

## THE *IN VITRO* DIFFERENTIAL REACTION OF EIGHT GENOTYPES OF BIRD'S FOOT TREFOIL (*LOTUS CORNICULATUS* L.) UNDER THE ASPECT OF GERMINATION CAPACITY

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### Abstract

We have studied the differential reaction of the eight genotypes of bird's foot trefoil (*Lotus Corniculatus* L.) in order to show the biological material that has got the best answer in what the germination capacity is concerned. We have used 30 seeds in three repetitions. The seeds have been inoculated on the basic Murashige-Skoog (1962) environment improved with 30g/l of sucrose and 7g/l of agar with a pH of 5.8. The culture medium has been distributed in tubes and then sterilized through autoclaving at 121°C for 20 minutes. The sterilized seeds have been inoculated in tubes, on the Murashige-Skoog medium without using growing hormones and in each tube three seeds have been inoculated. After the inoculation the ex plants have been maintained in the growing room at a photoperiod of 12/12 hours, a light intensity of 2500 lux and at a temperature of 22°-24°C. The first observations related to the seed germination has been done at seven days and then at 14 days. At a period of 14 days after the *in vitro* inoculation of the seeds observations were made concerning the growing of the plantlets. For the Nico and Danitim types of bird's foot trefoil the germination percent has been very low, of only 10% after 14 days from the inoculation. After 14 days of germination the Alina genotype has remarked itself through its developing dynamics in comparison with the other genotypes, a premise for an excellent *in vitro* multiplication capacity.

**Key words:** germination, *in vitro*, *Lotus corniculatus* L., genotypes of bird's foot trefoil, explants, seeds.

### INTRODUCTION

The *Lotus corniculatus* genotype includes a great diversity of species, approximately 100 spread in Asia, Africa, Australia, America and Europe, especially in the Mediterranean basin. The large spreading of these species in the Mediterranean area shows that this region is probably the origin centre of the bird's foot trefoil (Seaney B., B., 1975).

In our country the bird's foot trefoil is greatly spread and it can be seen in almost all the ecological areas of the permanent lawns and meadows from the Black Sea beach up to the mountain areas.

The expansion of this species has got wider after 1980 when the first Romanian type of bird's foot trefoil has been created (Livada, 1984) and when the specific technology for seed production and seed multiplication has been issued, on a hierarchy manner, based on superior biological links (Dragomir N., 1998).

Currently, if we talk about the leguminous plants, the bird's foot trefoil is on the third place in what surface is concerned (after the lucerne aka the Spanish trefoil - and the red trefoil), being cultivated in pure culture as well as in different types of mixtures in order to form temporary meadows. The surface cultivated with bird's foot trefoil, in pure culture is of about 100.000 hectares (Dragomir N., 1998).

The relatively small surface occupied by the bird's foot trefoil is due greatly to the lack of sufficient seed quantities which could satisfy the needs for this species, a species which is sometimes of utmost necessity in the structure of the complex mixtures in order to create temporary meadows on hill and mountain areas.

The importance of the bird's foot trefoil as a feeding plant relies mainly in the fact that it can replace lucerne and trefoil from some areas less favorable for these cultures. From this point of view, the opinion of many researchers and cultivators/growers is unanimous when they assert that the bird's foot trefoil is the feeding plant having the highest capacity to adapt to different climate and soil conditions: to drought, to high humidity, to acid or superficial soils, to salty soils or with to ones with low fertility, to disforested fields, etc. (Pop M., 1972; Dragomir N., 1981; Winch J., E., MacDonald H., A., 1961; Laskey G., B., Wakefield B., C., 1978; Dionne J., L., 1969).

The bird's foot trefoil has got a great capacity of autoinsemination even in the conditions of a long lasting graze. In the same time the rustic aspect offers the bird's foot trefoil special qualities in comparison with other perennial feeding plants because the meteorization process does not appear while it is consumed under the form of a green mass and because it is very resistant to diseases and to pests (Zamfirescu N. et al., 1965; Rieunau M., 1969).

The *in vitro* vegetative multiplication allows the reproduction of the specimen chosen for a series of interesting features in a big number of identical samples and in a very short time. This fact made many species be multiplied exclusively through the *in vitro* method.

The impact of this technique upon the improvement of the plants is illustrated by the results obtained by different researchers who studied forage plants (Murashige T., 1974; Parrott W., A., Collins G., B., 1983; Zăpârțan Maria et al., 2006).

