

*Morphogenesis potential of interspecific hybrid (*Lupinus albus*/*termis* x *Lupinus mutabilis* MUT.628) and its parents in the tissue culture*

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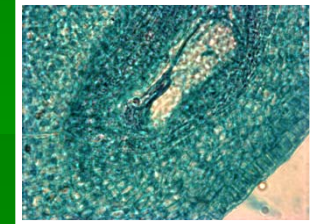
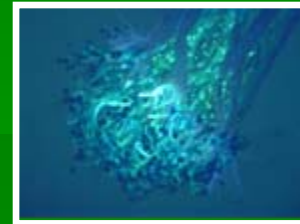


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*The possibilities of increasing the genetic variability in the genus *Lupinus* through the use of interspecific hybrids are reduced due to the differences in the number of chromosomes and existence of cytogenetic barriers developed in the course of evolution.*

The difficulties experienced during crossing make efforts to increase the genetic variability rarely successful. Research is pursued in search of suitable methods, in vitro culture among others, which would overcome the barriers mentioned as well as to obtain vital and fertile interspecific hybrids



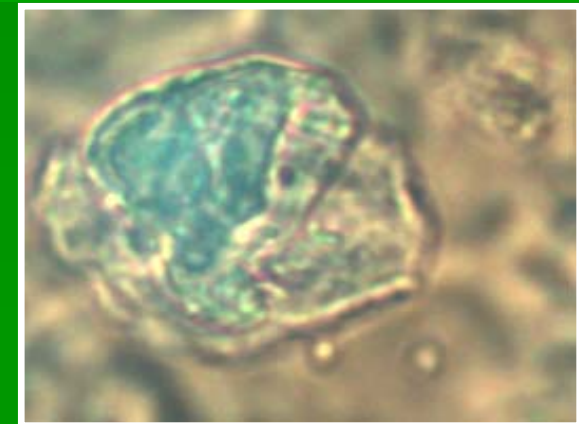
- *Application of tissue culture methods in the breeding of lupin could be a chance to expand the genetic variability through interspecific hybridization in the genus *Lupinus*, as it is the case in many other crop plants such as tobacco, cereals, fruit trees and horticultural plants.*



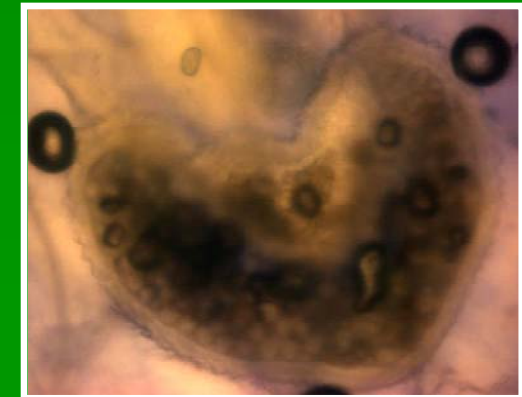
- *In spite of numerous studies on the lupin micropropagative abilities in in vitro culture, the plants that belong to the genus *Lupinus* are regarded as difficult to cultivate in tissue culture. Of the 300 species representing this genus only a few have so far been the subject of investigations which involve in vitro techniques.*



- *For regeneration of lupins in tissue culture the most frequently used parts have been apical and stem meristems, immature embryos, embryo axes, callus, hypocotyls, cotyledons and leaves, anthers, microspores, ovules or protoplasts*



- *Attempts to induce regeneration in hybrid embryos at different stages of development have also been undertaken (Przyborowski 2003, Kasten and Kunert, 1991, Kasten et al. 1991, Sonntag et al. 2005).*
- *As a source of explants, fully developed embryos have been acknowledged to be the most favourable stage.*



- *The aim of this study was to assess the capability for organogenesis of a hybrid*

L. albus/termis \times *L. mutabilis* Mut.628,

which was obtained in 1998 and propagated during a few subsequent years in field conditions,

and of its parental forms

L. albus/termis and L. mutabilis Mut.628 in meristem culture.

MATERIALS AND METHODS

- The material used for setting up the experiment were apical meristems, after sterilization placed on agar medium B₅, of the following:
- *Lupinus albus* var. *albus* Gladst. – *L. termis* Forsk., – population no. 095631 – Wiatrowo Plant Breeding Station
- *Lupinus mutabilis* Sweet MUT.628 – own mutant of the Department of Genetics, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences
- Interspecific hybrid – *L. albus* var. *albus* (*L. termis*) × *L. mutabilis* (MUT.628) – Department of Genetic, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences

