THE CYTOLOGY OF POLLEN GERMINATION PROCESS AT *LOTUS CORNICULATUS* L. AND *CORONILLA VARIA* L.

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Abstract

The paper presents the characteristics of the germination process in *Lotus corniculatus* L. and *Coronilla varia* L. pollen. We made estimates on the length of pollinic tubes in the dynamics of germination (after 1, 2, 24, 48 and 72 hours since the inoculation on medium), according to the glucidic concentration of the nutritive mediums used in this experiment. We pointed out the maximum lengths of the pollinic tubes, correlated to the lengths of flower stiles from the two taxons. Achieving a certain length of the pollinic tubes is very important, because the fertilization of ovules is thus ensured and, implicitly, the fructification of the respective taxons. We also referred to the way of the emergence of pollinic tubes from the two taxons. The bicellular pollen-type of the two *Fabaceae* is shown by pointing out the generative cell in the pollinic cell, which, after the mitotic division, produces two spermatic cells having the value of immobile male gametes. We have also shown the type of anomalies of the pollinic tubes and the frequency of these anomalies during the process of pollen germination at the two taxons from the *Fabaceae* family. The obtained results were correlated to pollen germinating potential of the two taxons, to geographic spreading area, and to native resistance of the two taxons to various abiotic factors. Although the phylogenetic connection between the two investigated taxons is very tight, there are distinctive traits of the germinating process, which have a diagnosis value and may represent valuable genetic and physiological indicators.

Key words: pollen tube, generative cell, vegetative cell, anomalies of pollen tubes

A direct correlation between pollen size (volume) and style length has likewise been observed. This may show the importance of the pollen food reserves in sustaining the growth of its pollen tube along the style (Ortega O. et al., 1997). On the other hand, we know that pollen ranges from 2.5% to 61% protein content. Most pollen proteins are likely to be enzymes that function during pollen tube growth and subsequent fertilization. Pollen protein concentrations are highly conserved within plant genera, families, and divisions. It was found that the need for growing pollen tubes plays a important role in determining pollen protein content (Roulston H. et al., 2000). Has been shown in some cases at fabacee, that self-sterility can be: prezygotic mechanism, when pollen tubes are rejected in style and ovary; postzygotic mechanism, when the fertilized ovules aborting (Valtueña F.J. et al., 2010). Vital issues related to achieving fertilization in fabacee focused: durations of stigmatic receptivity, pollen viability and pollen tube growth. At *Vicia faba* L., pollen tubes at up to three days took to reach the ovules furthest from the stigma (Stoddard F.L. et al., 1986). In this paper we propose to complete studies of pollen germinating potential of two fabacee - *Lotus corniculatus* and *Coronilla varia* (Pădureanu S. 2010; Pădureanu S., 2011) with investigations of peculiarities of the male gametophyte development. *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 (2n=4x=24) (Grant W.F., 1999; Da Silva Neide, Pagliarini M.S., 2002). Biochemical and genetic evidence indicates that this species is an allotetraploid (Grant W.F., Small E., 1996). *Lotus corniculatus* not prefers own pollen, ie is self-incompatible (Dobrofsky S., Grant W.F., 1980). *Coronilla varia* is originally a autoploid namely a autotetraploid (2n=4x=24) (Berchtold D.J. et al.,1973). *Coronilla varia* L., is relatively self-incompatible. The self-incompatibility is manifested partly in the stigma, style and ovary (Baluch S.J. et al.,1973).

