MORPHOLOGICAL CHARACTERIZATION AND THE GERMINATING POTENTIAL OF *LOTUS CORNICULATUS* L. POLLEN

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Abstract

This scientific paper presented the morphological characterization and characteristics of pollen germination process in *Lotus corniculatus* L. The samples from which pollen was taken came from two stationaries: a polluted one and an unpolluted one. „In vitro" pollen germination tests were carried out in „van Tieghem" wet medium. Nutritive mediums that served for pollen grain inoculation had different glucide concentrations, until 300%. This paper presents the concentration of glucidic elements from mediums allowing pollen germination at minimum percentage, shows the sucrose concentrations for developing the germination process under best conditions and analyses the germination process in dynamics (after 2, 24, 48 and 72 hours since pollen inoculation in nutritive mediums). The proportions of germinated pollen were very high both on mediums lacking glucides and on glucide hyperconcentrated ones, irrespective of the stationery from which samples were taken. The obtained results showed that *Lotus corniculatus*, known as a natural tetraploid (2n = 4x = 24), of hybrid origin, which regularly forms bivalents at metaphase I of meiosis, has a genetically balanced diploid type meiosis. Very high pollen germination potential in mediums with different glucide concentrations is a proof of meiosis normality in this tetraploid. Furthermore, the fact that pollinic tubes keep growing in length six days after pollen inoculation on all nutritive mediums of the trial is a proof sustaining the eco-physiological plasticity of *Lotus corniculatus.* Pollen germination potential in *Lotus corniculatus* was not influenced by polluted environment, showing that major genes controlled this trait; therefore, it is very well genetically consolidated. Also, the pollen morphological traits were not influenced by polluted environment.

Key words: *Lotus corniculatus*, pollen grain, germinating potential of pollen, medium which sucrose.

Lotus corniculats (birdsfoot trefoil) is a fabacee, grass, perennial, common pastures and meadows where sometimes reach a dominant species, spontaneous, rarely cultivated forage value, melliferous and pharmaceutical (Barry T. & all. 2003). This species is widespread, managing satisfactory on other grounds (acid), where other more valuable legume (alfalfa, clover) fail. It is the only legume species adapted to different climatic and soil conditions. Shows a high resistance to cold, drought and even the excess moisture. It is considered good production and high quality hay compared to other legumes (Grant W.F., Niizeki M., 2009).

Studies based on: tannin content, phenolic compounds, cyanide production, morphology, cytogenetics studies, specificity in the *Rhizobium* and self-incompatibility in group corniculatus, suggests that *Lotus corniculatus* was born by hybridization between *L. alpinus* and/or *L. tenuis* (likely female parent), with *L. uliginosus* (likely male parent), followed by doubling the number of chromosomes. So, *Lotus corniculatus* is a hybrid, so a allopolyploid, namely a allotetraploid (Ross M.D., Jones W.T., 1985; Davies A., Jenkins G., Rees H. 1990; Grant, W. F., 1999).

In this paper, we proposed to establish the variability of pollen morphological traits, the potential of germinating pollen of *Lotus corniculatus*, and its influence by noxious pollutants from the environment. Germinative potential of pollen may be a valuable index eco-physiological which may correlate with other valuable characteristics of this fabacee species.

MATERIAL AND METHOD

The biological material is represented by a vegetal taxon – *Lotus corniculatus* L., taken from two stationary placed in the surroundings of the Ceahlău National Park: an unpolluted control stationary called Potoci village and a polluted stationary called Taşca-marshalling yard. Last stationary is affected by polluting noxa, which come from the cement factory of Taşca.

From the each stationary we took pollen at the anthesis phase. The pollen was studied as concerns the morphological specific features and the germinating potential. In order to define the pollen morphology, we determined shape of pollen grains, exine ornamentation, size of pollen grain and number of germinative pores/pollen grain. For determining the shape of pollen grains and of exine ornamentations, we have used the Tesla electron-scan microscope, at which we took microphotographs.

For determining the size of pollen grains we did micromeasurements at 1000 grains/stationary. We measured the longitudinal and the equatorial diameter.

