MORPHOGENESIS POTENTIAL OF INTERSPECIFIC HYBRID (*L UPINUS ALBUS* TERMIS X *L UPINUS MUTABILIS* MUT. 628) AND ITS PARENTS IN THE TISSUE CULTURE

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ABSTRACT

The aim of these studies was to determine conditions for organogenesis of hybrid (*L. termis* x *L. mutabilis* Mut.628) and its parents in in vitro culture of meristems. The apical meristems were used for tissue culture. Sterilised explants were placed on the medium B^5 containing growth regulators: B_5+1.5 BA, B_5+1.0 NAA, B_5+3.0 IBA, B_5+1.0 NAA. B_5+1.5BA + 3.0 IBA (expressed in mg L^{-1}). The genotypes studied showed different reactions of organogenesis to changed tissue culture conditions. In long-term culture of meristems of the *L. termis* explants auxiliary and adventitious shoot and flower development was observed on the B_5. Complete morphogenesis (roots, lateral and adventitious shoots development) was observed on the medium containing auxin (B_5+3.0 IBA). Plant regeneration was also observed (shoots, leaves and roots) in the meristems culture of *L. mutabilis* Mut-628 on media B_5, B_5+1.5 BA and B_5+3.0 IBA. In this species a strong callus was formed. Meristems explants of the hybrid were regenerated. The hybrid showed morphological affinities to the maternal parent, but some significant differences were also observed. The best shoot development was observed on the medium with growth regulators (B_5+1.5 mg L^{-1} BA + 3.0 mg L^{-1} IBA), but no roots formation was noted. On the B_5, both the hybrid and the maternal parent produced flowers. The hybrid produced callus but less abundantly than the male parent.

KEY WORDS

Lupinus albus, *Lupinus mutabilis*, interspecific hybrid, tissue culture

INTRODUCTION

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 (Babaoğlu, 2000, Przyborowski, 1994; Przyborowski and Packa, 1997, Przyborowski 2003, Sawicka *et al*. 2006). Application of tissue culture methods in the breeding of lupin could be a chance to expand the genetic variability through interspecific hybridisation 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 (Nadolska–Orczyk, 2000, Przyborowski, 2003 and Rybczyński, 2001). 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 (Babaoğlu, M., 2000; Rybczyński, 2001).

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. termis* x *L. mutabilis* Mut.628, which was obtained in 1998 and propagated during a during a few subsequent years in field conditions, and of its parental forms *L. albus*/termis and *L. mutabilis* Mut.628 in meristem culture

MATERIAL AND METHODS

The material used for setting up the experiment were apical meristems, after sterilisation placed on agar medium B_5, of the following: *Lupinus alb*us var. albus Gladst. – *L. termis* Forsk. – population No. 095631–

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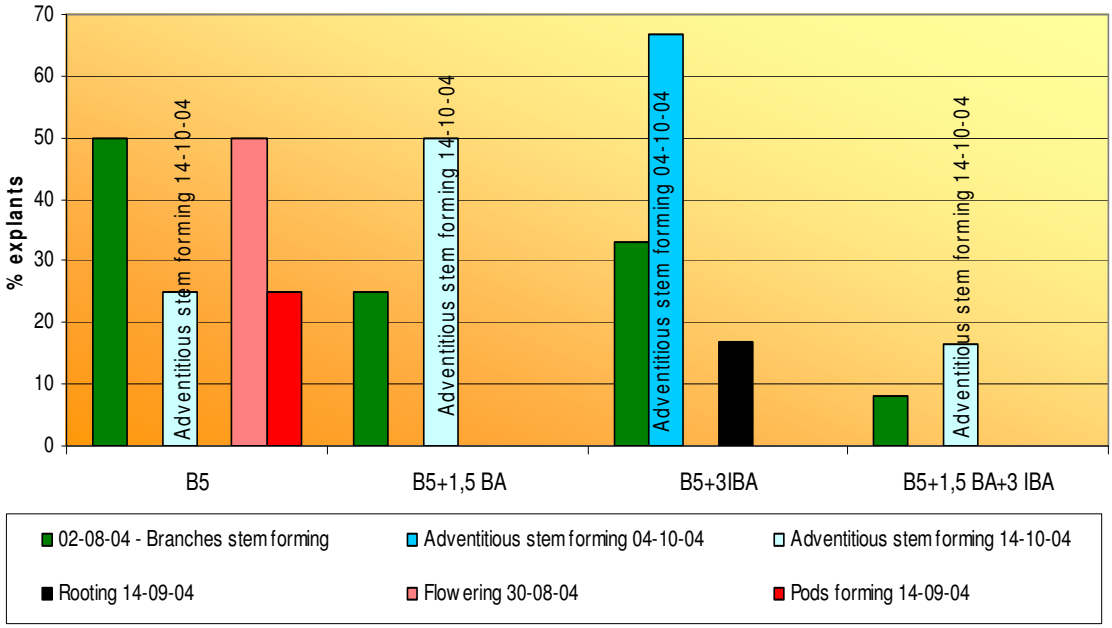