MATERIALS AND METHODS

The biological material is represented by pollen collected by *Lotus corniculatus* L. and *Coronilla varia* L. taken from two stationary placed in the surroundings of the Ceahlău National Park:

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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. Pollen was sampled from the two taxons, at the anthesis phase, and was analysed from the viewpoint of the germination process. Pollen grains have been inoculated on agar nutritive mediums, at which sucrose was added at different concentrations, from 0 to 200%. The quantity of pollen inoculated on these mediums was the same in all the cases. For each experimental variant, we have done 15 preparations. In order to maintain a wet medium, which was vital for pollen viability, we have used "van Tieghem" wet chambers. Micromeasurements for determining the dynamics for the extension of pollen tubes were carried out every 2, 24, 48 and 72 hours since the inoculation of pollen grains on nutritive medium for *Lotus corniculatus* and 1, 24, 48, 72 hours for *Coronilla varia*. For pointing out the characteristics of pollen tube from this taxon, photographs were taken at Hund Wetzlar optical microscope and drawings at camera lucida.

RESULTS AND DISCUSSIONS

The cytological analysis of the germination process was done at the same time with the analysis of the germinating potential.

After 2 hours since the inoculation of the *Lotus corniculatus* pollen on artificial medium, pollinic tubes emerged at different rates, on the variants of nutritive mediums with 0-200% sucrose. Their ratio (8-55%) (Pădureanu S., 2010) is directly correlated to the germinating potential. The mean length of pollinic tubes was comprised between 56 and 728 μm for unpolluted stationary (control) and 55 – 729 μm for polluted stationary (*fig. 1*). On the mediums more concentrated than 55% sucrose, the pollen not formed tubes in the first two hours after inoculation.

After an hour since the inoculation of the *Coronilla varia* pollen on artificial medium, pollen tubes occurred on medium with 0% sucrose and on medium with the addition of up to 70% sucrose (*fig. 2*). Pollen tubes formed in the first hour after inoculation have lengths ranging between 67 and 199 μm, the longest are on the medium with 25% sucrose. No significant differences between control and polluted stationary. It noted a direct relationship between % germination and pollen tube length (Pădureanu S., 2011).

After 24 hours since the inoculation, the pollinic tubes of *Lotus corniculatus* were elongated up to 3414 μm for control and 3413 μm for polluted stationary (*fig. 3*). After 24 hours appeared pollen tubes and on mediums up to 200% sucrose. The longest tubes were formed on medium with 25% sucrose.

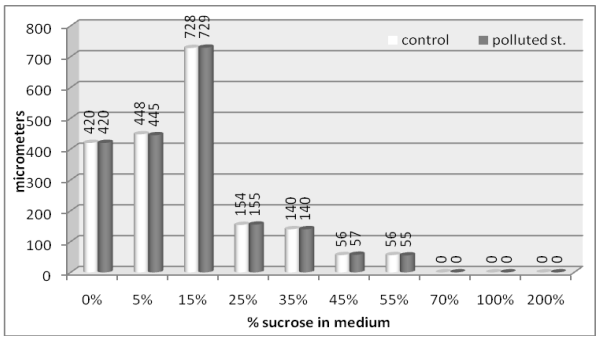

Figure 1 **The average lenght of the pollen tube at *Lotus corniculatus*, 2 hours after inoculation**

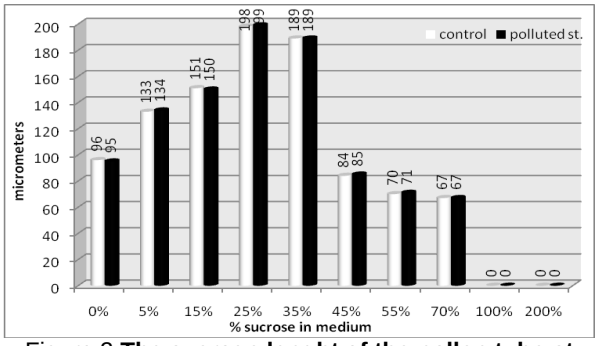

Figure 2 **The average lenght of the pollen tube at *Coronilla varia*, 1hour after inoculation**

In *Coronilla varia*, after 24 hours, the pollinic tubes emerged on mediums with very high concentrations in sucrose (200%). The longest tubes were formed on medium with 45% sucrose, when the average length was 4691 μm for control and 4690 μm for stationary polluted (*fig. 4*).