For establishing the number of germinative pores/pollen grain, we have done determinations on 1000 pollen grains/taxon/stationary. The method consisted in introducing the pollen in a mixture of concentrated sulphuric acid (one part) and acetic acid (two parts) and 3% methylene blue.

For determining the germinating potential, we have used the so-called van Tieghem "wet rooms". The nutritive mediums necessary for the germination of pollen grains consisted in distilled water, agar 1% and sucrose at different rates: 0%, 5%, 15%, 25%, 35%, 45%, 55%, 70%, 100%, 200%, 300%. Thus, eleven experimental variants resulted for each stationary. For each experimental variant, we have used 10 "wet rooms". The amount of inoculated pollen per each medium was the same in all cases. Readings at the Hund Wetzlar optic microscope were done at 2, 24, 48 and 72 hours since the pollen inoculation on mediums, thus, being established the percent dynamics of the germination capacity.

The germination capacity was expressed as percentage, by reporting the number of germinated grains to total pollen grains.

RESULTS AND DISCUSSIONS

Pollen morphology of *Lotus corniculatus* L.

Lotus corniculatus pollen is pale yellow, prolate, tricolporat and exine is finely reticulate *(fig. 1, 2)*.

Length of polar axis has values from 16.38 to 21.06 µm and equatorial axis has values between 10.53 and 14.04 µm *(tab. 1, 2)*. The ratio between the two diameters was around 1.6. These data are consistent with the literature (Diez M.J., Ferguson I.K., 1994; Knight C.A. & all. 2010; Prenner G., 2003; Tarnavschi I.T & all., 1990).

Lotus corniculaus pollen grains collected from the two stationary have three germinative pores *(fig. 3)*. There is an insignificant percentage of granules with one or two germinative pores *(tab. 3)*. Yet, the number of germinating pores does not reflect the polyploidity in *Lotus corniculatus*. Palinological data from literature show the presence of 3 pore-pollen in *Lotus corniculatus* (Diez M.J., Ferguson I.K.,1994; Knight C.A. & all. 2010; Prenner G., 2003; Tarnavschi I.T & all., 1990). We suggest that not always polyploidity may be correlated to an increase in the number of germinating pores/pollen grain, as in case of *Lotus corniculatus.*

No difference in shape, size of pollen and number of germinative pores/pollen grain taken from the two stationary.

