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Research Article

STERILIZATION AND STUDY OF GERMINATION OF ASTRAGALUS KNORRINGIANUS SEEDS IN VITRO CONDITIONS

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Daniyorova Shakhnoza Olimjon's daughter
Jizzakh state pedagogical university, Uzbekistan

ABSTRACT

The global decline of plant species exerts a significant detrimental impact on the stability of natural ecosystems. Endemic species with restricted geographical distributions are at risk of extinction in their natural habitats. Consequently, the investigation of rare plant species, the analysis of their current status, and the development of conservation measures are of substantial scientific and practical significance. Astragalus knorringianus Boriss. represents one such critical plant species.

KEYWORDS

In vitro technology, rare species, nutrient medium, sterilization, seed germination.

INTRODUCTION

Astragalus knorringianus Boriss. is a perennial herb belonging to the Fabaceae family. It flowers in March-April, and its seeds mature in May-June. The peduncle measures 2-5 cm in length, while the corolla is 2-2.5 mm long. The plant produces 2-3 flowers, situated at the terminus of a single stem, exhibiting pale yellow or dark red coloration, and measuring 32-37 mm in length. The calyx is 18-21 mm, with teeth 1-3 mm long, predominantly 0.2-0.3 mm long, dark pubescent, and tubular in form. The vexillum component measures 33-36 mm in length, with an arcuate lamina 13 mm in width. The upper portion is dentate, while the lower portion tapers. The wings are approximately 30 mm long, with an elongated lamina of about 11 mm. The keel measures 28 mm in length, featuring a sharp lamina of 9 mm in the upper section. Pollination occurs

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via insects. The pod attains a length of up to 7 cm and a width of 4 mm, possessing a short beak and covered with white and black trichomes. The pod morphology is arcuate or straight, narrowing towards both extremities. Upon maturation, the seed coat assumes a brown coloration. Reproduction occurs through seeds. The seeds are of moderate size, with a thousand-seed weight of 5.95 g.

Astragalus knorringianus is a rare endemic species that occurs in northwestern Pamir-Aloy. Its distribution encompasses the mountains of Nurota, Molguzar, and Turkestan in the Jizzakh, Samarkand, and Navoi regions. In addition to its presence in Uzbekistan, the species is also found in Tajikistan and Kyrgyzstan.

The Red Book of Uzbekistan editions (2016, 2019) enumerate 54 species from the sedge family, of which 34 belong to the Astragalus genus. The flora of certain mountainous regions in Central Asia is characterized by an abundance of Astragalus L. species. Significant research has been conducted on rare and endemic species of the Astragalus genus. Notably, Kamelin (1990) identified 14 specific endemic taxonomic features of Astragalus in Syrdaryo Karatov. Populations of some rare Astragalus species, including Astragalus abolinii, have been observed to be in satisfactory condition in the region [5].

Astragalus centralis, as reported by Sh.U. Saribaeva [6] in South-West Kyzylkum. Sheld., has been the subject of senopopulation studies. The current state and viability of this plant species were evaluated, and the primary factors contributing to the reduction of its range were identified, along with the development of protective measures. In recent years, new species (Astragalus belolipovii Kamelin ex F. O. Khass. et N. Sulajm., A. russanovii F. O. Khass., Sarybaeva et Esankulov, A. zaaminensis F. O. Khass. & Esankulov) have been described in the Kokhistan region, enriching the flora inventory with new discoveries. Information regarding the assessment of vitality status, morphogenesis, ontogenesis periods, and types of senopopulations of Astragalus holargyreus Bunge is reflected in the works of K.F. Shomurodov (2018) and Sh.U. Saribaeva (2009). Astragalus belolipovii was initially discovered by I.V. Belolipov near the Kulsay forest cottage and subsequently cultivated in the Botanical Garden in 1975.

Plant biotechnology facilitates the preservation of rare and endangered plant species.

Regarding in vitro technology, it is crucial to emphasize its efficacy:

1. Rapid propagation: The process of plant propagation utilizing the in vitro method is significantly more expeditious and efficient than conventional methods. This technique enables the production of millions of seedlings within a single year.

2. Genetic stability: This technology maintains the genetic stability of plants, ensuring that the resulting seedlings possess identical quality and characteristics.