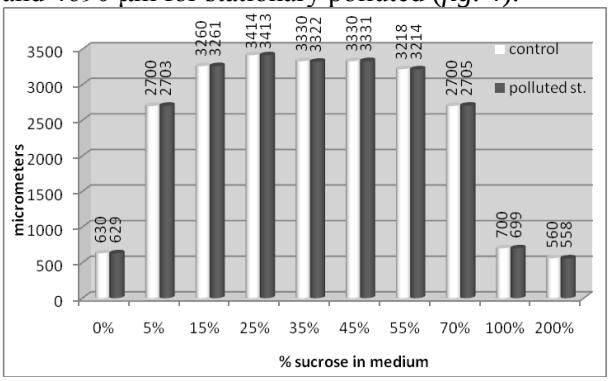

Figure 3 **The average lenght of the pollen tube at *Lotus corniculatus*, 24 hours after inoculation**

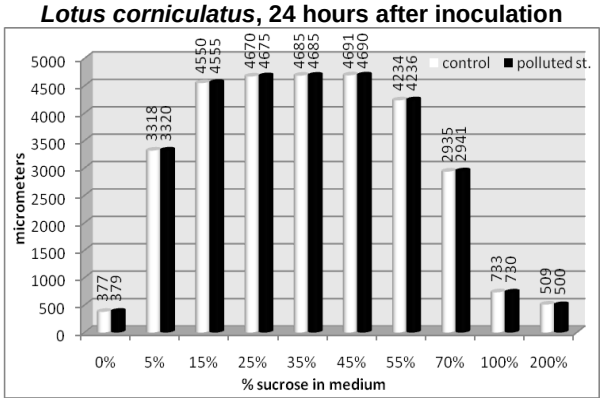

Figure 4 **The average lenght of the pollen tube at *Coronilla varia*, 24 hours after inoculation**

In *Lotus corniculatus*, after 48 hours since inoculation, the pollinic tubes increased in length in all the experimental cases, achieving values comprised between 1700 µm and 3890 µm for control and 1771 µm and 3891 µm for polluted stationary (*fig. 5*). The longest tubes formed on medium with 25% sucrose.

In *Coronilla varia*, after 48 hours since inoculation, also, the pollinic tubes increased in length in all variants. Their length was between 2311 µm and 5457 µm for control and 2310 µm and 5460 µm for polluted stationary (*fig. 6*). The longest tubes formed on medium with 35% sucrose.

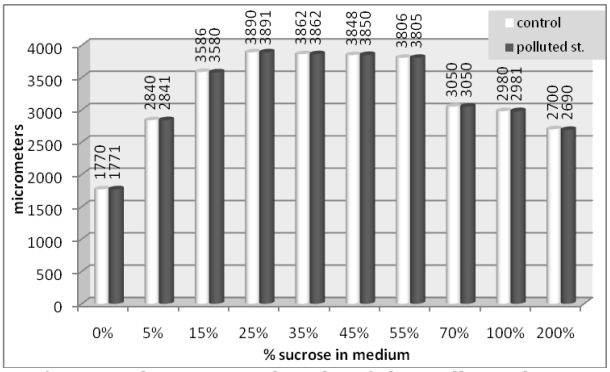

Figure 5 **The average lenght of the pollen tube at *Lotus corniculatus*, 48 hours after inoculation**

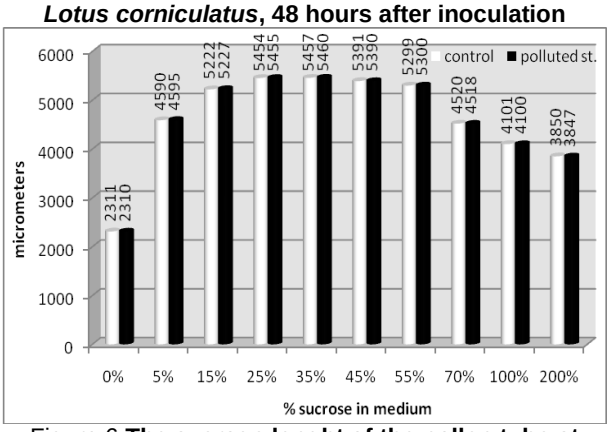

Figure 6 **The average lenght of the pollen tube at *Coronilla varia*, 48 hours after inoculation**