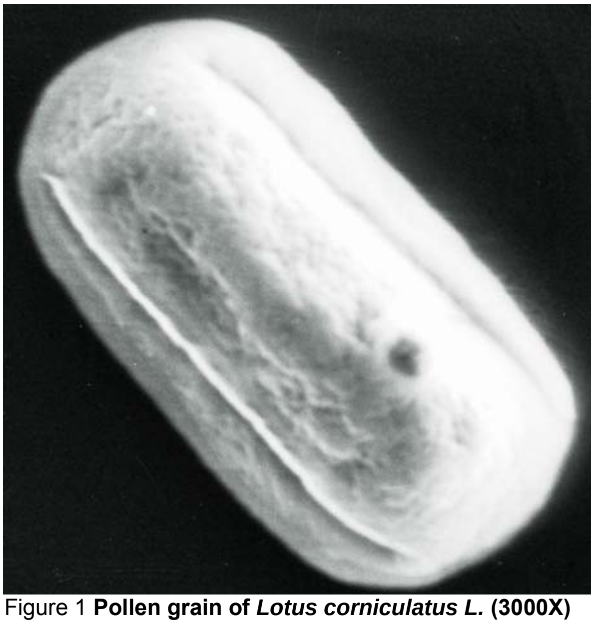

Figure 1 **Pollen grain of *Lotus corniculatus L.* (3000X) (Original)**

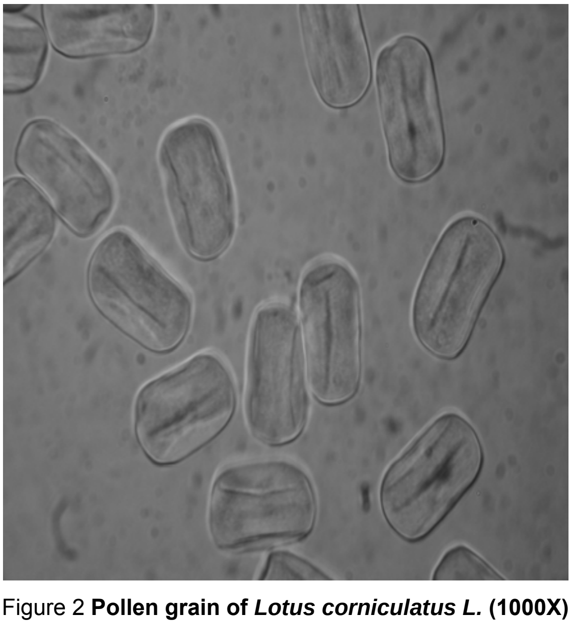

Figure 2 **Pollen grain of *Lotus corniculatus L.* (1000X) (Original)**

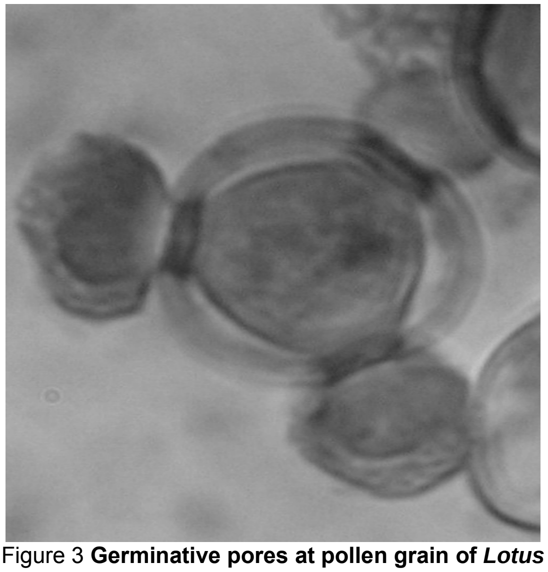

Figure 3 **Germinative pores at pollen grain of *Lotus corniculatus L.* (1500X) (Original)**

After 2 hours since inoculation on nutrient mediums, control pollen indicates that the germination process can occur even in medium completely lacking of sucrose, germination manifested on nutrient enriched substrates up to 55% sucrose. Within two hours, the highest percentages of germinated pollen recorded on 25-35% sucrose media. The same situation is found in the case of stationary polluted pollen *(fig. 4)*.

After 24 hours since inoculation, the percent values of control *Lotus corniculatus* pollen germinability increase significantly al all the variants, and the palette of sugar concentration, which allows pollen germination is larger, reaching 300%. After this time interval of inoculation on medium, the highest rate of germinated pollen was also registered on 25 and 45% sugar mediums *(fig. 10)*. In these cases, the germination was 89-91%. The same situation is found in the case of stationary polluted pollen *(fig. 5)*. After this time interval, germination was also possible on mediums hyperconcentrated in glucidic elements (100-300% sucrose), in quite high proportions.

After 48 hours since inoculation, in both cases of stationary, we found an increase in the percentage of germinated grains on all the variants of sucrose mediums, especially on 200 and 300% mediums the values have significantly increased, to 79%. On 25, 35 and 45% sucrose mediums, the maximum rate of germinated pollen is maintained (91-94% pollen germinated) *(fig. 6)*.

Germinating potential of *Lotus corniculatus* L. pollen

After 72 hours since inoculation, the germinating potential of *Lotus corniculatus* pollen from both stationary, insignificant increase at all the experimental variants, even on sucrose lacking medium (64-65% pollen germinated) and hyperconcentrated in sucrose (55-81% pollen germinated). The highest values of germinability are maintained on 25, 35, 45 and 55% sucrose mediums (90-97% pollen germinated) *(fig. 7)*.