Fig. 1. *L. albus/termis* regeneration on B_5 medium with additional growth regulators after month earlier meristem culture.

Fig. 2. Flowering plants on B5 medium – *L. albus/termis*.

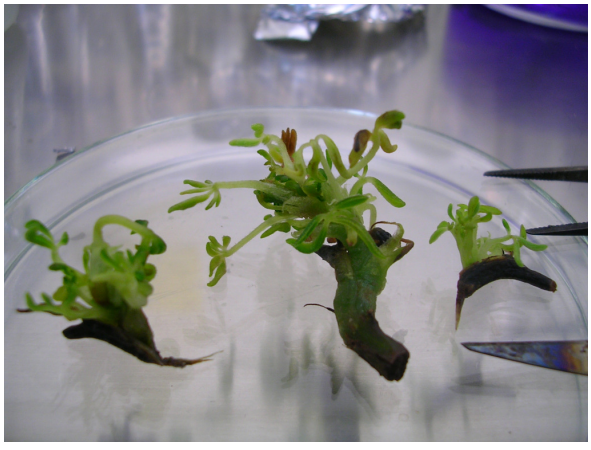

Fig.3. Adventitious stem forming of *L. albus/termis* plants on B5+3IBA medium.

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.* termis x *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 Wiatrowo Plant Breeding Station *Lupinus mutabilis* Sweet MUT.628 – own mutant of the Department of Genetic, Plant Breeding and Seed Production, Wrocław University of Wiatrowo Plant Breeding Station *Lupinus mutabilis* Sweet MUT.628 – own mutant of the Department of Genetic, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences. Interspecific hybrid – *L. albus* var. *albus* (*L. termis*) x *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 measured and transferred onto medium B_5 (control) and B_5 containing growth regulators (mg dm-3): B_5 + 1.5 BA, B_5 + 1.0 NAA, B_5 + 3.0 IBA, B_5 + 1.0 NAA. B_5 + 1.5BA + 3.0 IBA. The meristems were put into flasks, 5 specimens in each. Thirty explants fell on each combination of the medium. 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. After observations, 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

After a week of development on the basic medium B_5, the highest growth was recorded for the hybrid *L. termis* x *L. mutabilis* MUT.628 and for *L. termis*. In the paternal form *L mutabilis* MUT.628 the size of explants did not exceed 0.$_5$ cm. In explants of all three objects, formation of the first leaf was observed on the control medium. After four weeks of culture, for all analysed objects the greatest growth dynamics was recorded on the basic B_5 medium and also on this medium with the addition of 1.5 IBA combined with 1.0 NAA (mg L^{-1}). The mean size of explants with leaves, cultured on the above mentioned substrata, ranged from 2.7 to 3.5 cm, the number of leaves reaching 5. In the case of explants of *L. mutabilis* Mut.628 put on the medium with 1.0 NAA added, the meristem culture was found decaying. Except for the medium containing 1.5 BA, on the remaining combinations the Andean lupin meristems displayed a comparable growth relative to that on the control medium. The meristems of *L. termis* showed the poorest growth on the medium with 1.5 BA and 3.0 IBA (mg L^{-1}).

When tracing further the course of regeneration of *L. termis* on different combinations of medium B_5, appearance of branch stems was noted after five weeks of culture, whereas adventitious stems developed after a considerably longer period of three months (Fig. 1). In 68% of the studied explants, development of numerous adventitious stems was noted for the medium with auxin (B_5 + 3.0 IBA), and 17% of the explants on this particular combination successfully rooted. On the basic medium B_5, in 50% of the *L. termis* explants flowering was observed (Figs 1, 2, 3).