After 72 hours since inoculation, the pollinic tubes of *Lotus corniculatus* were elongated to 3862 µm for control and 3863 µm for polluted stationary (*fig. 7*). The pollen tubes of *Coronilla varia* have increased to 5534 µm for control and 5539 µm for polluted stationary (*fig. 8*). After this time, at the *Lotus corniculatus* remains directly proportional relationship between % germination and pollen tube length, while at *Coronilla varia* is an inverse relationship between % germination and pollen tube length (Pădureanu S., 2010, 2011).

The dynamic analysis of pollen tube length in *Lotus corniculatus* show that in the first 24 hours after inoculation, pollen tubes grow significantly, and the increase is less significant until 72 hours after inoculation. The longest tubes are formed on 25-45% sucrose in medium. Regarding the dynamics of pollen tube growth, there are no differences between control and polluted stationary (*fig. 9, 10*).

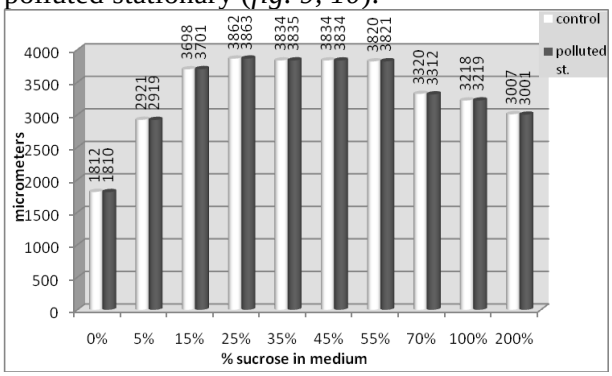

Figure 7 **The average lenght of the pollen tube at *Lotus corniculatus*, 72 hours after inoculation**

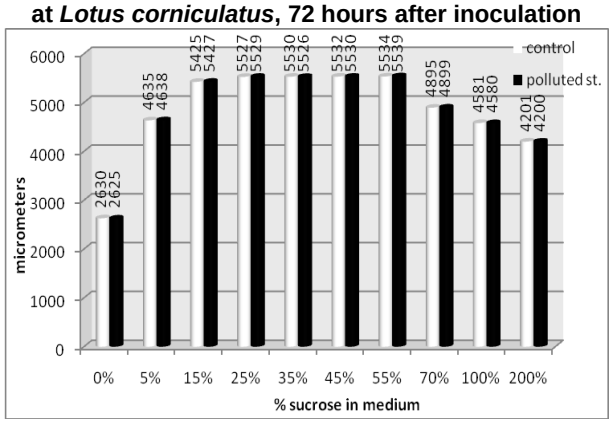

Figure 8 **The average lenght of the pollen tube at *Coronilla varia*, 72 hours after inoculation**

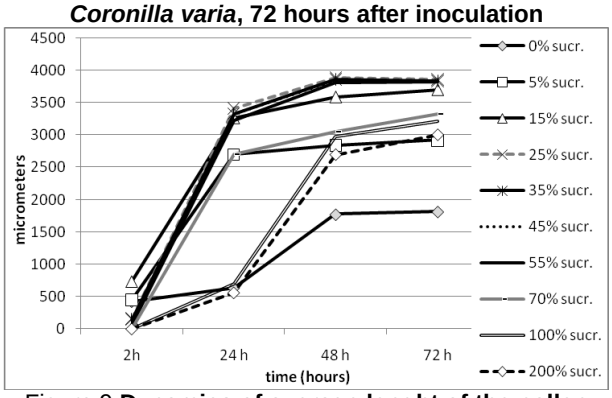

Figure 9 **Dynamics of average lenght of the pollen tube at *Lotus corniculatus* - control**

