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RESTORATION OF LATVIAN ALFALFA (Medicago sativa) GENETIC RESOURCES PERSPECTIVE FOR BREEDING

SELEKCIJAI PERSPEKTĪVU LATVIJAS LUCERNAS (Medicago sativa) ĢENĒTISKO RESURSU ATJAUNOŠANA

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Abstract. *We have carried out restoring of some Latvian alfalfa genetic resources from seeds of accessions that did not germinate in soil at all. Two of them were repatriated from the N. Vavilov All-Russian Institute of Plant Industry (multiplied more than 40 years ago) and three from the Research Institute of Agriculture (Latvia) (multiplied 15-20 years ago). For germination of old seeds we used early elaborated* in vitro *culture conditions. Germination rate ranged 2-60%, depending from the genotype and seeds storage conditions. Plantlets with well developed roots and 2-3 leaves were planted in the substrate in small pots and grown in a greenhouse about a month, then replanted in the soil in field conditions and grown till the maturity. Restored accessions are available now for evaluation and involving in the alfalfa breeding programs.*

Keywords: *aged seeds, alfalfa, germination,* in vitro.

Introduction

Alfalfa is a perennial legume widely grown throughout the world as forage for cattle. Most often it is harvested as hay but can be processed into silage, grazed, or fed as a green crop. One of the most important characteristics of alfalfa is high nutritional quality as the animal feed [1]. Alfalfa contains 15 to 22% crude protein as well it can serve as an excellent source of vitamins (A, B, C, E and K) and minerals (calcium, phosphorus, copper, potassium) therefore it is also used for human consumption and as a nutritional supplement [2]. When grown in areas where it is well adapted, alfalfa is among the highest yielding forage plants. Alfalfa nitrogen-fixing abilities make it valuable for use in crop rotations, by increasing the productivity of crops grown after it. *M. sativa* is a perspective crop for organic farming but locally adapted varieties are needed for this purpose.

Currently, only one alfalfa variety bred in Latvia ('Skrīveru') is registered for cultivation in Latvia [3; 4]. In Latvian conditions foreign varieties give low seed yield. Using local alfalfa genetic resources is important for the breeding new varieties for the Latvian agroecological conditions. Our goal was to restore old Latvian alfalfa genetic recourses by elaborating an *in vitro* method for germination of alfalfa seeds that did not germinate in soil at all.

Materials and methods

In the experiment were used 5 alfalfa accessions of the Latvian origin. Because those accessions were not multiplied for the long time all available seeds have lost germinating ability in the soil. Two accessions were repatriated from the N. Vavilov All-Russian Institute of Plant Industry (VIR), three were kept in the Latvian Research Institute of Agriculture (RIA) (Table 1).

Accessions repatriated from the VIR had only 1.5 g seeds per accession and it is not enough to test different germination methods. Therefore experiment was divided in two parts. In the

first step accessions from the RIA were used to determine the best seeds pretreatment and cultivation conditions. In the second step seeds of accessions from the VIR were germinated using the best method worked out in the first part.

Table 1.

Alfalfa accessions selected for germination *in vitro*

Accession name	*Year of reproduction*	*Age of seeds involving in the experiment*	*Source collection*
Local-k-31068	1964	44	VIR
Local k-31069	1964	44	VIR
Mentu kalna	1989	19	RIA
Lucerna Nr. 2	1993	15	RIA
Mežotnes	1994	14	RIA

In the first experiment part seeds pretreatment with 0.07% $KMnO_4$ solution or 0.1% $KMnO_4$ solution were performed both for 40 or 60 minutes [5]. Then they were sterilized by 50% solution of the commercial bleach "Belizna" for 20 minutes. Seeds after each pretreatment method were placed in Petri dishes on three different media:

1. Murashige and Skoog (MS) basal media [6].
2. MS basal media supplemented with 10 mg/l $AgNO_3$.
3. MS basal media supplemented with 1 g/l activated carbon.

Seeds were cultivated at 20-26 °C in day (16 h)/night conditions during 3–4 weeks.

Plantlets with good developed roots and 3-4 leafs were planted in the soil in plastic pods. Roots before planting in soil were flush with water. For better acclimatization pods with plantlets were covered by plastic film and cultivated in a greenhouse. After acclimatization the plastic film was removed and plants were grown for 2-3 months. Good developed plants were planted in field conditions for seed production.

