CAPACITY OF GERMINATION IN VITRO OF BIRDS' FOOT TREFOIL SEED (LOTUS CORNICULATUS L), DONOR MATERIAL OF EXPLANTS FOR CULTURE AND I PROPAGATION OF THE SPECIES IN VITRO

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Abstract

The Present study aims to make known Lotus corniculatus L species in matters of biological and morpho-physiological traits, highlighting the in vitro behavior. After disinfection, the seeds were passed to the simple average: MS-Go MS1/2- G1; MSC1/2- G2; MS1/2-G3. Recorded in each decade the rate of germination in all environments, but MSC1 / 2 and MS1 / 2 fluid was higher. After 30 days after inoculation on the germination percentage of G_2 and G_3 was between 99-100%, while the G_0, G_1, much smaller, 48% and 78%. After another 30 days of culture in vitro, the number of seedlings from germination plants have evolved with the training, in number about equal to the number of germinated seeds of various heights. From these seedlings were seconded peaks, tissues that ensures the propagation of the species at a very high percentage of the G_2, G_3 and somewhat lower G_0, G_1.

Key words: Lotus corniculatus L, in vitro, germination, explants, percentage of germination rate of propagation.

INTRODUCTION

The center of origin of Lotus corniculatus L species is the Mediterranean, then spread to Asia, Africa, Australia and Europe (Seaney B.B., 1975). Birds' foot trefoil is considered one of the most important forage plants together with alfalfa and clover. In Europe there are many countries that are growing Birds' foot trefoil, but worthy of note is that the plant can be found in Switzerland up to 3000 m altitude in the Swiss Alps (Dragomir N., 1997).

In our permanent grassland is widespread near the coast by Black Sea and in the Alpine area. As the importance and scope of culture, Birds' foot trefoil is on the third rank among the perennial feed legumes after alfalfa and red clover grown in pure culture or be mixture, in order to ensure our space for temporary grasslands. Around the years 1995 – 1998 the cultivated surface with Birds' foot trefoil was approx. 100.100 ha. (Varga P., et all., 1998), even systematic studies are related to the early nineteenth century, namely in 1957 (Flora RSR Vol V). Relatively small cultivated areas in Romania is mainly due to lack of required quantity of seed culture expansion, common trefoil complex mixture is essential to ensure the establishment of protected grasslands in hill and mountain (Dragomir N., 1993).

In terms of economic importance, Birds' foot trefoil can be replaced with alfalfa and clover in less favorable areas of perennial forage legume species because of high adaptability to different climatic conditions (drought, high humidity) and soil acidity, salt or soils with low fertility (Dragomir N., 1992). Tracking the state of vegetation at harvest, is not only important for the production of hay and green mass production of selected seed. The flowering is not enjoyed for animals, but the yellow flowers send some high quality to the milk and butter. (Teodorescu S., 1976). The morphological and agrobiological features of the common Birds' foot trefoil are studied in detail in terms of training nodule (depending on the depth of roots in soil), the degree of variability in the size and shape of leaves and flowers, perennial studies, physiology, etc. (Dragomir N., 1982).

Cytogenetic is identified the variability of the species based on the number of chromosomes, and as a consequence of evolution, the number of chromosomes can be x = 6 x = 7, x = 8 (Borsos Olga Sz. 1973), DNA content increases from 1.48 to 2.92 depending on the number of chromosomes. Lotus corniculatus L is considered a Tetraploid species (2n = 24). Chromatographic studies on DNA content showed that the species with absorption values greater than the number of chromosomes is higher than those with fewer, the final objective of these studies on the development of genetically ideal type will apply the appropriate method to improve. In our research we started from the idea that between the methods for improvement, enhance production of selected seed is an important objective, while maintaining the value of biological species, in vitro culture techniques (Cachița D., 1987) are essential.

MATERIALS AND METHODS

The behavior in vitro of common Birds' foot trefoil seeds (Lotus corniculatus L) and seed germination ability was monitored. The experiment started in early March. The seeds were disinfected as follows: they were passed through a 70% alcohol bath for 10 seconds, then were maintained for 25-30 minutes in hypochlorite solution 5% + 2 drops of Tween 20, followed by repeated rinses (3-4 rinses) with double distilled water (surgery performed at blowing sterile air mass). After sterilization seeds are inoculated on simple culture media, solid or liquid (Murashige T., A. Skoog, 1962), with composition and additional additives specified in Table 1. The literature indicates that for in vitro germination of any species of plant are simple average efficiency, average biotechnologists called basic and often halved dose of macroelements, microelements, FeEDTA and vitamins (Cachiță D., 2007; Cachiță D. and A. Ardeleanu, 2009).

The behavior in vitro of perennial forage legume species has been much explored in an effort to induce the production of mutations and genetic variability to red, white clover, sainfoin, Birds' foot trefoil etc. with remarkable results (Savatti M., et all., 2006; Zăpârțan M. et all., 2006), and the reaction of mentioned species at the hormonal balance of a specific culture medium, and certain dose of hormones (Zăpârțan M., 1990).