If we analyse in dynamics the pollen germinating potential of the two stationary, we find that within 24 hours after inoculation, there is a significant jump in growth rates of pollen germinated, and then in the next 48 hours, germinating process continues to grow, but insignificant, on all nutrient mediums, especially on mediums rich in sucrose *(fig. 8, 9)*. Also note that the nutrient mediums which they allow the highest percentage of pollen germination potential are those mediums enriched with 25-45% sucrose. But after 72 hours since inoculation, and 55% sucrose concentration is very suitable for showing a high rate of germination capacity. Analyse in dynamics the pollen germination potential found in the process of pollen taken from the two stationary is the same, following the same pace during the 72 hours of observations. The dynamic of the germination potential is the same for the pollen of *Lotus corniculatus* taken from two stationary, during the 72 hours of observations.

The investigations prove that germination capacity of pollen of *Lotus corniculatus* is very high on all nutrient media used in the experiment: medium lacking of sucrose, mediums with sucrose in low concentrations (5%), mediums usual concentration of sucrose (15-45%), and mediums extremely concentrate in sucrose (over 55% sucrose). Ability of pollen to germinate on substrates with various sucrose concentrations, could explain that *Lotus corniculatus* is only *fabaceae* adapted to different climatic and soil conditions, high resistance to cold, drought and even the excess moisture. Be it should mentioned that *Lotus corniculatus* is native to Europe and western Asia. Furthermore it is widely naturalised throughout the temperate regions of South and North America, Asia Minor, North Africa, Australia and New Zealand (Garcia de los Santos & all. 2001).

Also, we find that germination pollen of *Lotus corniculatus* does not change if the environment is polluted.

Impressive high viability of pollen of *Lotus corniculatus*, which refers to the survival of viable pollen tubes even after 144-168 hours since inoculation, on the mediums hyperconcentrated (over 55% sucrose). At concentrations below 55% sucrose mold develops after a few hours since inoculation, so the pollen germinated can not survive long.

Table 1

Variability of pollen grain size in *Lotus corniculatus* L. - unpolluted control stationary

Type of diameter	Mean value (µm)	Minimum value (µm)	Maximum value (µm)	Variation height (µm)	S (µm)	S%	S $\bar{x}$ (µm)	Rate high diameter/ equatorial diameter (µm)
high diameter	18.9774	16.38	21.06	4.68	0.833	4.39	0.0833	1.629
equatorial diameter	11.6474	10.53	14.04	3.51	0.681	5.847	0.0681	

Table 2

Variability of pollen grain size in *Lotus corniculatus* L. – polluted stationary

Type of diameter	Mean value (µm)	Minimum value (µm)	Maximum value (µm)	Variation height (µm)	S (µm)	S%	S $\bar{x}$ (µm)	Rate high diameter/ equatorial diameter (µm)
high diameter	18.9540	16.38	21.06	4.68	0.899	4.74	0.0899	1.625
equatorial diameter	11.6649	10.53	14.04	3.51	0.680	5.829	0.068	

Table 3

Number of germinative pores/pollen grain in *Lotus corniculatus* L.

No. stationary	Mean value ($\bar{X}$)	% pollen grains with … germinative pores			
		0	1	2	3
Control	2.88	0	2	8	90
polluted stationary	2.87	0	3	7	90

