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OPTIMIZATION OF LINSEED FLAX (*LINUM USITATISSIMUM* L.) *IN VITRO* CULTURES

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Abstract

Linseed (*Linum usitatissimum* L.) is an important crop in Lithuania used for oil and fibre production. However, no Lithuanian cultivars of linseed have been developed yet. The breeding program of linseed was started at the Agrobiotechnology Laboratory of the Lithuanian University of Agriculture in 2002. The objective of our experiments was to study the effects of various factors influencing linseed flax morphogenesis *in vitro*.

Different media compositions were employed to develop regenerants from various somatic tissues. Our study demonstrated that hypocotyls and stem segment of linseed flax are competent for adventitious shoot organogenesis.

The effect of genotype, growth regulators and preconditioning of donor plants on callus induction in anther and ovary cultures of flax was investigated. Our study indicates that there is a strong genotype effect on callus production from generative explants in flax, and therefore specific medium composition must be designed for each genotype in order to elicit optimum results.

Optimized somatic and generative tissue techniques will be used for creation of initial breeding material of linseed flax.

Key words: linseed flax, morphogenesis, somatic and generative tissues.

Introduction

Breeding of linseed flax is practiced in many countries of the world: India, Hungary, Uzbekistan, Argentina, Russia, Canada, Romania, France, Finland, Czech, Ukraine. In Lithuania field tests of the linseed flax cultivars were started in 1998 at the Upyte Experimental Station of the Lithuanian Institute of Agriculture. Breeding of flax even nowadays is a long and complicated process, based on interspecific hybridization and selection of the best plants, therefore the development of genetically stable lines takes a very long time – 10–12 years /Chen et al., 1998/. Only advanced selection technologies can help to develop new cultivars, valuable for the local industry, which could open a new market for this crop. Developing genetic diversity of the initial genetic material of plants, the role of biotechnology methods in plant selection is constantly increasing. Tissue culture technologies in flax selection may be used seeking to provide cultivars with new and useful characteristics (resistance to diseases, improved oil quality and tolerance to herbicides) through somatic hybridization and somaclonal variation

/Marshall, Courduries, 1992; Rutkowska-Krause et al., 2003/. One of the most perspective biotechnological methods, used in plant selection, is anther culture.

All biotechnological approaches like genetic engineering, haploid induction, or somaclonal variation to improve traits of important crops strongly depend on an efficient recovery of plants through *in vitro* systems. However, the successful application of these methods is determined by the genotype, donor plant quality, developmental stage of explant, and composition of the culture medium resulting in inefficient *in vitro* culture protocols /Chen, Dribnenki, 2002; Mundhara, Rashid, 2002; Tang et al., 2003/. Therefore, it is important to study biological requirements for *in vitro* system of plants and to select conditions ensuring a sufficient output of plant regenerants of desirable genotypes.

This paper presents prime results obtained with linseed flax somatic and generative cultures at the Lithuanian University of Agriculture. Furthermore, improved *in vitro* culture methods for callus initiation and plant regeneration are described.

Somatic tissue culture

In the process of *in vitro* regeneration the growth of easy dividable embryogenic callus is very important. Morphogenetic process *in vitro* in tissue cultures is determined by several factors. The most important are the genotype and the ratio of auxins and cytokinins in the induction medium /Dewitte, Murray, 2003/.

In most cases for somatic tissue culture of linseed flax are used cotyledons, hypocotyls, meristems and stem segment as explants /Friedt, 1990/. In our studies we used leaf, stem segment and hypocotyls of linseed flax cultivars 'Lirina', 'Barbara' and 'Szaphir'. Among the tested explants, the leaf tissue showed the highest potential for cell dedifferentiation. The tested genotypes had different intensity of callus induction. Cultivar 'Barbara' had the highest frequency of callus formation, which was obtained in all media treatment, where 100 % isolated explants had formed callus. The complex MSB_5 (Murashige and Skoog (MS) and Gamborg (B_5)) medium with 1.0 mg l^{-1} kinetin was the most suitable for callus induction for all tested genotypes. On this medium stem segments and hypocotyls of all tested genotypes were established to have the highest potential of callogenesis. Hypocotyls from all tested genotypes had lower responsibility of callus induction than stem segments on all media types /Blinstrubienė et al., 2004a; Blinstrubienė et al., 2004b; Blinstrubienė et al., 2004c/.