Table 1

Composition of culture media for seed germination *Lotus corniculatus* L

Culture medium	Components	Observations
MS(G_o)	Macroelements, microelements, FeEDTA, vitamins: Thiamine HCl, Pyridoxine HCl and nicoting acid by 1 mg/l; sucrose 20 g/l; agar 7g/l, pH = 5.9	Components with whole content of elements
MS1/2(G_1)	Macroelements, microelements, FeEDTA, vitamins: Thiamine HCl, Pyridixine HCl and halved nicoting acid ;sucrose 20 g/l; agar 7g/l, pH = 5.9	Components with halved elements
MSC1/2(G_2)	Same with G_1. + 5 g/l coal plant	MS1/2 + coal plant
MS1/2(G_3)	Same with G_1,liquid medium with filter paper	MS1/2, without agar + filter paper bridge

Factors which control the regenerative capacity in vitro of crop plants are very little known, in case of forage legumes it is considered that the priority factors involved in regeneration, multiplication and genetic variability of the tissue, the chemical structure of substances contained in the environment, quantity, quality phytohormones and their nature (Phillips G., and Collins G.B., 1984).

RESULTS AND DISCUSSION

During periods of two months have pursued three aspects: germination rate decade, the development from germination seedlings, rate of propagation of the species by the number of explants (apex) contributed by each of Birds' foot trefoil seedlings germinated *in vitro* and treated as donor explants. *Germ rate* was followed each ten days (after 10, 20 and 30 days).

The percentage of germinated seeds is shown in table 2. Following the table we see that the liquid medium in each decade of the greatest number of germinated seeds (G_3).

Table 2

Percentage of seeds germinated in common trefoil (after 30 days)

Var.	% germination (after 10 days)	% germination (after 20 days)	% germination (after 30 days)	Relief
G_o	5	18	48	XXX
G_1	20	42	78	XXXX
G_2	20	59	99	XXXXX
G_3	25	70	99-100	XXXXX

On the G_0 (MS) version after 30 days only 50% of the seeds are germinated, on average halved components, MS1 / 2 (G_1), germination percentage of 78% is good but inferior to G_2 and G_3. The G_2 and G_3 variants have sprouted around the same percentage, 99% - 100%.

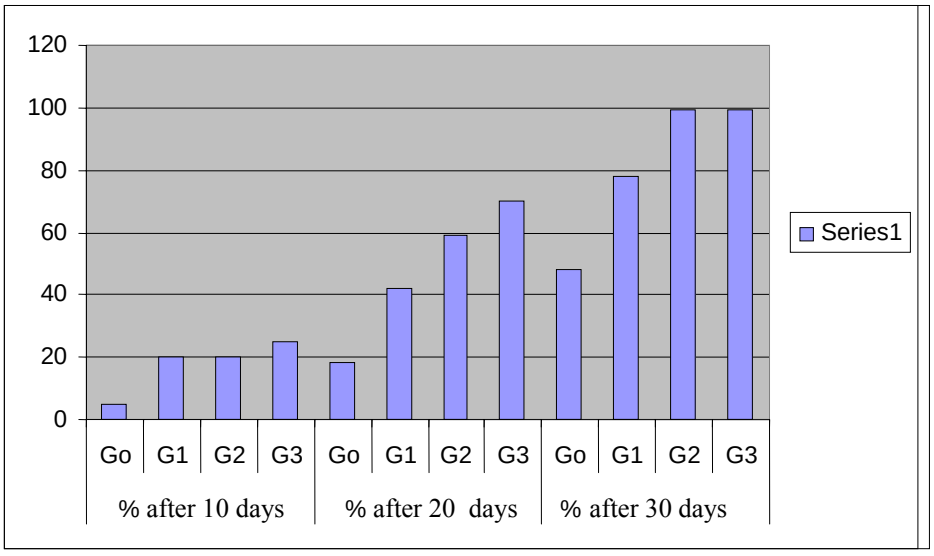

Figure1 The percentage of germinated seeds per decade

Percentage of germination on the medium variant coal plant and the liquid medium (G_2 and G_3) from the first decade is superiors to other environments. In fact we believe that the environment MS1 / 2 (G_1) gives good results (78%) but lower than the two mentioned (see figure 1).

The evolution of seedlings obtained from seed germination were examined after approx. 60 days (an additional 30 days after germination), setting the number of seedlings and their height. Were initially inoculated approx. 50 seeds / vial, and the calculation was reported in 100 seeds, and the highest average seedlings / explants was obtained on natural variants with the highest number of seeds germinated (G_2 and G_3), the number being directly proportional to the number peaks seedling (see figure 2).

