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Long-term Protection of Germplasm Millet (*Panucum miliaceum* L.) Native to the Northwest of Iran

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Introduction

Preservation of plant genetic resources is of particular importance. By applying ultra-cold seed storage techniques, seeds can be stored for a long time at a much lower cost. In the present study, the cryopreservation of millet seeds by a verification method was investigated. Anyway, in Cryopreservation conditions at -196 degrees Celsius, in liquid nitrogen, plant organs or seeds can be stored for a long time (Naderi Shahab, et al., 2017). During freezing (Cryopreservation), all biochemical activities are significantly reduced and biological deterioration is stopped (Kaviani, et al., 2011).

Freezing protection (Cryopreservation) of plants has been studied in laboratories around the world for years. And it should also be said that the methods of kept as seeds, pollen, dormant buds and shoot tips of plant are widely being developed (Withers, 1991; Bajaj, 1995 and Reed, 1998).

Many researchers have conducted research on the Cryopreservation of other plants, such as Reed et al., 1998 in Pear (*Pyrus* L.) germplasm; Issac et al., 2021 in *Xerophyllum asphodeloides* (L.); de Figueiredo, et al., 2017 in *Coffea arabica L.* seeds and Naderi Shahab, et al., 2017 in *Smirnovia iranica*).

Considering the agricultural, edible and medicinal importance of millet, in the present research, in order to find a suitable method for protecting the germplasm of this plant, millet seeds were treated with solutions used in cryogenic protection.

Materials and Methods

First, millet seeds were collected and prepared from the fields of Asadabad city (Hamadan). In order to find a suitable method of cryopreservation of millet seed germplasm, first the seeds were disinfected with the help of 3%

sodium hypochlorite for 15 minutes. Then they were washed with distilled water and re-sterilized with 70% alcohol for 50 seconds and finally washed with distilled water three times. After drying, 10 seeds were transferred to 2 ml freezer vials at laboratory temperature and treated with preservative solutions.

The solutions used are two widely used solutions in the cryopreservation of plant tissue named PVS2 (including 30% glycerol, 15% ethylene glycol, 15% dimethyl sulfoxide with 0.4 M sucrose MS liquid culture medium) and PVS3 (including 40% glycerol and 40% Sucrose (WV)). These solutions were sterilized with the help of a 0.22 micrometer filter.

The experiment in factorial format (with two factors, the first factor in two levels of de-watering solution (PVS2 and PVS3) and the second factor in de-watering times in five- time levels (20, 40, 60, 80, and 100 minutes) and with a completely randomized design and it was done with three repetitions.

Statistical operations were performed for the three traits of radicle length, shoot length and germination percentage with the help of SAS 9.1 software. The graphs were drawn using Excel 2016 software.

Result & Discussion

The results of the analysis of variance showed that all traits (root length, stem length, and germination percentage) were significant in terms of treatment time at the level of 1% probability. Also, the type of solution was significant for root length and shoot length and the interaction effect of treatment time in solution type was significant only for shoot length at the level of 1% probability. Treatment of millet seeds with protective solutions for 60 minutes indicated the highest germination rate compared to other treatments. The results showed that seeds treated with PVS3 solution had shorter stem length but longer root length than PVS2 solution.

Conclusion

In general, cryopreservation can be considered as an appropriate method to protect the germplasm of millet. PVS3 solution had the highest average germination percentage at 60 minutes, which is the best treatment composition for long-term storage of millet.

Keyword: Cryopreservation, Germplasm, Millet, Preservative Solution, Verification.

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