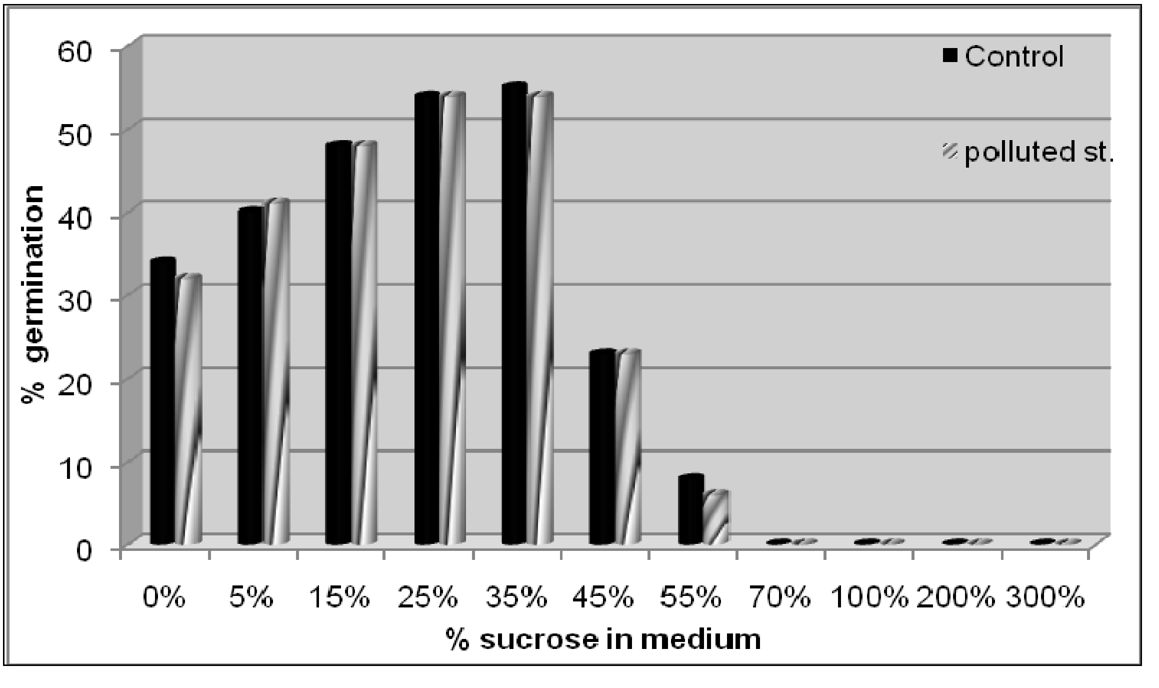

Figure 4 **Pollen germination after 2 hours since inoculation on medium**

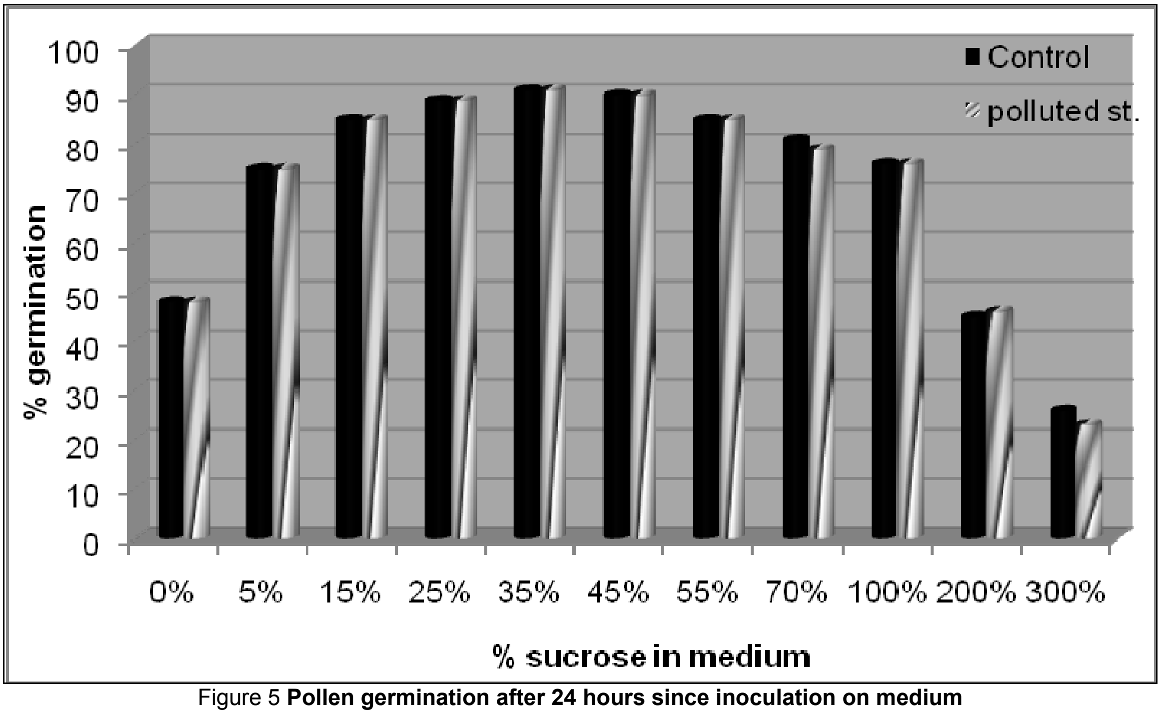

Figure 5 **Pollen germination after 24 hours since inoculation on medium**