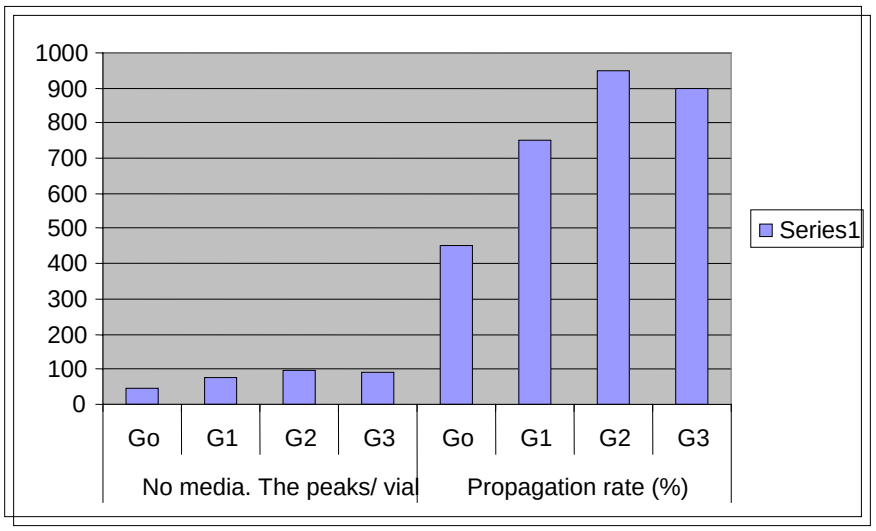

Figure 2 The average number of peaks / vial and average height of seedlings

Seedlings height evolves differently: G_0 and G_3, the average height reaches only 1.5 to 2.0 cm, while the G_1 and G_2 environment plantlets height exceeds 4.5 to 5.0 cm.

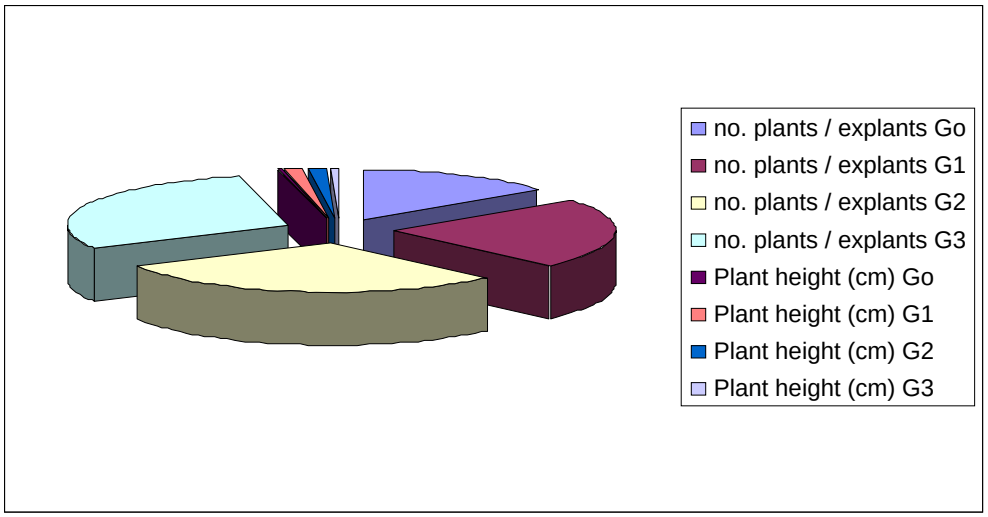

Figure 3 Rate by multiplying the average number of peaks

It is known that the presence of an coal plant in the medium MS1 / 2, produces elongation so the seedlings are higher (Cachița D., 1987), providing a much higher number of explants as it provides because multiplication can be ensured not only from apex but also from node. Multiplication rate reported at the number of apical tissues (apex) is suggestively depicted in figure 3, there was reported to 100 bottles / variant.

We can notice the multiplier note on G_2, G_3, G_1 and G_0 variants, in order of value, namely: that 990 explants / variant (G_2), 900 explants / variant (G_3), 750 explants /variant (G_1) and 450 explants / variant (G_0). But the multiplication rate can be infinitely greater if we start a culture of copying of nodes, taking into account the number of nodes from each seedlings.

CONCLUSIONS

The ten days rhythm of germination of *Lotus corniculatus* L seeds after 30 days is between 48-99%. On MS medium only half of seeds are germinating while the variants MS1 / 2, MS1 / 2 5 g / l coal plant and MS1 / 2 fluid, the percentage is between 78 and 99%.

We recommend to use Murashige-Skoog medium, solid or liquid with halved components, with the addition of coal or filter paper bridge to get 100% germination rate.

After 60 days obtained seedlings from seeds were fully developed providing a number of explants for multiplication. Seedlings average number is between: 49 seedlings / variant on the G_0, 78 seedlings / variant on the G_1, 99 and 100 seedlings / variant G_2 and G_3.

The number of detached apex is directly proportional to the number of germinated seedlings, the multiplication rate reaching 450 to 900 seedlings / variant.

We recommend the multiplicantion in vitro of Birds' foot trefoil species, based on explants detached from the seedlings obtained from seed germination and for the propagation rate they can provide.

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