Depending on the genotype and explant type, organogenesis started on the 21–28 day after explant isolation. Leaves-derived callus did not form any organogenic structure and subsequently became necrotic. Organogenesis has been observed only from hypocotyls-derived and stem segment-derived callus. The shoots were produced spontaneously from the green soft callus with or without subculture onto fresh medium. Murashige & Skoog (MS) and Gamborg (B_5) nutrient media are the widest and most successfully used nutrient media *in vitro* /Son, Bhojwani, 1999/. Our comparison of these media and their complex MSB_5 revealed that the most intensive rhizogenesis and shoot regeneration was observed in MSB_5 nutrient medium /Blinstrubienė et al., 2004b/.

The process of organogenesis appears to be complex, involving multiple internal and external factors. The reinitiating of cell division, considered one of the key factors during regeneration, appears to be controlled differently depending on the various model systems. The type of first division under inductive conditions can be different /Blervacq

et al., 1995/, often depending on growth regulators in the culture medium and the type of the primary explant used /Dedicova et al., 2000; Bonell, Lassaga, 2002/. Many plant species require both exogenous auxin and cytokinin in suitable balances in order for shoot formation to occur. The maximum shoot regeneration frequency in *Brassica* species was obtained in medium supplemented with 3.0 mg l^{-1} BAP and 0.15 mg l^{-1} NAA /Tang et al., 2003/. Hypocotyls of *Beta vulgaris* showed the best response on adventitious shoot regeneration in medium supplemented by BAP and NAA /Zhang et al., 2004/. The medium containing the combination of 2.0 mg l^{-1} 2,4-D and 1.0 mg l^{-1} BAP significantly improve shoot regeneration in anther culture of flax /Chen et al., 1998/. However, shoot formation on hypocotyls of *Linum* seedlings was marginally promoted by 6-benzylaminopurine (BAP) or thidiazuron (TDZ) /Mundhara, Rashid, 2002/. In our experiments we investigated effect of BAP, 2iP, kinetin, IAA and NAA on morphogenesis of linseed flax. According to some researchers for flax shoots induction *in vitro* nutrient medium should be supplemented by auxin and cytokinin at low ratios /Cunha, Ferreira, 1996/. However in our experiments the development of adventitious shoots of tested linseed cultivars was promoted by a low concentration of 2iP. Besides, our studies determined that kinetin, at least at concentration above 1.0 mg l^{-1}, inhibits shoots regeneration from linseed flax callus. In fact, the kinetin in combination with 0.1 mg l^{-1} IAA had lower inhibitory effect on the development of shoots /Blinstrubienė et al., 2004b/.

The same media were employed to induce callus and regeneration from stem segments and hypocotyls. Generally stem segments produced more callus than the hypocotyls, however hypocotyls-derived callus had better morphogenetic abilities in comparison with stem segment-derived callus /Blinstrubienė et al., 2004c/. Successful shoot regeneration was found to be dependent on genotype and culture media. This is in agreement with the results of other published work /Nichterlein et al., 1991; Chen et al., 1998/.

The three tested linseed flax cultivars exhibited different regeneration responses. The genotype ‘Szaphir’ demonstrated the highest frequency of morphogenesis on all analyzed types of nutrient media, in comparison with ‘Barbara’ and ‘Lirina’. It can be assumed that the differences in morphogenetic reaction of different linseed flax genotypes are determined by the balance of endogenous hormones. Combinations of growth regulators optimal for callus induction, root formation and shoot regeneration were different.

The number of shoots per explants was significantly affected by the culture media. The largest number of shoots was produced in MSB_5 supplemented with 2.0 mg l^{-1} 2iP. A smaller concentration of cytokinins reduced the number of regenerated shoots. Cultivar differences were significant for the shoots number per explants. Hypocotyls-derived callus from the cultivar ‘Szaphir’ gave the best results, while the cultivar ‘Lirina’ had the lowest organogenic response /Burbulis et al., 2005b/.

The age of explant also affected shoot regeneration in linseed flax. Four-weeks-old callus showed the maximum shoot regeneration frequency. The shoots regeneration frequency decreased with the increase in explant age from 4 to 36 weeks. In fact, the decreasing of regeneration frequency of stem segment-derived and hypocotyls-derived callus showed no obvious difference, except for cultivar ‘Szaphir’. Organogenesis

potential of hypocotyls-derived callus of this cultivar was reducing slower – shoot formation frequency from twenty-weeks-old callus was only 20 % less than that from four-weeks-old callus. Among the tested cultivars morphogenetic potential was most rapidly lost by the callus of the cultivar 'Lirina' /unpublished data/.