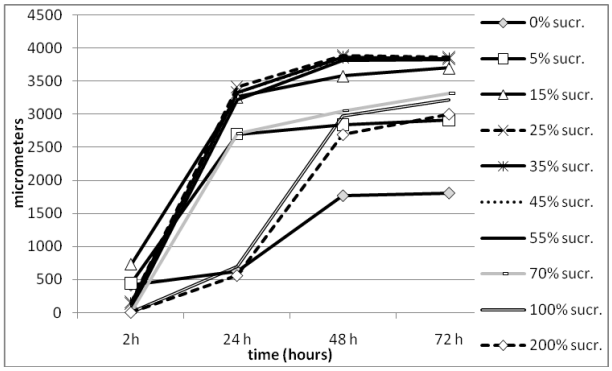

Figure 10 **Dynamics of average lenght of the pollen tube at *Lotus corniculatus* - polluted stationary**

Same aspect analyzed at *Coronilla varia* leads to the conclusion that in the first 24 hours after inoculation, the pollen tubes are elongated significantly and continue to grow in the next few hours, but insignificant. The longest tubes

are formed on 25-55% sucrose in medium. No differences between control and polluted stationary with regard to the pollen tube growth dynamics (*fig. 11, 12*).

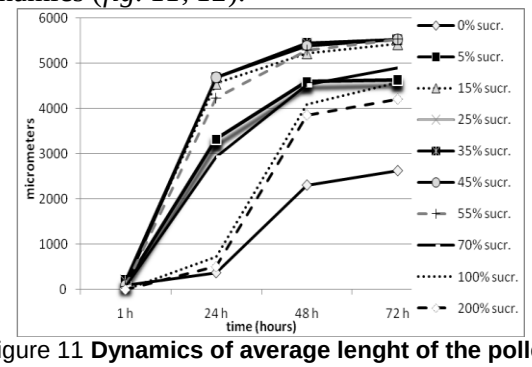

Figure 11 **Dynamics of average lenght of the pollen tube at *Coronilla varia* – control**

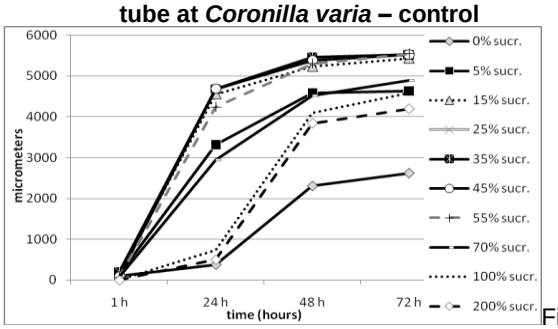

Figure 12 **Dynamics of average lenght of the pollen tube at *Coronilla varia* - polluted stationary**

The pollinic tubes from *Coronilla varia* are longer as compared to the ones of *Lotus corniculatus*. We can explain this phenomenon if we take into account the length of the flower stiles from the two taxons. The *Coronilla varia* flower stile has the mean length of 4.6 mm, while the same floral element from *Lotus corniculatus* has 3.8 mm (original values). There is a direct correlation, genetically determined, between the length of stile and that of pollinic tubes that must penetrate the tissues of the stile to the ovules (Ortega O. et al., 1997). It is very likely that "in vivo", the pollinic tubes from the two taxons should be longer than those obtained "in vitro". Another important phenomenon is the long term viability of *Lotus corniculatus* and *Coronilla varia* pollen tubes, which, even after 168 hours since inoculation, kept unchanged their vitality even on hyperconcentrated mediums.On low and hyperconcentrated sucrose in mediums, the inoculated pollen grains have known expulsions of the living content at high rates for the two taxons.