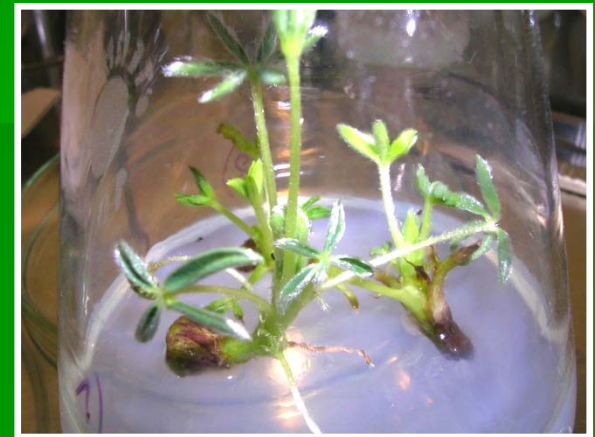


⇒ *After a week of growth, the explants were transferred onto medium B₅ (control) and B₅ containing growth regulators (mg dm⁻³):*

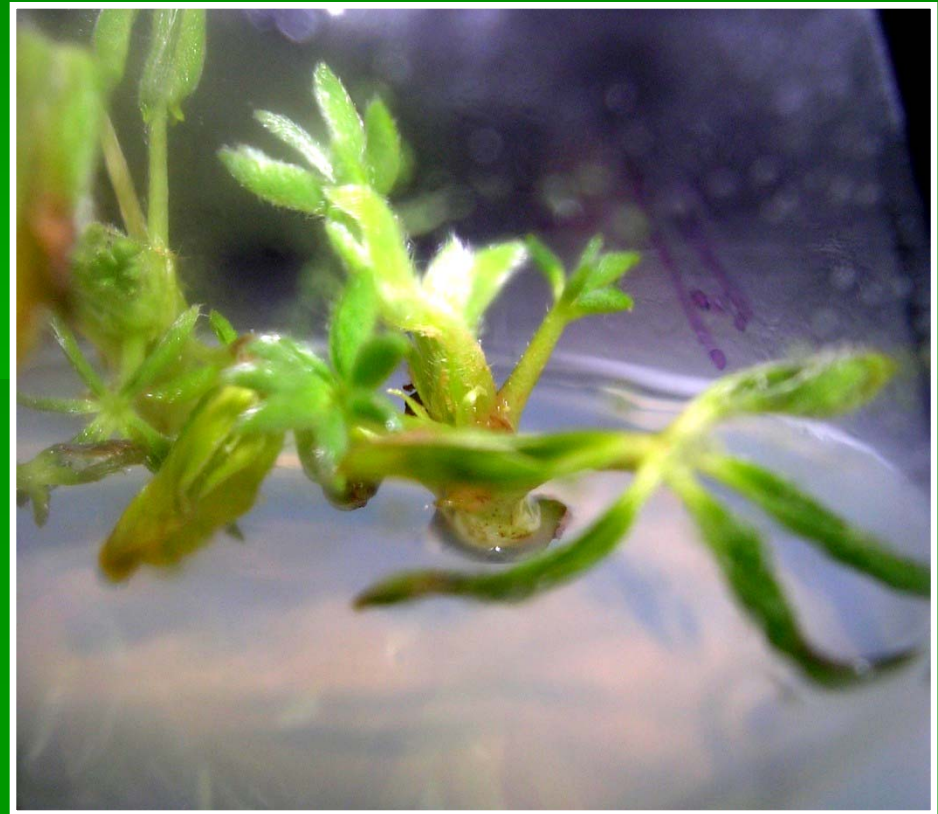
- *B₅ + 1.5 BA,*
- *B₅ + 1.0 NAA,*
- *B₅ + 3.0 IBA,*
- *B₅ + 1.5 BA + 1.0 NAA,*
- *B₅ + 1.5 BA + 3.0 IBA.*

⇒ *After four weeks, measurements of the developing meristems (the size of the explants with and without leaves) were performed and the number of leaves was determined.*

