Possibility of *Lupinus angustifolius* L. ovule development in *in vitro* cultures with applying wide crossing'

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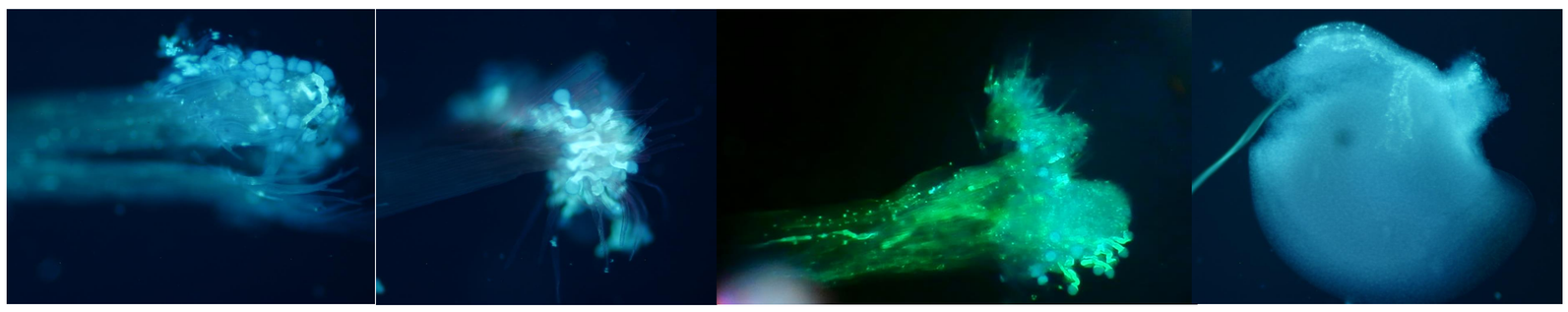
The aim of the study was to evaluate the development of the potential structures embryo sac of narrow-leafed lupin (2n = 40 chromosomes) after previous pollination using pollen grains originated from *Lupinis mutabilis* (2n = 48 chromosomes).
Four genotypes of narrow-leafed lupin were the objects of experiment: 'Graf', 'Emir', 'Karo' and line LAE-1. Presence on stigmas of *L. mutabilis* (*two genotypes* LM 13 and LM 34) pollen grains were checked. Germination of pollen grains tubes on stigmas and growing through the styles and reached ovules after 48 and 96 hours after pollination were observed.

Stigma of *L. angustifolius* after pollination

Narrow-leafed lupin pods - 4 days after pollination



The presence of pollen grains on the stigmas and germination

Growing of the pollen grains tubes through the style and reached ovary

LM 13

'EMIR'

LM 34

Pods before isolation of ovules

The ovules were isolated from young disinfected pods 7-10 days after pollination by *L. mutabilis* (LM13 or LM 34) and placed on two types of medium (P2_NLN, P3-ML6). Observations were performed after 7, 14, 21 and 30 days. The percent of ovules with different structure of tissue callus was founded. Development of three type of callus (I- needles, II - crystal, III hydrated) has been noticed. Callus measurements - height (H) and width (W) have been specified.

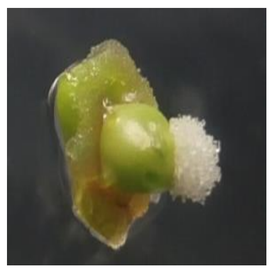
LM 13

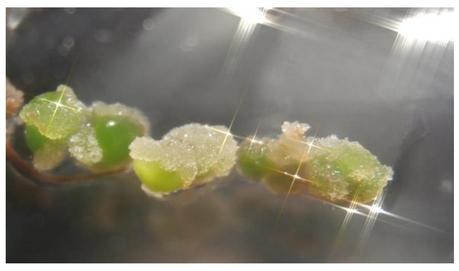
GRAF

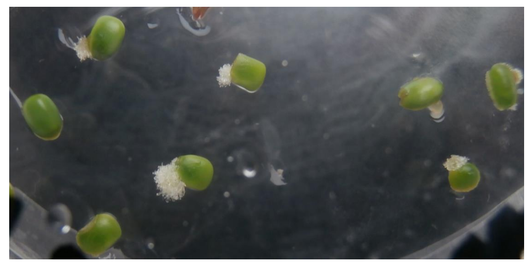
LM 34

Pods before isolation of ovules

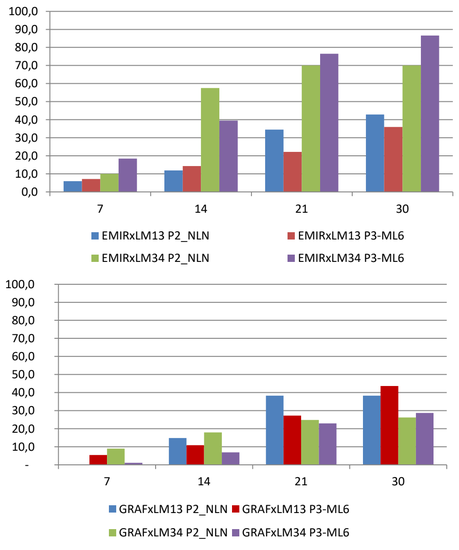
Regeneration of ovules after wide crossing