The subsequent phases of regeneration in *L. mutabilis* MUT.628 (Fig. 8) differed from the course of this process in *L. termis* (Fig. 1). Flowering was not recorded for any combination of the medium with growth regulators, adventitious stems appearing only in 8% of the explants on the medium with cytokinin (B_5 + 1.5 BA). On the other hand, most explants of this object produced branch stems on the basic medium B_5, on B_5 + 1.5 BA and B_5 + 3.0 IBA. On the substrata mentioned, adventitious roots developed, although the greatest number of explants (30%) rooted on the basic medium B_5. Also, abundant production of the callus tissue was noted for these combinations of the medium (Figs 8, 9, 10).

Observations of explants of the hybrid *L. albus/termis* x *L. mutabilis* MUT.628 during further regeneration (Fig. 4) clearly revealed similarity in the course of this process as compared with the maternal form *L. termis* on medium B_5, where flowers and branch stems were produced. Like in the paternal form *L. mutabilis* MUT.628, appearance of the callus tissue was observed (Figs 5, 6, 7).

These experiments resulted in fully regenerated plants of parental forms were obtained in meristem culture. The rooting process had to be induced in vivo only in the case of the hybrid since this form had not developed roots in vitro. Although regeneration of roots in the genus *Lupinus* can easily be induced, each of the species may require a different combination of growth substances added to the medium (Rybczyński, 2001). For initiation of lupin rooting, Rybczyński and Podyma, (1993) used medium B_5 with an addition of activated carbon and then transferred the explants to perlite, thus obtaining rooted plants.

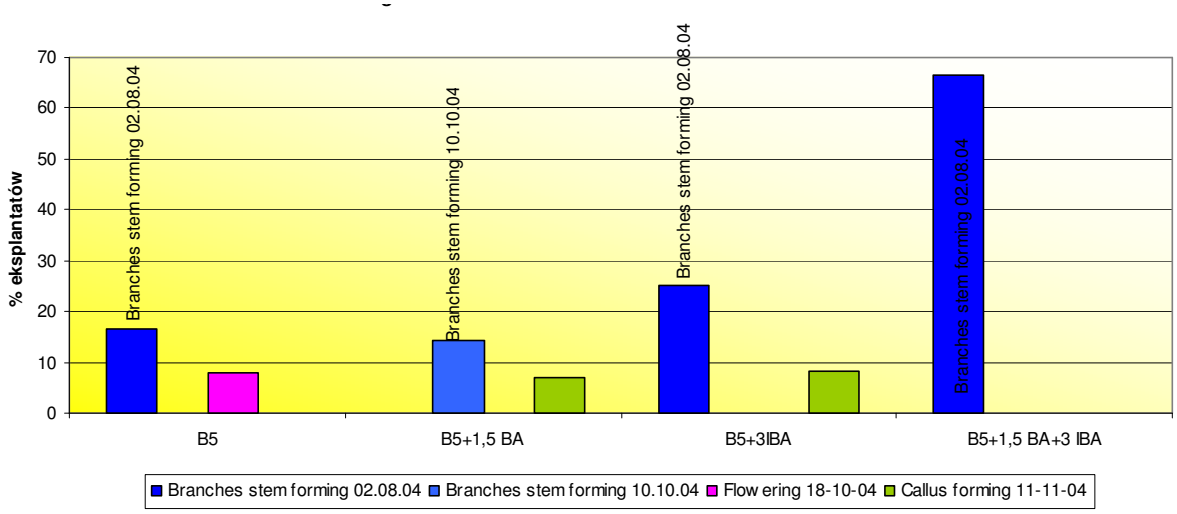

Fig. 4. *L. albus/termis* x *L. mutabilis* Mut628 regeneration on B_5 medium with additional growth regulators after month earlier meristem cultire.

Fig. 5. Callus forming on medium- B_5 + 1,5 BA – *L. albus/termis* x *L. mutabilis* Mut.628.

Fig. 6. Branches stem forming on medium B_5+ 1,5BA+3,0 IBA-*L. lbus/termis* x *L. mutabilis* Mut.628.