⇒ *Next, the developing plants were transferred onto the earlier used combinations of the medium – with the exception of the combinations containing NAA – in order to trace the process of morphogenesis.*



Results



Observation after four weeks of culture on B₅ medium with addition of chosen growth regulators

Medium	Genotype	A	B	C
B ₅	<i>L. albus/termis</i>	1.6	3.1	<u>6.1</u>
	<i>L. mutabilis</i> Mut.628	2.9	2.2	3.9
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	1.7	3.3	4.3
B ₅ +1.5 BA	<i>L. albus/termis</i>	2.2	3.9	4.4
	<i>L. mutabilis</i> Mut.628	<u>3.6</u>	<u>4.5</u>	1.7
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	1.9	3.3	4.5
B ₅ +1.0 NAA	<i>L. albus/termis</i>	1.4	3.1	4.8
	<i>L. mutabilis</i> Mut.628	-	-	-
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	0.8	2.2	4.0
B ₅ +3.0 IBA	<i>L. albus/termis</i>	<u>2.8</u>	<u>4.3</u>	3.5
	<i>L. mutabilis</i> Mut.628	1.5	2.5	4.0
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	2.6	3.3	3.3
B ₅ +1.5 BA+1.0 NAA	<i>L. albus/termis</i>	1.3	2.5	4.8
	<i>L. mutabilis</i> Mut.628	1.5	3.0	4.5
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	1.3	2.5	<u>4.8</u>
B ₅ +1.5 BA+3.0 IBA	<i>L. albus/termis</i>	2.2	2.9	2.75
	<i>L. mutabilis</i> Mut.628	2.0	3.7	4.3
	<i>L. albus/termis</i> x <i>L. mutabilis</i> Mut.628	3.2	4.4	<u>4.6</u>

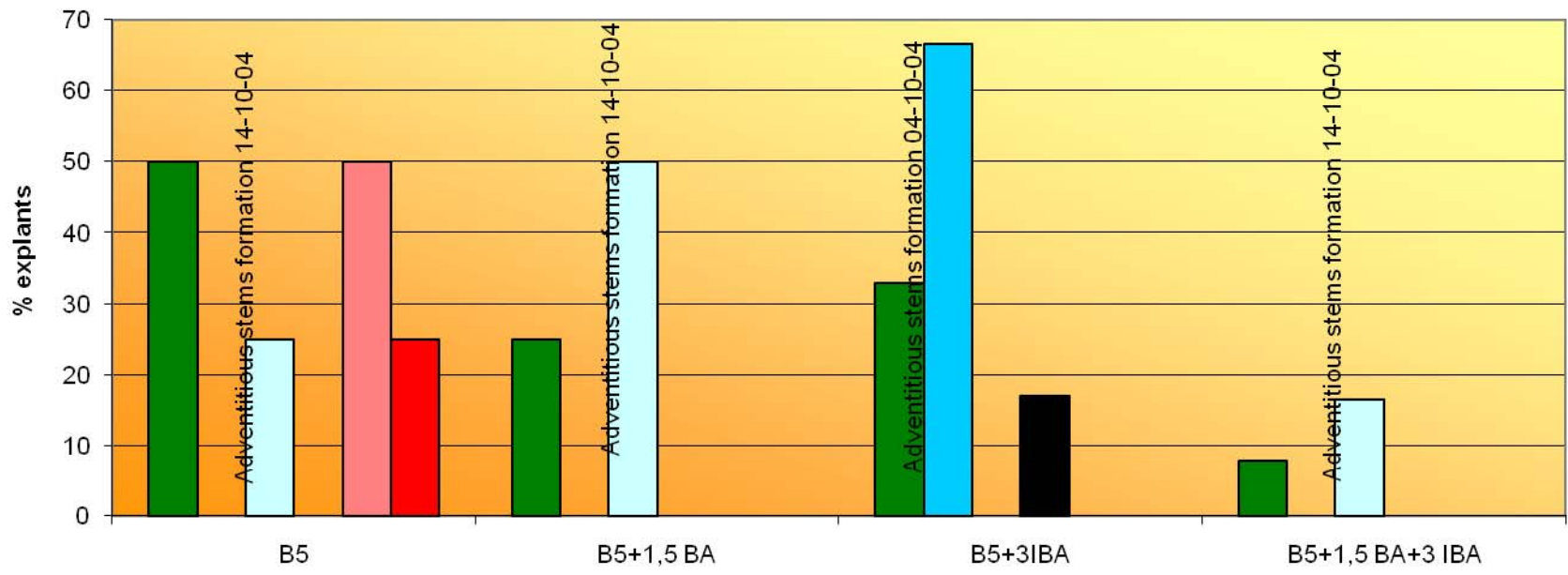


A - explants height without leaves (mean)

