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Study Methods of Dormancy Breaking and Germination of Common Madder (*Rubia tinctorum* L.) Seed in Laboratory Conditions

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Abstract: Seed dormancy is a mechanism that insures the survival of the species. Madder (*Rubia tinctorum* L.) seeds present low germination due to the high degree of seed dormancy. Different methods to overcome seed dormancy were compared: mechanical scarification (sanding), chemical scarification (dilution in concentrated sulfuric acid for 10, 15 or 20 minutes, dilution in hot water at 70°C and 90°C for 5 and10 minutes, soaking of seed in gibberelic acid (0.05% GA_3), light (24 hour), pre chilling at 4°C for 4, 6 or 10 week and germination test (control). According to results mechanical scarification (sanding), chemical scarification (treatment with acid for 15 minutes) and hot water were efficient in promoting germination. For practical purposes, mechanical scarification is highly recommended.

Key words: Common madder *rubia tinctorum* L. · Dormancy breaking · Seed germination · Chilling · Scarification

INTRODUCTION

Seed dormancy is a mechanism that insures the survival of the species. To "Mother Nature", dormancy is a blessing that insures the continuation of the species over time and through periods of environmental stress. Madder is the common name of the plant genus Rubia, the type genus of the madder family Rubiaceae. *Rubia tinctorum* L., also known as Madder is a herbaceous perennial belonging to Rubiaceae family which grow widely in Iran. The roots of madder (*Rubia tinctorum* L.) contain a certain number of anthraquinones, such as lucidin primeveroside, ruberythric acid, pseudopurpurin, munjistin, alizarin (1,2-dihydroxyanthraquinone) and purpurin. The hydrolysis of ruberythric acid gives alizarin, which is the parent form of many dyes and pigments, including mordants. Anthraquinone dyes occur in the Free State in the plant as glucosides (1) .In addition, it is known that this plant is used as a diuretic (2), as a treatment for kidney and bladder stones (3) and as fodder (4). This plant prefers to be propagated vegetatively. Both low germination rate and difficulty in cultivating this plant using vegetative parts (such as problem in gathering and storage of rhizomes) make *Rubia tinctorum* L. production difficult. Madder seeds present low germination due to the high degree of seed dormancy. The causes of seed dormancy in plants are varied and include such things as a hard seed coat, an undeveloped embryo, requirement of a particular environmental factor, or the presence of an inhibitor. The inhibition of germination caused by hardseededness and embryo dormancy are common types of dormancy in plants. Scarification use to overcome hardseededness and low temperature (stratification) use to overcome embryo dormancy of seed.

The objective of this investigation was to identify the best pre-sowing seed treatment to break seed dormancy and to promote germination of *Rubia tinctorum* L. seeds.

MATERIALS AND METHODS

Ripe seeds were collected from plants of *Rubia tinctorum* L. growing in Iranian Institute of Medicinal plant (IMP)-Karadj, Iran fields.

All equipment was sterilized in an autoclave and only sterilized water was used. To further minimize the risk of infestation by fungi and bacteria, seed samples surface sterilized in 2.5% sodium hypochlorite (NaOCl) for 5 min. Treatments to break seed dormancy included: 1- untreated seed (control); 2- mechanical scarification (the seed coat was sanded with a # 80 wood sandpaper at an area opposite from the embryo until the cotyledon was exposed); 3- imbibition in hot water (Surface-sterilized seed was immersed in hot water at 70°C and 90°C for 5 and 10 minutes. Following hot water immersion, the seed was

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rapidly cooled by placing it under running cold water); 4-chemical scarification (Two volumes of acid to one volume of seed were used [5]. Seed samples were placed in concentrated sulphuric acid (36N) for 5, 15, 30, 60, 120 or 180 minutes. Following acid treatment seed was washed in running water until the pH was neutral and then rinsed in sterile water; 5- pre-germination chilling (4°C) of seeds for 2,4 or 6 weeks in moist sand (stratification); 6-soaking of seeds in gibberellic acid (0.05% GA_3); 7- continuous light. 25 seeds were placed in 10 cm Petri dishes lined by two sheets of Whatman No. 1 filter paper and containing 7cc-distilled water, except GA_3 treatment containing 7cc GA_3 solution. There were four replicates at each treatment. The seeds were left in an incubator to germinate at constant temperature of 25°C in dark except of continuous light. The number of seeds germinated was counted from 3 to 24 days after treatments. A seed was counted as germinated when it showed a radicle that was at least the length of the seed. Obtained numbers were analyzed according to complete randomized design using SAS program. Differences among treatments were tested by Duncan's multiple range test (DMRT).