For rhizogenesis induction nutrient media are often modified by adding different contents of vitamins and growth regulators. The scientists have no common opinion on this issue as some of them recommend for rooting *in vitro* medium without growth regulators, whereas others advise to use auxins, mostly IAA or NAA /Rutkowska-Krause et al., 2002/. Our experiments showed that linseed rhizogenesis mainly depends on the concentration of micro and macro elements in regeneration medium. The most intensive rhizogenesis process of tested genotypes was observed when the concentrations of macro and micro salts were reduced by 50 %. In this case 45 % of shoots formed roots. Further lowering of macro and micro salts concentration decreased the shoots rooting even 6 times /Blinstrubienė et al., 2004b/.

Regenerants were acclimatized to greenhouse conditions and resembled true linseed flax in growth habit.

Generative tissue culture

The physiological conditions under which donor plants are grown may influence the final number of haploid embryos from cultured anthers. Varying temperature and light conditions during the growth and development of donor plants also affect anther response. Nichterlein with co-authors recommended growing flax donor plants at 14/8 °C day/night temperature /Nichterlein et al., 1991/. Tejklova reported that a minimal day temperature above 15 °C and a maximal day temperature above 30 °C completely stopped callogenesis in anthers /Tejklova, 1996/. We demonstrated a clear relationship between the specific temperature in which anthers were developing on donor plants and the subsequent effect on callogenesis in isolated anthers. It was found that a temperature even few degrees lower (from 22/18 ^{o}C to 18/14 ^{o}C) was favourable for anther callogenesis /Burbulis et al., 2005a/.

The presence of an appropriate concentration of growth regulators in the medium plays a critical role in callus formation in anther culture. Some genotypes responded quite well to different combinations of auxins and cytokinins /Chen, Dribnenki, 2002; Chen et al., 2003; Rutkowska-Krause et al., 2003; Soroka, 2004/. The reports available so far on anther culture suggest that, in the majority of cases, an auxin or/and cytokinin was been required as a component of the medium. Growth regulators have widely been used for callus enhancement in anther culture of flax. A significant effect of the combination of 1.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA on callus formation in flax anther culture has been reported by Obert with co-authors /Obert et al., 2005/, while higher callogenesis in flax anther in medium with 1.0 mg l^{-1} BAP and 2.0 mg l^{-1} 2,4-D was observed in experiments reported by Chen with co-authors /Chen et al., 1998/. In our study, the combination of 1.0 mg l^{-1} BAP with 2.0 mg l^{-1} 2,4-D in induction medium produced the higher percent of callus in the cultivar 'Mikael', F_1'Barbara' x 'Lirina' and F_1'Lirina' x 'Barbara'. This combination of growth regulators can be used for more efficient callus production also in 'Lirina', 'Barbara' and 'Szaphir' when the induction medium is supplemented with an increased level (9 % or 12 %) of sucrose. Furthermore,

results show that the auxin, 2,4-D, can be successfully replaced by IAA in the induction medium with 6 % sucrose for genotypes 'Atalante' and 'Szaphir'. However, anther of 'Lirina', F_1'Barbara' x 'Mikael' and F_1'Mikael' x 'Barbara' showed the better response on medium supplemented with 2.0 mg l^{-1}BAP and 1.0 mg l^{-1} NAA. Response to androgenesis for a number of crops including flax is known to be strongly genotype dependent and influenced by numerous exogenous factors. Our study showed significant variation in callus producing ability between the genotypes. The cultivar 'Mikael' was found to have the highest callus induction rate, while cultivar 'Barbara' was only able to produce calli in medium supplemented with 1.0 mg l^{-1} BAP and 2.0 mg l^{-1} 2,4-D containing 9 % sucrose. Variability in callogenesis and differences in growth regulators necessary for callus production in each genotype suggest that explants have different endogenous hormone levels. Differences in responsiveness among flax genotypes were also found by another research groups /Chen, Dribnenki, 2002; Rutkowska-Krause et al., 2002; Obert et al., 2004/. This suggests that growth regulator combinations and sucrose levels in induction medium for flax anther culture must be modified and optimized for specific flax genotypes within each particular breeding program. We made crossing experiments involving responsive ('Mikael' and 'Lirina') and poor/non-responsive ('Barbara') genotypes. This study showed that appropriate combination of growth regulators for hybrids and their parental form is different. Anther of cultivar 'Lirina' showed higher level of callogenesis on medium with 2.0 mg l^{-1}BAP and 1.0 mg l^{-1} NAA, while reciprocal hybrids showed the better response on medium containing 1.0 mg l^{-1} BAP and 2.0 mg l^{-1} 2,4-D. In contrast, cultivar 'Mikael' showed the higher value of induced anthers on medium with 1.0 mg l^{-1} BAP and 2.0 mg l^{-1} 2,4-D, whereas combination 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA promoted callus formation in anthers of this genotype reciprocal hybrids /Burbulis et al., 2005a; Burbulis, Blinstrubiene, 2006/.