B - explants height with leaves (mean)

C - no. of leaves (mean)

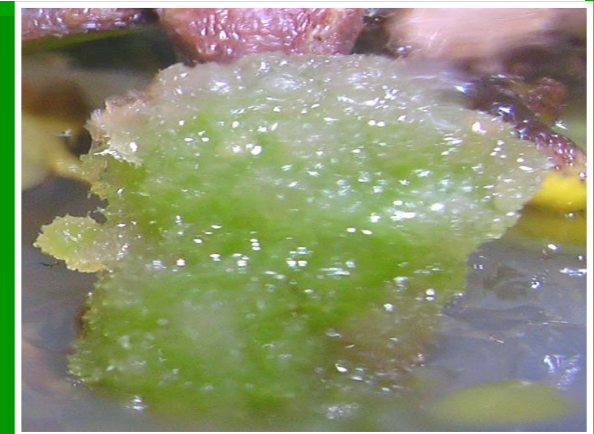
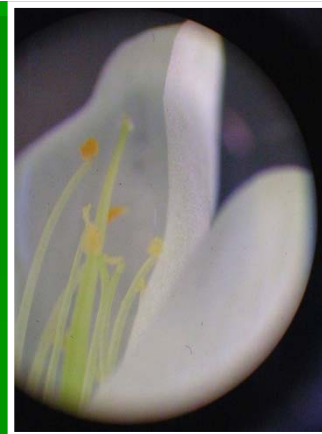
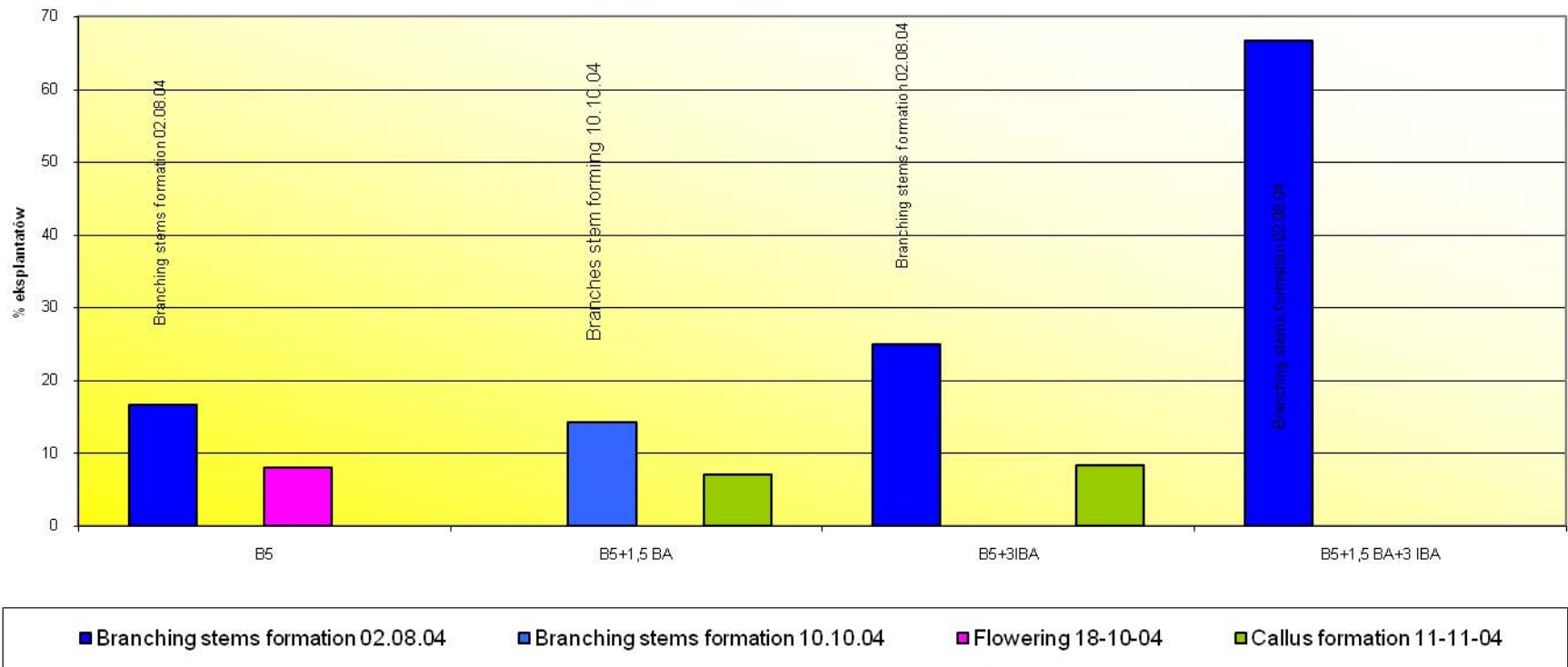
Fig.1. *L. albus/termis* regeneration on B5 medium with additional growth regulators after month earlier meristem culture