The *in vitro* cultures have found, in a very short period of time, a lot of practical applications in the improvement of many culture species among which the multiplication of the valuable genotypes, propagation of virus free genetic stocks, conservation of germoplasma, etc.

The current study has in view to determine the germination percent for eight genotypes of bird's foot trefoil, the genotypes being the following:

Alina, Nico, Danitim, Suceava1, Suceava2, Suceava3, Suceava4 and Suceava5.

## MATERIAL AND METHOD

The seeds have been disinfected with a solution of sodium hypochlorite, commercial product Domestos 30%, for 15 minutes, to which we added a few drops of TWEEN-20. The operation has been performed in a laminary flow hood, in aseptic conditions. After 15 minutes, while from time to time it is indicated to shake the pots, the seeds had been washed three times in sterile distilled water to eliminate the remaining action of the disinfection agent. We have used 30 seeds in three repetitions. The seeds have been inoculated on the basic Murashige-Skoog medium (1962) supplemented with 30 g/l sucrose and 7 g/l agar, pH 5,8. The culture medium has been distributed in tubes and then sterilized through autoclaving at 121°C for 20 minutes.

The main Murashige-Skoog medium (MS) (1962) has got as basic components the following: macroelements, microelements, vitamins and aminoacids.

The sterilized seeds have been inoculated in tubes on the Murashige-Skoog medium without growing hormones, in each tube three seeds being inoculated.

After the inoculation the explants have been maintained in the growing room at a photoperiod of 12/12 hours, a light intensity of 2500 lux and at a temperature of 22°-24°C.

The tubes containing infested or dead explants have been removed after the first three days from the inoculation.

## RESULTS AND DISCUSSIONS

The first observation concerning the seed germination has been done at seven days (Table 1).

*Table 1*

Germination percentage of *Lotus corniculatus* L. varieties after seven days from inoculation

Criteria No.	Genotype	Number of inoculated seeds	Number of germinated seeds	Percentage of germination
1	Suceava1	30	7	23.33
2	Suceava2	30	5	16.67
3	Suceava3	30	13	43.33
4	Suceava4	30	14	46.67
5	Suceava5	30	6	20.00
6	Nico	30	1	3.33
7	Alina	30	24	80.00
8	Danitim	30	2	6.67

After seven days of inoculation it can be noticed that the highest number of germinated seeds is obtained at the Alina genotype with a number of 24 such seeds followed by genotypes Suceava4 and Suceava3 with 14 and with 13 samples respectively. A very small number of germinated seeds have been registered for the Nico and Danitim genotypes, between 1 and 2 seeds.

After 14 days of seed germination observation, the following results have been registered for the varieties of *Lotus corniculatus L* (Table 2).

Table 2

Germination percentage of *Lotus corniculatus L* varieties after 14 days from inoculation

Genotype	Number of inoculated seeds	Number of germinated seeds	Percentage of germination
Suceava1	30	14	46.67
Suceava2	30	13	43.33
Suceava3	30	17	56.67
Suceava4	30	21	70.00
Suceava5	30	14	46.67
Nico	30	3	10.00
Alina	30	30	100.00
Danitim	30	3	10.00

After two weeks of inoculation it is still the Alina genotype that stands out with a number of 30 germinated seeds. A high number of germinated seeds have been registered for Suceava4 and for Suceava3 genotypes with 21 and with 17 germinated seeds respectively. A small number of germinated seeds have been obtained for the Nico and Danitim genotypes, with 3 seeds.

## CONCLUSIONS

After having analyzed the data obtained from the experiment, data related to the differentiated reaction of eight *in vitro* genotypes of bird's foot trefoil (*Lotus corniculatus*) in what the germination aspect is concerned, the following can be concluded:

- After seven days it is noticed that three genotypes have got the highest germination percents: Alina 80.00%, Suceava4 46.67% and Suceava3 43.33%;

- the lowest germination percents, after seven days, belong to the following genotypes: Nico 3.33%, Danitim 6.67%, Suceava2 16.67%, Suceava5 20.00% and Suceava1 23.33%;

- After two weeks of inoculation Alina genotype has got the highest germination percent, of 100.00%; Suceava4 genotype has also got a high percent, of 70.00%;

- Nico and Danitim genotypes have got very low germination percents, with one percent of 10.00%;

- Alina genotype has got a very good development dynamics in comparison with the other genotypes, a premise of a good *in vitro* multiplication capacity;

- We consider that the alternation of the genotypes' germination percent is not conditioned by a biological deficiency of the seeds, this deficiency being influenced by their preservation duration.

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