The germination process starts (for both taxons) at by forming a small hill. In 1-2 hours, the small hill is lengthened, and thus the pollinic tube emerges. The nucleus of the vegetative cell is cantoned on the top of the tube, and the generative cell penetrates in the tube, being maintained at a certain distance from the nucleus of the vegetative cell. Therefore, it results that *Lotus corniculatus* and *Coronilla varia* pollen is bicellular.

The different aspects of *Lotus corniculatus* and *Coronilla varia* pollinic tubes, formed on different nutritive mediums are presented in *figures 13- 19.*

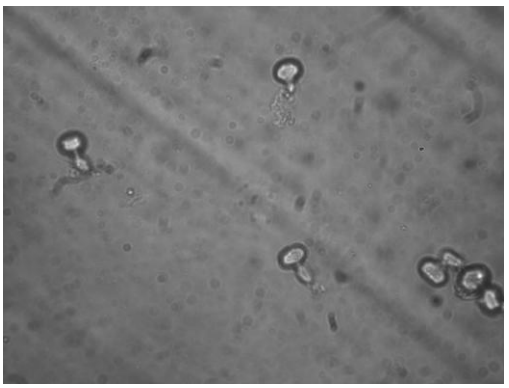

Figure 13 **The debut of edification of the pollen tube at *Lotus corniculatus* (400X) (Original)**

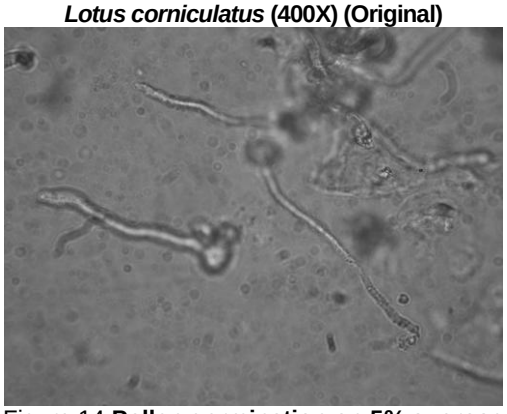

Figure 14 **Pollen germination on 5% sucrose medium, 2 hours after inoculation at *Lotus corniculatus* (400X) (Original)**

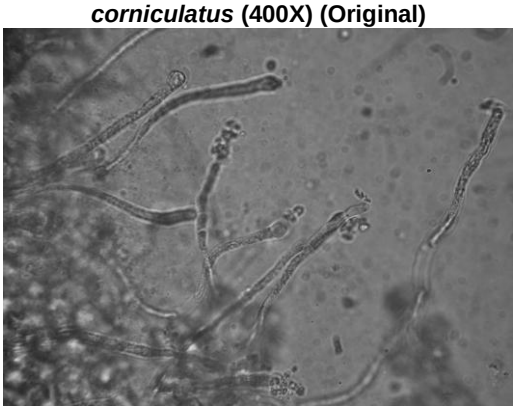

Figure 15 **Pollen germination on 25% sucrose medium, 24 hours after inoculation at *Lotus corniculatus* (400X) (Original)**

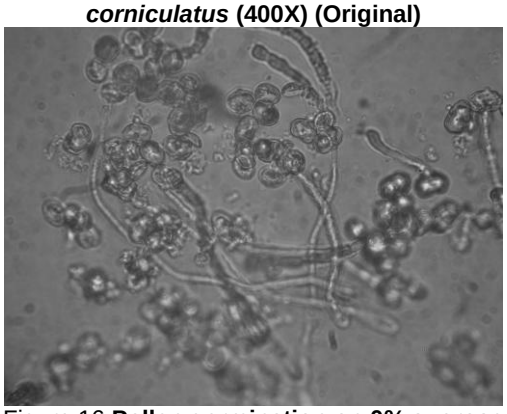

Figure 16 **Pollen germination on 0% sucrose medium, 24 hours after inoculation at *Lotus corniculatus* (400X) (Original)**