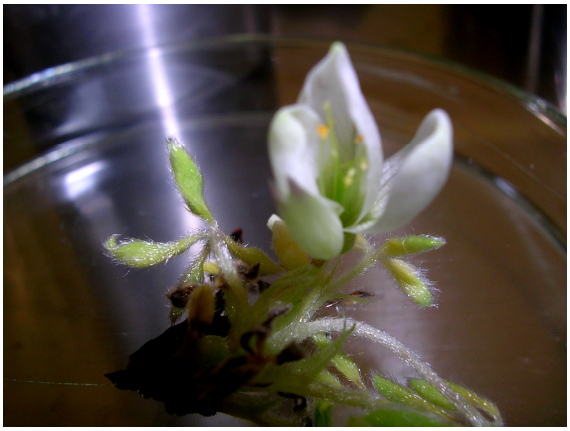

Fig. 7. Flowering plant on based medium B_5 – *L. lbus/termis* x *L. mutabilis* Mut.628.

This research highlighted different reactions of the studied genotypes to different media used in in vitro cultures (Kasten and Kunert, 1991; Przyborowski, 1994; Rybczyński, 2001; Zgagacz and Rybczyński, 1994; Sawicka *et al*. 2006). Flowering of regenerated plants in meristem culture indicates a possibility of inducing regeneration of interspecific hybrids in in vitro culture.

CONCLUSIONS

In long-term culture, explants of Lupinus termis were observed to flower as well as to produce branch and adventitious stems on basic medium B_5, whereas on B_5 + 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: B_5, B_5 + 1.5 BA, B_5 + 3.0 IBA (mg L^{-1}). For this form, production of a strong callus tissue was recorded.

Explants of *L. termis* x *L. mutabilis* MUT.628 regenerated best on medium B_5 + 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.

LITERATURE CITED

Babaoğlu, M. 2000. Protoplast isolation in Lupin (*Lupinus mutabilis* Sweet): determination of optimum explant sources and isolation conditions. Turk. J. Bot. 24: 177-185.

Kasten, W. and R. Kunert. 1991. A culture method for isolated immature embryos of different *Lupinus* species. Biologische Zentralblatt 110: 290–300.

Kasten, W., T. Paradies, R. Kunert and P. Straka, 1991. Progress in realisation of interspecific hybrids in the genus *Lupinus* by an embryo rescue technique. Biologische Zentralblatt 110: 301–309.

Nadolska-Orczyk, A. 2000. Regeneracja *in vitro* i transformacja genetyczna roślin motylkowych grubonasiennych. Monografie i Rozprawy Naukowe IHAR 6: 9–63.

Przyborowski, J.A. 1994. Kultura i*n vitro* dojrzałych zarodkow i roslin *Lupinus albus* L., *L. angustifolius* L., *L. mutabilis* Sweet. Prace Ogrodu Botanicznego PAN Zeszyt 5/6: 457–464.

Przyborowski, J.A. and D. Packa. 1997. Embryo development after interspecific hybridisation of *Lupinus albus* L., *L. mutabilis* Sweet and *L. angustifolius* L. J. App. Genet. 38 (2): 131–141.

Przyborowski, J.A. 2003. Pre i postzygotyczne bariery przy krzyzowaniu wybranych gatunkow z rodzaju *Lupinus.* Wydawnictwo UWM Olsztyn.

Rybczyński, J.J. 2001. Biotechnologia lubinów (Przegląd literatury). Zeszyty Naukowe AR Wrocław 427: 93–114.

Rybczyński, J.J. and E. Podyma. 1993. Micropropagation of some *Lupinus* species from seedling explants. Genetica Polonica 34(3): 237–247.

Sawicka-Sienkiewicz, E., M. Buzar and R. Galek. 2006. Rozwój izolowanych dojrzałych zarodków mieszańcowych *Lupinus albus* sensu lato x *Lupinus mutabilis* i ich form rodzicielskich w kulturze *in vitro*. Pod red: *W. Sodkiewicz, T. Sodkiewicz i M. Surma,* PAGEN – Centre of Excellance in Plant Agrobiology and Molecular Genetics, Mieszańce Oddalone Roślin Uprawnych, 2006, Vol. 7, 177–184.

Sonntag. K., E. Rudloff and B. Ruge-Wehling. 2005. Wide crosses to improve pH-tolerance in narrow-leafed lupin. Proc.11th International Lupin Conference, Guadalajara, Mexico 4-9 May 2005: 17-19.

Zgagacz, S.E. and J.J. Rybczyński. 1994. Wykorzystanie niedojrzałych zarodkow lubinu zmiennego (*L. mutabilis* Sweet) do wegetatywnego mnozenia. Prace Ogrodu Botanicznego PAN Zeszyt 5/6: 445–4.