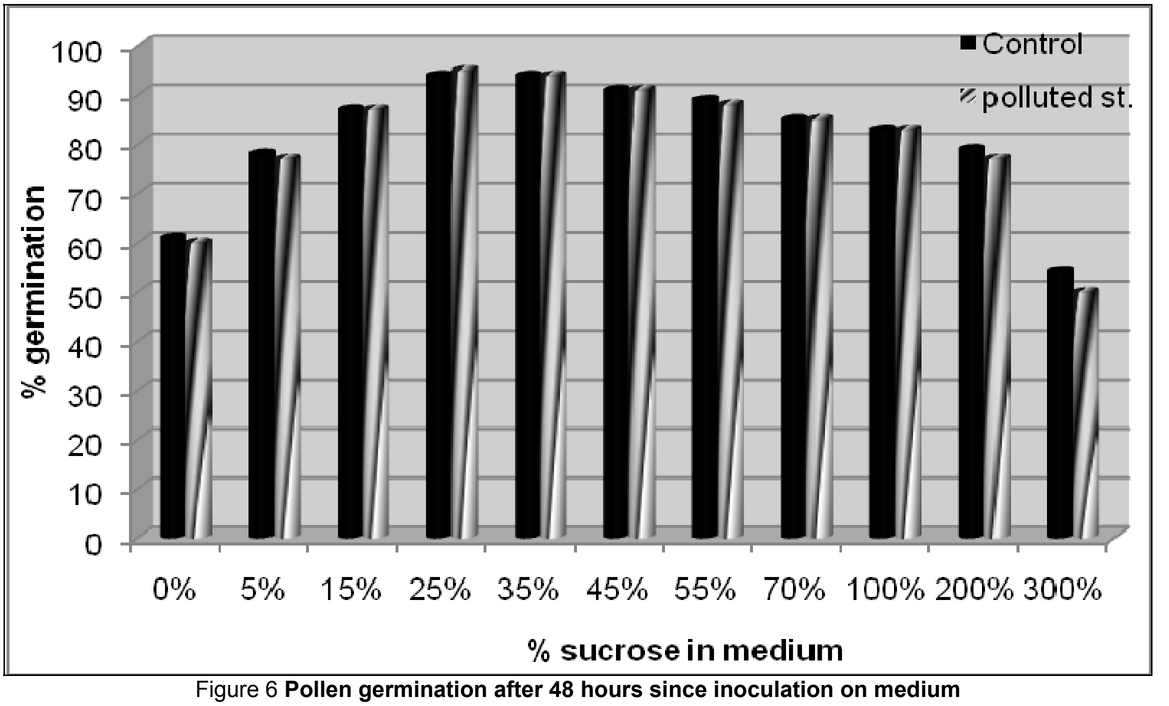

Figure 6 **Pollen germination after 48 hours since inoculation on medium**

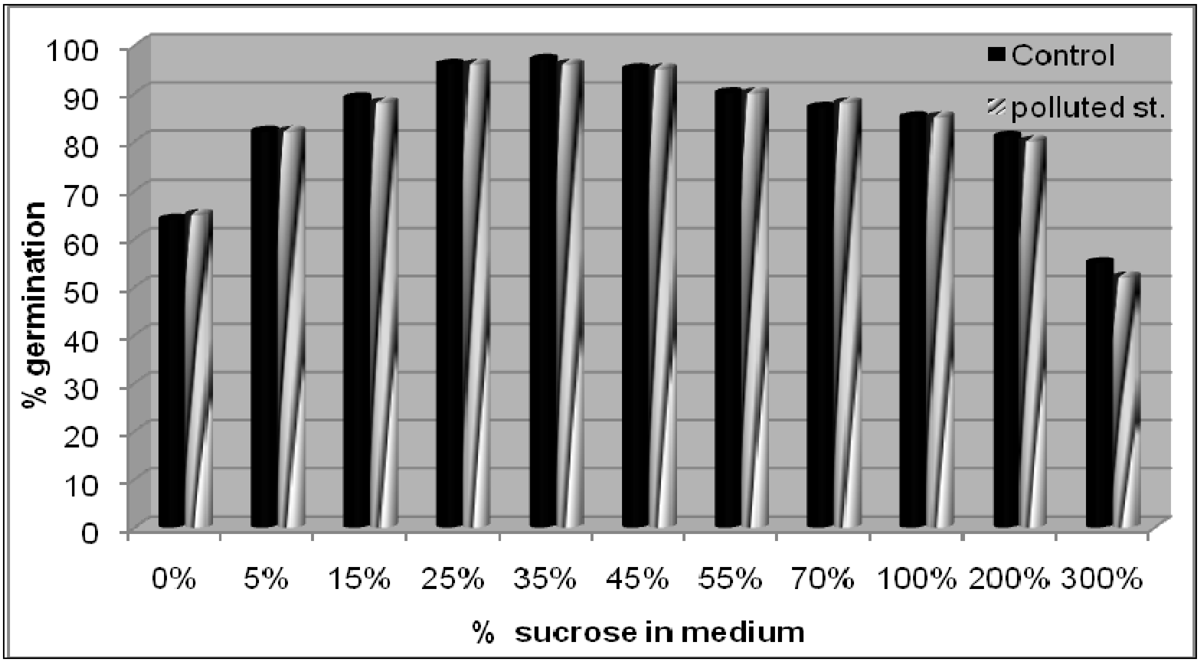