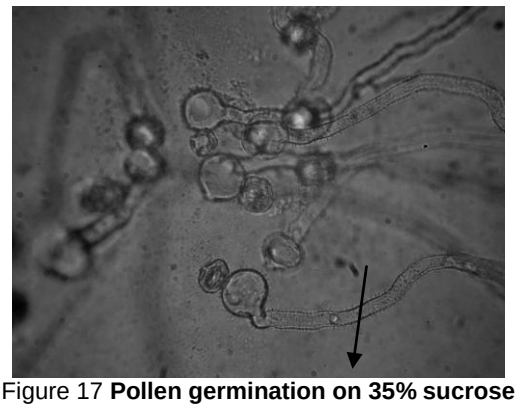

Figure 17 **Pollen germination on 35% sucrose medium, 24 hours after inoculation at *Coronilla varia.* Arrow indicates the generative cell (1000X) (Original)**

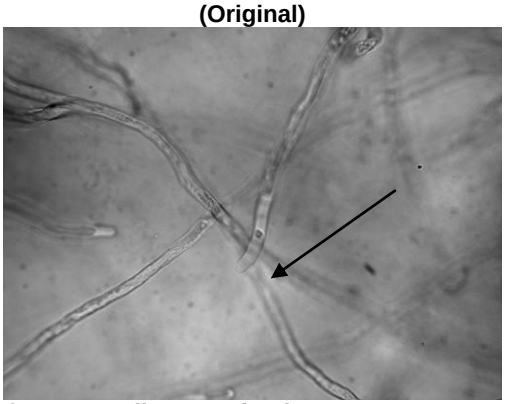

Figure 18 **Pollen germination on 35% sucrose medium, 24 hours after inoculation at *Coronilla varia.* Arrow indicates the nucleus of the vegetative cell (1000X) (Original)**

The pollen germination process of the two *Fabaceae* was marked by the emergence of atipic forms of pollinic tubes, known in literature as anomalies. These anomalies consisted for *Lotus corniculatus* in tubes which were branched at the top. For *Coronilla varia* these anomalies consisted in pollen grains with two tubes and dilatations at the top the pollen tube. In case of the pollen with two tubes, the generative cell was cantoned in a single tube. These anomalies are not correlated with sucrose concentration in mediums or with the environmental pollution. But the frequency of anomalies was higher in *Coronilla varia* pollen (19%), as compared to *Lotus corniculatus* (8%) (*fig. 20-24*).

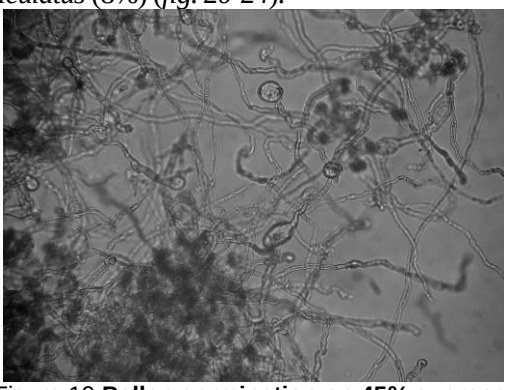

Figure 19 **Pollen germination on 45% sucrose medium, 24 hours after inoculation at *Coronilla varia* (100X) (Original)**

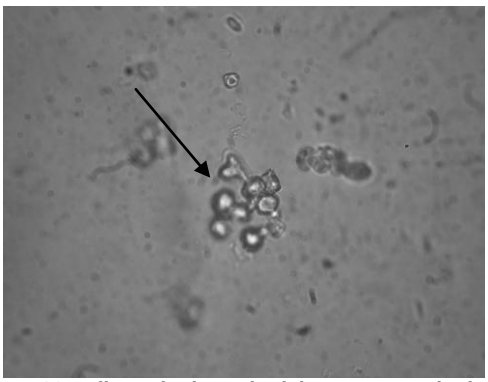

Figure 20 **Pollen tube branched, in *Lotus corniculatus* Arrow indicates the branching at the top of the tube (400X) (Original)**