The concentration of carbohydrates is also a very important factor directly influencing anther culture. Sucrose has generally been used as the major carbohydrate source in culture media and the effect of various sugars on anther culture has been investigated in a number of species. Generally, sucrose is supplied at a concentration of 2–3 % in a tissue culture medium. In flax it was reported that high sucrose concentrations in the induction medium inhibited callus formation in anther culture /Chen et al., 1998; Chen, Dribnenki, 2004/, and similarly Rutkowska-Krause with co-authors indicated that sucrose above 5 % resulted in the decrease in the number of callusing anthers of flax /Rutkowska-Krause et al., 2002/. Furthermore, according to Tejklova experiments did not show any significant differences in flax anther callogenesis between media with 6 % or 10 % sucrose in the induction medium /Tejklova, 1996/. In contrast, our experiments clearly show that a higher level of sucrose (9 % or 12 %) in induction medium is more suitable for at least three of the five flax cultivars tested. Furthermore, our study results show a different influence of sucrose level on callus induction of cultivars and their hybrids. Increased concentration of sucrose (9 %) promoted higher callogenesis of the cultivars 'Lirina' and 'Barbara', while lower sucrose level (6 %) was more suitable for their reciprocal hybrids. In contrast, 'Barbara' x 'Mikael' hybrid showed the better response in medium supplemented with 9 % of sucrose, as and cultivar 'Barbara', while the some sucrose level significantly decreased callus induction of cultivar 'Mikael' in comparison with 6 % of sucrose. The high heritability for anther

response estimated in our study suggests that relatively rapid genetic gain can be made in transferring this trait from responsive to poor/non responsive germ plasm /Burbulis et al., 2005a; Burbulis, Blinstrubiene, 2006/.

Genotype was the most significant factor influencing callus organogenic differentiation in culture, since only one of the five cultivars investigated produced shoots. Nichterlein with co-authors also observed differences in genotypic responses to shoot regeneration between different flax cultivars /Nichterlein et al., 1991/. It is interesting to note that callus induction from 'Mikael' anthers occurred using various auxin/cytokinin combinations, but shoot regeneration was found only when induction medium was supplemented with 2.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA /Burbulis, Blinstrubiene, 2006/.

As in androgenesis, gynogenic haploids may develop directly or indirectly via regeneration from callus. The major problems affecting the use of gynogenesis are the lack of established protocols for most species, poor yields, and production of diploid or mixoploid plants.

We used unfertilized ovaries of 9 flax cultivars and their hybrids for callus induction *in vitro*. While gynogenesis was induced in a few species without the use of growth regulators, most species required auxins and/or cytokinins in the medium. A significant effect of the combination 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA on callus formation in flax ovary culture has been reported by Obert with co-authors /Obert et al., 2005/. In our studies four different combinations of auxins and cytokinins were tested. Variable callogenic responses were expressed by all genotypes tested on the different induction media. It has been documented that isolated ovaries of some genotypes induced statistically reliably more callus on medium supplemented with 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA ('Linola', 'Barbara', 'Mikael'), while other genotypes ('Lirina', 'Mikael', 'Norman') showed the better response on medium with 1.0 mg l^{-1} BAP + 2.0 mg l^{-1} 2,4-D. However genotypes 'Atalante', 'Symphonia' and 'Avangard' did not shown significant differences among growth regulators combinations tested. From the tested genotypes the highest frequency of responding explants was shown by the cultivars 'Lirina', 'Barbara' and 'Linola' (unpublished data).