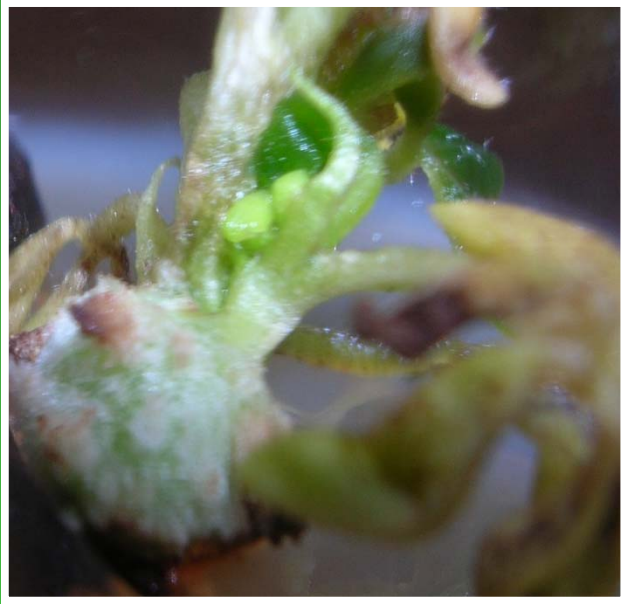


- 02-08-04 - Branching stems formation
- Adventitious stems formation 04-10-04
- Adventitious stems formation 14-10-04
- Rooting 14-09-04
- Flowering 30-08-04
- Pods formation



Fig.2. *L. albus/termis* x *L. mutabilis* Mut.628 regeneration on B5 medium with additional growth regulators after month earlier meristem culture