RESULTS

According to the results of variance analysis, the effect of different treatments on germination rate was significant.

Effects of Chemical Scarification on Seed Germination: This experiment showed that acid scarification significantly increased seed germination. Generally all concentrated sulphuric acid treatments tested enhanced germination rate compared to control treatment. It also indicated that the optimum period for acid scarified seed germination was 15 min (89%) compared to 10 min (81%) and 20 min(82%). Emerson of seeds in concentrated sulphuric acid may lead to the rapture of the coat wall allowing water to permeate the seed tissues causing physiological changes and subsequent germination of the embryo. Germination decreases when seeds were allowed in acid for more than 15 min, suggesting that embryo may get destroyed on contact with concentrated sulphuric acid for a prolonged period. The fact that concentrated sulphuric acid gave the highest percentage of germination within 15min as compared 10 and 20min, indicate that the more rapidly the seed coat is ruptured the faster the rate of germination, however, prolonged Emerson may be injurious to the seeds as the acid may rapture vital parts of the embryo.

Effects of Mechanical Scarification on Seed Germination: Results indicated that germination of seeds mechanically scratched with sand paper significantly increased of 83%. However, mechanical scarification turned out to be an excellent treatment to overcome seed dormancy. This agrees with results from several authors [6] for *Erythrina speciosa*; Bianchetti and Ramos, [7] for *Peltophorum dubium*; Candido *et al*., [8] for *Enterolobium contortisiliquum*; Nassif and Perez, [9] for *Pterogyne nitens*; and Jeller and Perez, [10] for *Cassia excelsa*; Lopes *et al*., [11] for *Caesalpinea ferrea, Cassia grandis* and *Samanea saman*) and Malavasi, [12] for *Enterolobium contortisiliquum*).

Effects of Imbibition in Hot Water on Seed Germination: Generally all hot water treatments tested enhanced germination rate in compared to control treatment. Significant differences were also found to exist among all the treatments. Results also indicated that seed germination increased with increasing water temperature and soaking period. It was observed that the maximum number of germinated seed (78%) was found with imbibition in hot water at 90°C for 5 whereas imbibition in hot water at 70°C for 5 minutes showed the minimum number of germinated seed (23%). this indicated that germination decreases when seeds were allowed in water (90°C) for more than 10 min, suggesting that embryo may get destroyed on contact with boiling water for a prolonged period. Imbibition in hot water may lead to the rapture of the coat wall allowing water to permeate the seed tissues causing physiological changes and subsequent germination of the embryo [13-16].

Effects of Pre-germination Chilling on Seed Germination: Results indicated that seed germination significantly increased with pre-germination chilling for 6 week (47%) and 8 week (76%). It was observed that the effect of 2 week pre-germination chilling on the percentage of germination was non-significant (8%). Similar results were also reported on *Accasia farnesiana* [17] that is related to crevice in seed coats on chilling.

Effects of Soaking in Gibberellic Acid and Continuous Light on Seed Germination: Among the treatments, soaking in gibberellic acid was the most inefficient (5%) in breaking dormancy and germination. In case of control the percentage of emergence was also 5%. The data revealed that continuous light treatment has non-significant influence on percentage of germination.

The effect of light on seed germination is various. Light has no effect on germination of many crops seeds but some of them need light to germinate at the highest level.

DISCUSSION

This experiment showed, mechanical scarification, chemical scarification and hot water yielded significantly higher total germination percentages than the other treatments involved. It was observed that the percentage of germination was significantly different at all treatments of breaking dormancy. The maximum number of germinated seed was found with concentrated sulphuric acid within 15min whereas soaking in gibberellic acid showed the minimum number of germinated seed. Two types of seed dormancy have been recognized, coat-imposed dormancy and embryo dormancy. Coat-imposed dormancy is dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extra floral organs. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues are either removed or damaged.

From the investigations carried out, such treatment as soaking in hot water, mechanical scarification and application of sulphuric acid were found to induce germination of seeds of *Rubia tinctorum*. From the above one can infer that dormancy of the seeds of *Rubia tinctorum* was probably associated with the seeds coat, since the treatment that induce germination were those that can effect disruption of the seed coat. Where seed coat is softened, the process of hydrolysis could commence to release simple sugars that could be readily utilized in protein synthesis. Release of hormones such as auxins and ethylene, which could increase nucleic acid metabolism and protein synthesis (Irwin, 1982 and Jackson, 1994).

Softening the seed coat by means of imbibing in concentrated sulphuric acid greatly enhance the germination percentage of the seed. Where such technology is not available and imbibition in hot water at 90°C for 5min appears to be an effective way of improving germination percentage.

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