It has been observed, that in contrast to anther culture most of the F_1 hybrids, were less responsible in comparison with parental forms /Burbulis et al., 2007/.

The level of sucrose in the nutrient medium is also an important factor for gynogenic structures induction. For gynogenesis induction sucrose concentration varied from 3 % to 12 % in a culture medium. However, higher sucrose concentration (10 %) have been found to be important for gynogenic induction in onion /Kamštaitytė, Stanys, 2002/. Our study shows that higher levels of sucrose (12 %) significantly decrease callus induction frequency. It has been observed that in most cases ovaries of tested genotypes should be cultivated on a nutrient medium supplemented by 6 % sucrose /Burbulis et al., 2007/.

After transfer of the calli onto the regeneration medium, bud regeneration was obtained from the genotypes 'Mikael', 'Szaphir', 'Barbara'. However it is important to note, that the callus of each cultivar had originated from different induction medium. Buds regeneration was not initiated at the same time in all callus and after initiation they continued to develop at different rates (unpublished data).

Conclusions

Our experimental results indicate that genotype, growth regulators combination, sucrose level and their interaction are important for linseed morphogenesis *in vitro*. Since the response of flax genotypes to *in vitro* cultures and plant regeneration is still comparatively low, further investigations of induction medium modification and optimization for specific flax genotypes within each particular breeding program should be carried out.

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REFERENCES

1. Blervacq A. C., Dubois T., Dubois J. et al. First division of somatic cells in cichorium hybrids "74"// Protoplasma. – 1995, vol. 186, p. 163–168

2. Blinstrubienė A., Burbulis N., Sliesaravičius A. Factors Affecting Callogenesis and Organogenesis in Tissue Culture of Oilseed Flax (*Linum usitatissimum* L.) // Vagos: mokslo darbai / LŽŪU. – Akademija (Kauno raj.), 2004a, t. 64 (17), p. 20–25

3. Blinstrubienė A., Sliesaravičius A., Burbulis N. Effect of Genotype and Medium Composition on Linseed (*Linum usitatissimum* L.) Morphogenesis in Tissue Culture // Žemdirbystė: mokslo darbai / LŽI, LŽŪU. – Akademija (Kėdainių raj.), 2004b, t. 86 (2), p. 13–21

4. Blinstrubienė A., Sliesaravičius A., Burbulis N. Factors Affecting Morphogenesis in Tissue Culture of Linseed Flax (*Linum usitatissimum* L.) // Biology:Acta Universitatis Latviensis. – University of Latvia, 2004c, vol. 676, p. 149–152

5. Bonell M. L., Lassaga S. L. Genetic analysis of the response of linseed (*Linum usitatissimum* L.) somatic tissue to *in vitro* cultivation // Euphytica. – 2002, vol. 125 (3), p. 367–372

6. Burbulis N., Blinstrubienė A., Sliesaravičius A. et al. Influence of genotype, growth regulators, sucrose level and preconditioning of donor plants on flax (*Linum usitatissimum* L.) anther culture // Acta Biologica Hungarica. – 2005a, vol. 56 (3–4), p. 323–331

7. Burbulis N., Blinstrubienė A., Venskutonienė E. et al. Organogenesis in callus cultures of *Linum usitatissimum* L. // Biology:Acta Universitatis Latviensis. – University of Latvia, 2005b, vol. 691, p. 129–135

8. Burbulis N., Blinstrubienė A. Comparison of anther culture response among *Linum usitatissimum* L. cultivars and their hybrids // Biology: Acta Universitatis Latviensis. – University of Latvia, 2006, vol. 710, p. 131–138

9. Burbulis N., Blinstrubienė A., Sliesaravičius A. et al. Some factors affecting callus induction in ovary culture of flax (*Linum usitatissimum* L.) // Biologija: mokslo darbai / LMA. – LMA leidykla, 2007, t. 53 (2), p. 21–23

10. Chen Y., Kenaschuk E., Dribnenki P. High frequency of plant regeneration from anther culture in flax, *Linum usitatissimum* L. // Plant Breeding. – 1998, vol. 117, p. 463–467

11. Chen Y., Dribnenki P. Effect of genotype and medium composition on flax *Linum usitatissimum* L. anther culture // Plant Cell Reports. – 2002, vol. 21, p. 204–207