*Callus formation on medium B5 + 1.5 BA
L. albus/termis x L. mutabilis Mut.628*

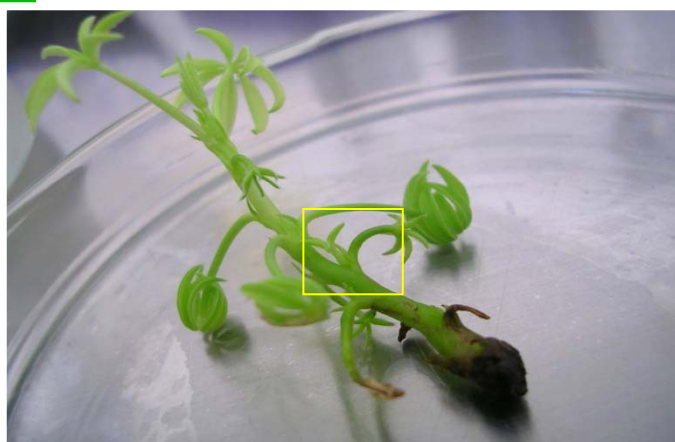
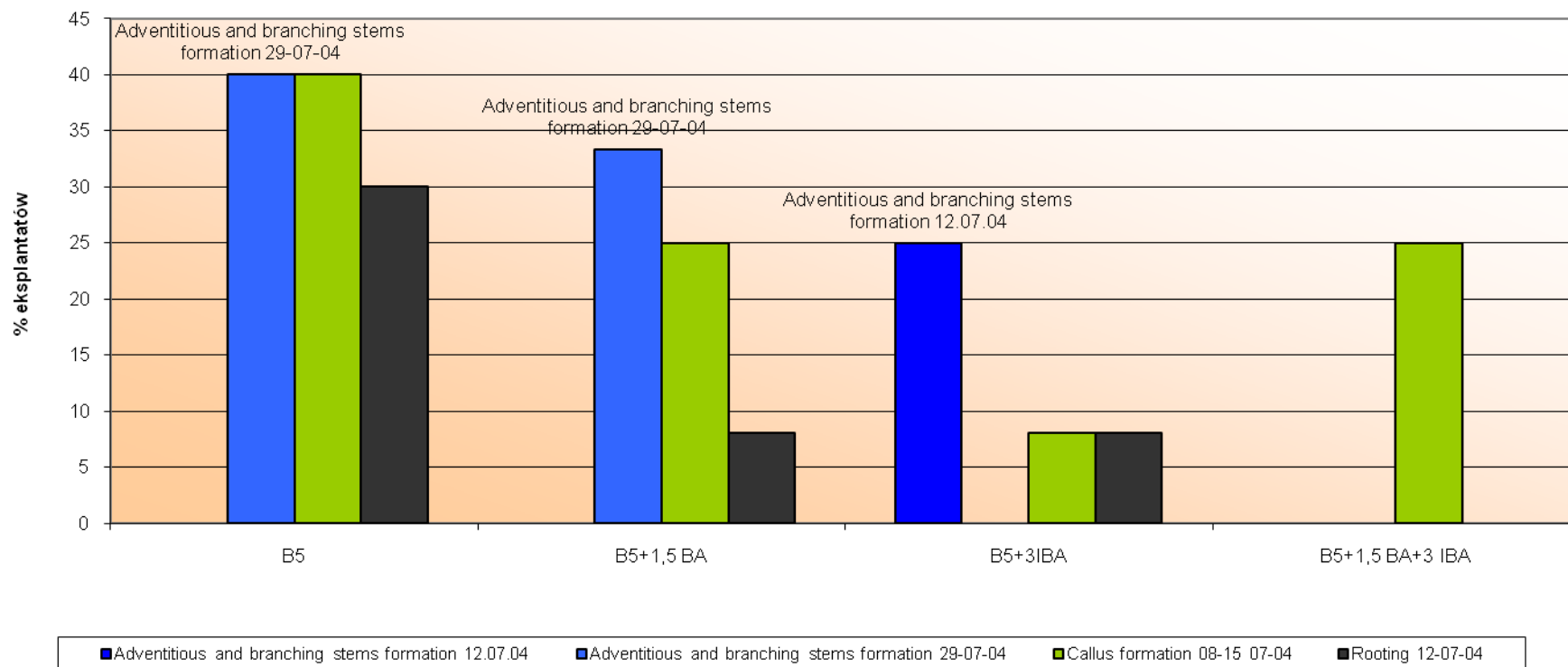


*Flowering plant on control medium B5 - L.
albus/termis x L. mutabilis Mut.628*



*Branching stems formation on medium B5+1.5 BA+3.0
IBA - L. albus/termis x L. mutabilis Mut.628*

Fig. 3. *L. mutabilis* Mut.628 regeneration on B5 medium with additional growth regulators after month earlier meristem culture



CONCLUSIONS

- *In long-term culture, explants of Lupinus termis were observed to flower as well as to produce branches and adventitious stems on basic medium B5, whereas on B5 + 3.0 mg L⁻¹ IBA complete regeneration occurred – development of roots, and branch and adventitious stems.*
- *In L. mutabilis MUT.628 full regeneration (development of stems, leaves and roots) was attained on: B5, B5 + 1.5 BA, B5 + 3.0 IBA (mg L⁻¹). For this form, production of a strong callus tissue was recorded.*
- *Explants of L. albus/termis x L. mutabilis MUT.628 regenerated best on medium B5 + 1.5 BA + 3.0 IBA (mg L⁻¹), although without developing roots. Like the maternal form, they flowered on the basic medium. These explants produced a callus tissue but not as strong as in the paternal form.*



*Thank you
for your attention!*



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