September breaks 4 months later, in January 2012. Germination tests indicated a good germination ability - 56 % and nondormant status of seeds, harvested in winter.

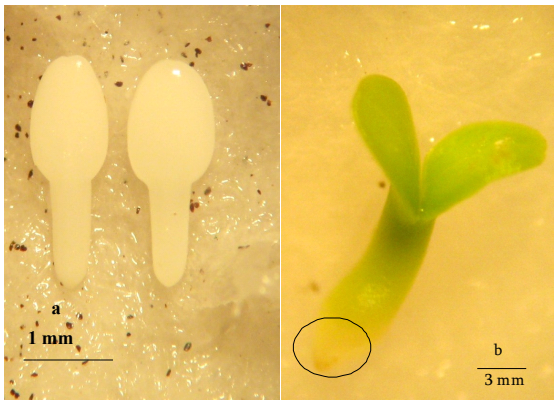

Fig. 2. A delayed initiation of true leaf primordia in the secondary epicotyl-dormant seedlings germinated at 20 °C.
a - embryo before radicle protrusion, after1month of cold stratification.
b - cotyledon opening (2 months of cold stratification); oval – root apical meristem.

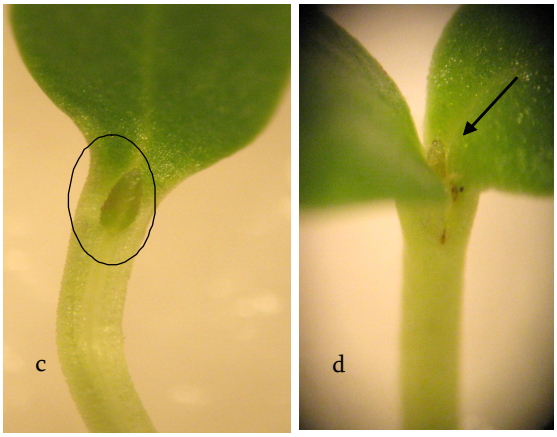

Fig. 2. c, d –True leaf bud primordium (4-5 months of cold stratification)

Thus, observations provide evidence that seeds of *A. andrachne* have intermediate type of physiological dormancy, which naturally overcome on the mother tree during winter season in ripe unharvested fruits.

However, dry afterripening causes the development of the secondary dormancy stage, when both the radicle and epicotyl become dormant. The latter also results in the development suppression of leaf primordia (Fig. 2). The asynchrony of root and shoot growth and delayed vegetative leaf emergence at post-germinative stages indicates the occurrence of deep complex secondary epicotyl morphophysiological dormancy in the case of long-term dry storage. Although these seeds exhibit radicle protrusion, they must be additionally stratified for 16-20 weeks at 5°C in order to obtain normal shoot growth. The number of days from sowing to radicle emergence (germination) and days from radicle emergence to plumule emergence were determined. The results indicate that the requirements to overcome complex secondary dormancy are different. Thus, 30 days of cold stratification breaks only radicle dormancy, whereupon additional 120 days of cold treatment just mitigates epicotyl dormancy in those seeds that were dry storaged for 4 months. In the case of more than 4 months of dry storage, the time span necessary for epicotyl dormancy breaking significantly increases. This may suggest that cold stratification removes dormancy more effectively in seeds stored for a short period.

Both primary dormancy breaking and secondary dormancy induction have been observed for seeds of many species. Our results are in agreement with previously published data showing that complex relationship between temperature and moisture content govern the nature of the mechanisms involved in secondary dormancy occurrence and release during dry storage [4-6]. Current knowledge of hormonal regulation of seed germination suggests that seed dormancy is controlled by the abscisic : gibberellin acid balance, rather than the absolute hormone content. Based on patterns of change in physiological responses to temperature during dormancy break, we suggest that the induction of seed dormancy in *A. andrachne* would also depend on metabolism and sensitivity of hormones.

Seeds of *A. andrachne* may demonstrate 3 different dormancy status: non-deep physiological dormancy (in ripe seeds harvested before winter frost), non-dormancy (in seeds harvested after winter frost) and deep secondary epicotyl physiological dormancy (in dry storage seeds), depending upon the extent of previous dry-afterripening.

ბოტანიკა

Arbutus andrachne L.-ს სტრატიფიცირებული თესლის გაღივება და ეპიკოტილური მოსვენების განვითარება

ნ. შაქარიშვილი*, ლ. ასიეშვილი**, ნ. ერაძე**, მ. სირაძე**

* *ილიას სახელმწიფო უნივერსიტეტის ბოტანიკის ინსტიტუტი, თბილისი*
** *საქართველოს ეროვნული ბოტანიკური ბაღი, თბილისი*

ნაშრომში შესწავლილია საქართველოს წითელი ნუსხის სახეობის, რელიქტური, მაღალდეკორატიული, თაფლოვანი მცენარის, ხმარწყვას *Arbutus andrachne* L. თესლის გაღივების პროცესი. მწიფე თესლს შემოდგომაზე ფიზიოლოგიური მოსვენება ახასიათებს, ამიტომ მისი გაღივება შესაძლებელია მხოლოდ სტრატიფიცირების შემდეგ + 5° C-ზე. მოსვენების ბუნებრივი შეწყვეტა დედა მცენარეზე ზამთრის პერიოდში ხდება. ასეთი თესლის გაღივების საშუალო დრო 13 დღეს შეადგენს. მშრალი შენახვის პირობებში ვითარდება ხანგრძლივი მეორადი ეპიკოტილური მოსვენება, რომელიც ფესვისა და ღეროს აპიკალური მერისტემების არათანაბარ აქტივობაში გამოიხატება. შემუშავებულია პროტოკოლი, რომლის გამოყენებით შესაძლებელია პირველადი და მეორადი მოსვენების ხელოვნური შეწყვეტა და ხმარწყვას თესლის გაღივება.

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