Figure 7 **Pollen germination after 72 hours since inoculation on medium**

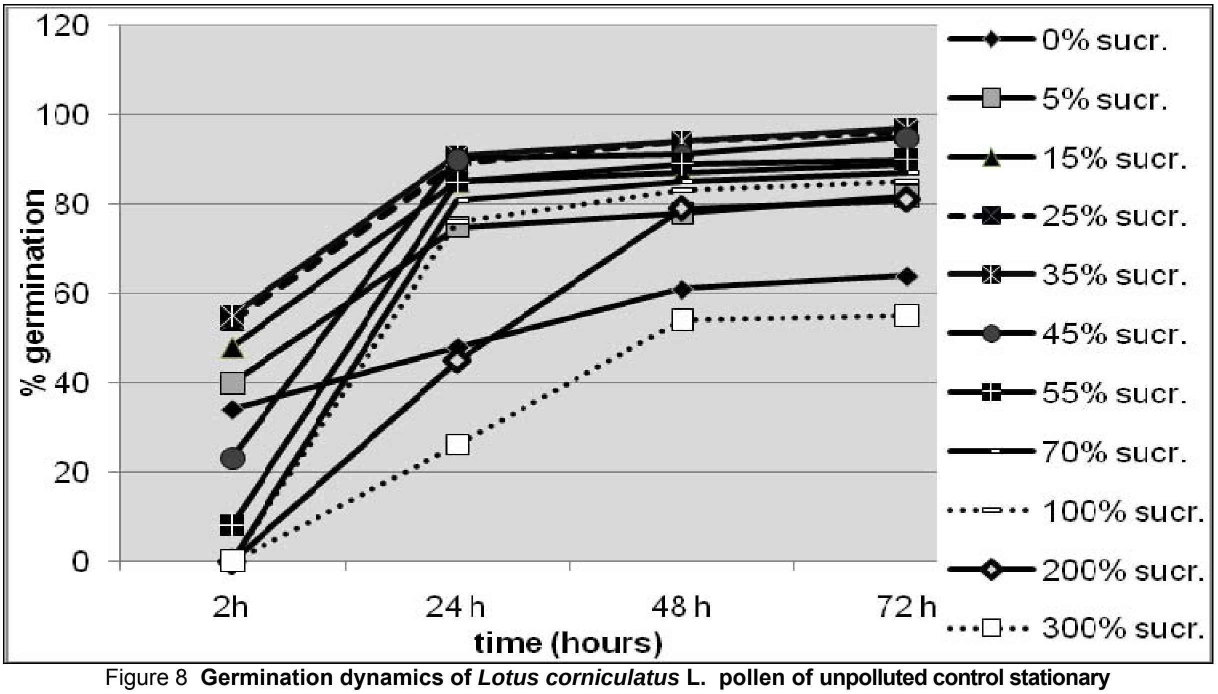

Figure 8 **Germination dynamics of *Lotus corniculatus* L. pollen of unpolluted control stationary**