Results and Discussion

Used seeds were phenotypicaly diverse, they varied both in shape and color, as well within and between accessions. There were visible signs of aging: seed coat color had been changed from yellow/orange brown to dark, red brown and seeds were flatter.

Seed germination started after 5-10 days of cultivation *in vitro* and continued for about four weeks. MS medium supplementation nor by $AgNO_3$ nor by activated carbon did not influence significantly germination rate. In addition, many plantlets obtained on the MS media supplemented with $AgNO_3$ were soft, without roots and/or leaves and with tendency to calli formation. Plantlets that show tendency to form calli are not useful for restoration of particular genotype because of possible somoclonal variation. Therefore we choose MS basal medium without supplement as the best suited media for the rest of experiment.

The total percent of germinated seeds on MS basal media significantly differed among accessions and depend of pretreatment method (Fig. 1).

The highest germination rate for Mežotnes and Mentu kalna was observed if as pretreatment 0.1% $KMnO_4$ solution for 40 minutes was used. In they turn, accession Lucerna Nr. 2 had highest germination rate if pretreatment with 0.07% $KMnO_4$ for 60 minutes were applied but in this case part of seeds were contaminated. Therefore, for the seed germination of aged accessions from the VIR pretreatment with highest concentration of $KMnO_4$ (0.1%) for 60 minutes on the MS basal medium without supplement were used.

Germination rate for both Local k-31068 and Local k-31069 was 61%. Seeds age of both accessions was 44 years, nevertheless germination rate for these lines was significantly higher than for seeds with age 14-20 years. It could be explained by different storage conditions: Mežotnes, Mentu kalna, Lucerna Nr. 2 were kept in the Research Institute of Agriculture in

unstable room temperature and humidity, while seeds of the VIR collection were also preserved in room temperature but in more stable conditions.
From all used accessions 20-220 fertile plants were obtained.

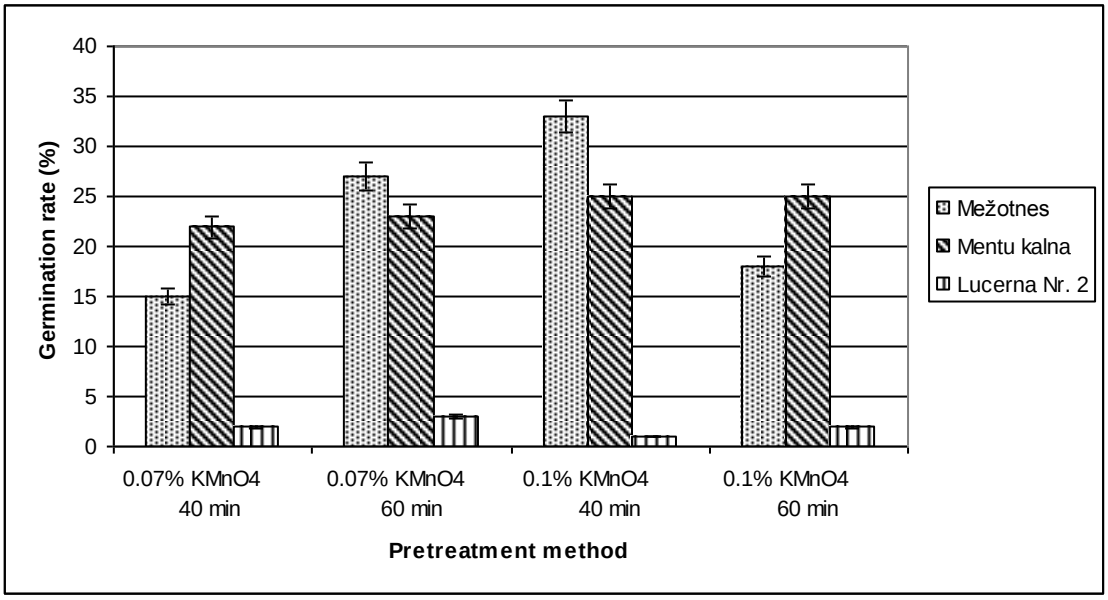

Fig. 1. **Old alfalfa seeds germination on MS basal media depending of the pretreatment method**

Conclusions

By using optimal pretreatment and cultivation media it is possible to obtain plants from seeds non-germinating in soil. The total percent of germinated seeds differed among accessions and did not correlate with age of seeds but with genotype and storage conditions. By recovering old alfalfa accessions of the Latvian origin we have obtained useful material for further investigations and breeding.

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