3. High yield: In vitro propagated plants exhibit high yield and enhanced resistance to diseases.

Numerous studies have been conducted on the cultivation of various Astragalus L. species under in vitro conditions [7,8]. However, research on the in vitro cultivation of Astragalus knorringianus from tissues and organs has not been undertaken.

Research results. For this purpose, we conducted studies on in vitro sterilization and fertility of Astragalus knorringianus seeds. A review of the literature indicated that sterilization and fertility determination of this species' seeds in vitro have not been previously investigated.

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Astragalus knorringianus seeds were collected from a community of plants from diverse herbaceous astragali almond orchards in the vicinity of Karasoy village (N 082738 E 739259 h=485 m) (near Temurlang gate) of Jizzakh region.

Seed germination biology is a critical stage in a plant's developmental cycle, wherein a seed emerges from dormancy and initiates growth to form a new plant. This process comprises several interrelated physiological and biochemical stages. The biology of seed germination is of significant importance in plant reproduction, ecological adaptation, and dispersal, facilitating the transfer of genetic material to subsequent generations.

For the cultivation of Astragalus knorringianus seeds, fully matured seeds were selected. The sorted seeds were sterilized in the in vitro scientific laboratory of Jizzakh State Pedagogical University to examine seed viability and viability.

In isolated tissue culture, strict adherence to sterility is essential. The nutrient-rich composition of the medium is conducive to microbial growth, which can readily damage plant parts (explants). Consequently, both explants and nutrient medium must be thoroughly sterilized. All tissue-related processes (culture transfer, transfer to new nutrient medium) are conducted in a sterile environment, utilizing laminar flow hoods and sterile instruments. Maintaining sterility during the growth period is crucial, as microorganisms can enter and contaminate the test tube through the moist stopper of the container due to temperature fluctuations or humidity [1]. Typically, seeds are sterilized for 10-20 minutes, while vegetative parts are sterilized for 5-10 minutes [2]. For the sterilization of plant organs, R.G. Butenko's method was employed.

Various methods were utilized for the in vitro sterilization of Astragalus knorringianus seeds.

The seeds stored for three weeks were prepared for collection in the Invitro scientific laboratory. For this purpose, the nutrient medium was first prepared. 276.25 mg of Murasiga - skuga feed was measured into 250 ml of distilled water. Subsequently, 1.875 g of sucrose was added to the mixture and homogenized with a magnetic stirrer until dissolved. The pH indicator of the prepared solution was adjusted to 5.8. At the conclusion of the process, 2 g of agar-agar polysaccharide obtained from seaweed was added, the container was covered with tissue paper and filter paper, and sent to the autoclave for sterilization. This nutrient medium was sterilized in an autoclave at a temperature of 120° C and a pressure of 0.75 atm for 15 minutes. In the subsequent step, the explant is sterilized.

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Figure 1. Germination of *Astragalus knorringianus* seeds in vitro conditions

For this study, mature seeds of Astragalus knorringianus were selected. Initially, the seeds were washed in distilled water, followed by a 10-minute wash in soapy water, and subsequently rinsed in distilled water 3-4 additional times. Further procedures were conducted in a laminar flow hood. The seeds were immersed in a 4 percent sodium hypochlorite solution for 10 minutes. They were then rinsed 5-6 times in distilled water and submerged in a 70% solution of ethyl alcohol for 30 seconds, followed by 2-3 rinses in distilled water.

Upon complete sterilization, the seeds were inoculated onto Murashige and Skoog nutrient medium in glass vessels within a laminar flow hood, adhering to aseptic techniques. The inoculated seeds were placed in a dark environment at a temperature of 4 C for 14 days. Subsequently, the seeds were transferred to an incubator maintained at a temperature of 22 C under light conditions.

Figure 2. Washing seeds in soapy water; Placing samples in the thermostat.

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Germination of seeds commenced 16 days after sowing. During the second week of seed germination, 55% of the sown seeds germinated.

CONCLUSION

The rate of seed germination exhibits variability. It was observed that the germination rate of the cultivated seeds attained 80 percent. The obtained results pertaining to the seed germination of this species will serve as foundational material for the investigation of ontogenetic morphogenesis of the species within a brief temporal period in vitro.

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