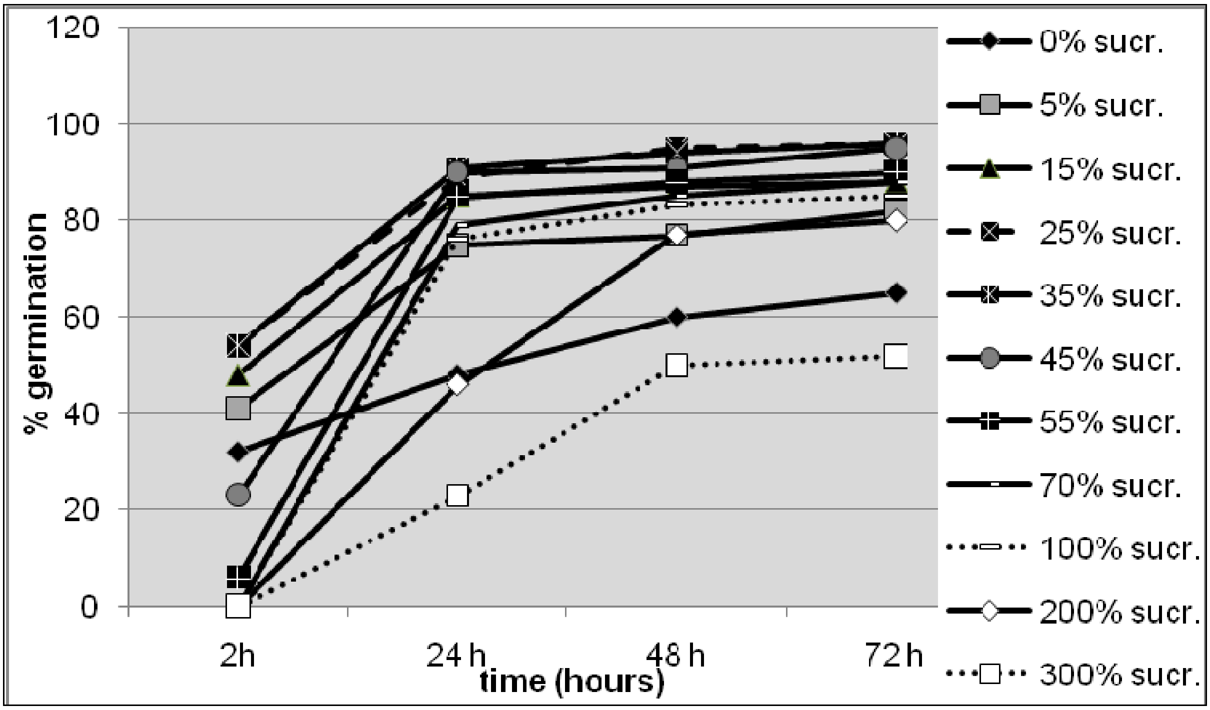

Figure 9 **Germination dynamics of *Lotus corniculatus* L. pollen of polluted stationary**

Figure 10 **Pollen germination on 25% sucrose medium, 24 hours after inoculation in control *Lotus corniculatus* L. (100X) (Original)**

CONCLUSIONS

The shape, the ornamentation of the exine, the size of pollen grains and number of germinative pores/pollen grain from *Lotus corniculatus* are genetically enhanced characters well.

The germinating potential of *Lotus corniculatus* pollen, very high, even under a polluted environment, is a proof that and this character is controlled by major genes, therefore, genetically enhanced well.

Very high pollen germination potential of *Lotus corniculatus* is a testament to the balance of this genotype tetraploid meiosis, amfiploida origin.

Pollen germination of *Lotus corniculatus* could be an argument of eco-physiological plasticity and the area of propagation of the species.

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