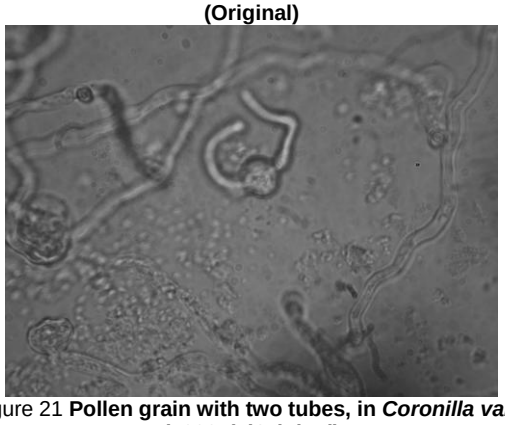

Figure 21 **Pollen grain with two tubes, in *Coronilla varia* (1000X) (Original)**

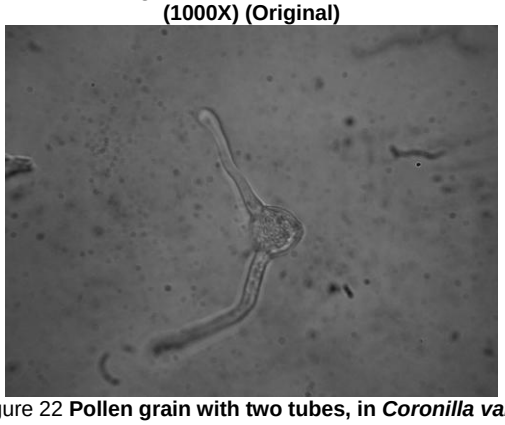

Figure 22 **Pollen grain with two tubes, in *Coronilla varia* (1000X) (Original)**

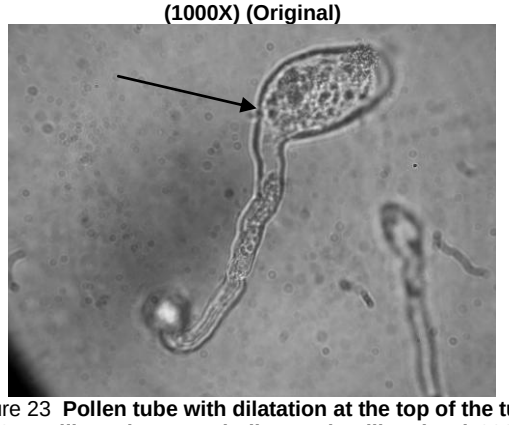

Figure 23 **Pollen tube with dilatation at the top of the tube in *Coronilla varia.* Arrow indicates the dilatation (1000X) (Orignal)**

Figure 24 **The dilatation at the top of the tube in *Coronilla varia.* Arrow indicates the dilatation (1000X) (Orignal)**

CONCLUSIONS

The pollen of *Lotus corniculatus* and *Coronilla varia* is bicellular.

The length of *Lotus corniculatus* and *Coronilla varia* pollinic tubes is, generally, positively correlated to the germination potential and especially, to the length of flower stiles.

The *Lotus corniculatus* and *Coronilla varia* pollen has the ability of making pollinic tubes on mediums which glucidic concentration reaches saturation. This demonstrates that the two taxons have a high native resistance at long term droughts.

Pollen tubes of *Lotus corniculatus* and *Coronilla varia* are viable "in vitro" up to seven days on mediums hyperconcentrated in sucrose.

In pollen germination process of *Lotus corniculatus* and *Coronilla varia* occur anomalies with nonsignificant frequency, that not influence very high germination potential of the two fabacee. These anomalies could be correlated to genetic origin of the two taxons.

The algorithm process of pollen germination at the two fabacee, the average of pollen tube length, the dynamics of pollen tube growth, the viability of the pollen tubes, the anomalies of pollen tubes were not affected by the polluted environment. This proves that all these issues have a strong genetic determinism.

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