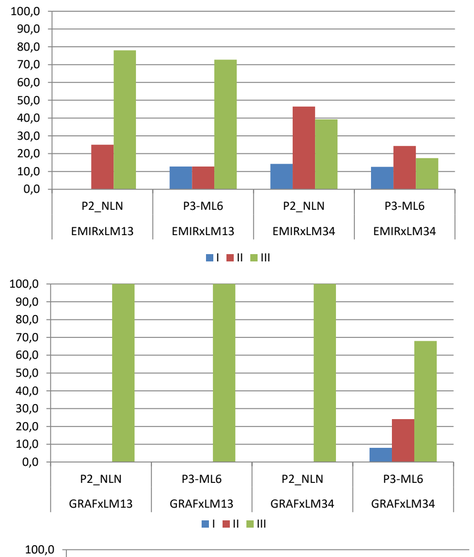




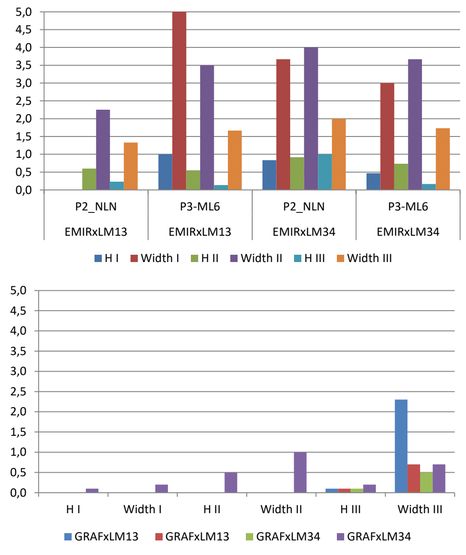
Figures representing % of ovules with callus (7, 14, 21 and 30 days)

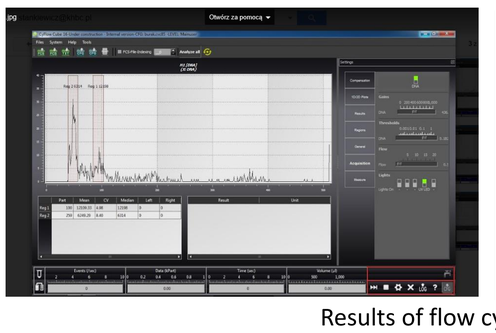


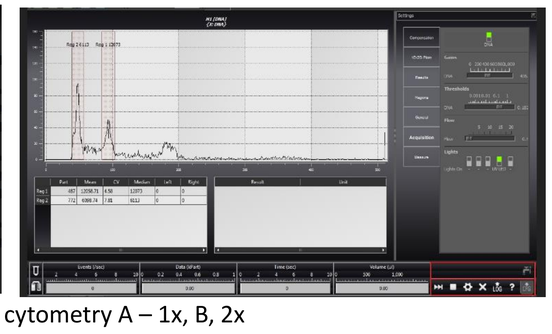
Figures representing % of type callus structures



Figures representing sizes of callus







Results of flow cytometry A – 1x, B, 2x

REASUME

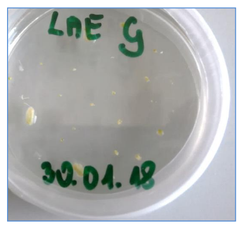
The use of wide crossing method has had positive influence on the development of pods. While isolated ovules showed induction of tissue callus. The ovules of *L. angustifolius* not treated with pollen of foreign species were characterised very rare regeneration.

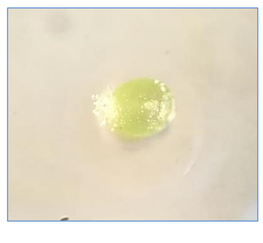
The LM34 pollen grains had a positive effect on the callus induction in the case of three analyzed forms of narrow-leafed lupin, especially in the Emir variety on both types of media. The weakest regeneration of callus tissue occurred in 'Graf'. Hydrated callus (type III) and crystal structure (type II) most often occurred in the analyzed explants.

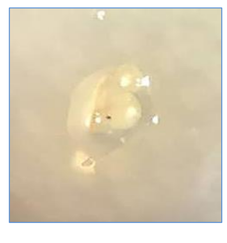
The results of the cytometric analysis indicated that in the most of regenerating ovules showed ploidy level 1x and 2x. Only in the case of regenerating ovules of the cv. Karo derived from wide crossing (LM13, on P2-NLN medium), the ploidy level was only 1 x.

The research will be continued to analyze the changes in the embryo sac, as a result of the wide crossing and further regeneration of callus tissue as well development of plants.

GYNOGENESIS







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