12. Chen Y., Lin S., Duguid S. et al. Effect of sucrose concentration on elongation of shoots from flax anther culture // Plant Cell, Tissue and Organ Culture. – 2003, vol. 72, p. 181–183

13. Chen Y., Dribnenki P. Effect of medium osmotic potential on callus induction and shoot regeneration in flax anther culture // Plant Cell Reports. – 2004, vol. 23 (5), p. 272–276

14. Cunha A. C., Ferreira M. F. Somatic embryogenesis, organogenesis and callus growth kinetics of flax // Plant Cell, Tissue and Organ Culture. – 1996, vol. 47, p. 1–8

15. Dedicova B., Hricova A., Samaj J. et al. Shoots and embryo-like structures regenerated from cultured flax (*Linum usitatissimum* L.) hypocotyl segments // Journal of Plant Physiology. – 2000, vol. 157, p. 327–334

16. Dewitte W., Murray J. A. The plant cell cycle // Annual Review Plant Biology. – 2003, vol. 54, p. 235–264

17. Friedt W. Biotechnology in breeding of industrial oil crops: the present status and future prospects // Fat Science of Technology. – 1990, vol. 90, p. 51–55

18. Kamštaitytė D., Stanys V. Pathways of onion regeneration via flower and ovary culture // Žemdirbystė: mokslo darbai / LŽI, LŽŪU. – Akademija (Kėdainių raj.), 2002, t. 78 (2), p. 245–250

19. Marshall G., Courduries P. An assessment of somaclonal variation in linseed (*Linum usitatissimum* L.) // Annals of Applied Biology. – 1992, vol. 120, p. 501–509

20. Mundhara R., Rashid A. Stimulation of shoot-bud regeneration on hypocotyl of *Linum* seedlings, on a transient withdrawal of calcium: effect of calcium, cytokinin and thidiazuron // Plant Science. – 2002, vol. 162, p. 211–214

21. Nichterlein K., Umbach H., Friedt W. Genotypic and exogenous factors affecting shoot regeneration from anther callus of linseed (*Linum usitatissimum* L.) // Euphytica. – 1991, vol. 58, p. 157–164

22. Obert B., Dedicova B., Hricova A. et al. Flax anther culture: effect of genotype, cold treatment and media // Plant Cell, Tissue and Organ Culture. – 2004, vol. 79 (2), p. 233-238

23. Obert B., Bartosova Z., Pretova A. Dihaploid production in flax by anther and ovary cultures // Journal of Natural Fibers. – 2005, vol. 1 (3), p. 1–14

24. Rutkowska-Krause I., Mankowska G., Poliakov A. V. Regeneration of androgenic flax (*Linum usitatissimum* L.) plants and their application in breeding programme // Natural Fibres. – 2002, p. 92–101

25. Rutkowska-Krause I., Mankowska G., Lukaszewicz M. et al. Regeneration of flax (*Linum usitatissimum* L.) plants from anther culture and somatic tissue with increased resistance to *Fusarium oxysporum* // Plant Cell Reports. – 2003, vol. 22 (2), p. 110–116

26. Son W. Y., Bhojwani S. S. Morphogenesis in plant tissue cultures. – Kluwer Academic Publisher, 1999, p. 133–204

27. Soroka A. I. Influence of nutrient medium composition on callusogenesis and regeneration in anther culture of oil flax // Cytology and Genetics. – 2004, vol. 38 (2), p. 20–25

28. Tang G. X., Zhou W. J., Li H. Z. et al. Medium, explant and genotype factors influencing shoot regeneration in oilseed *Brassica* spp. // Journal of Agronomy and Crop Science. – 2003, vol. 189, p. 351–358

29. Tejklova E. Some factors affecting anther culture in *Linum usitatissimum* L. // Rostlinna Vyroba. – 1996, vol. 42(6), p. 249–260

30. Zhang C. L., Chen D. F., Elliot M. C. et al. Efficient procedure for callus induction and adventitious shoot organogenesis in sugar beet (*Beta vulgaris* L.) breeding lines // Planta. – 2004, vol. 40, p. 475–481

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SĖMENINIŲ LINŲ (*LINUM USITATISSIMUM* L.) *IN VITRO* KULTŪRŲ OPTIMIZAVIMAS

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Santrauka

Sėjamasis linas (*Linum usitatissimum* L.) – svarbus pluoštinis ir aliejinis augalas Lietuvoje. 2002 metais Lietuvos žemės ūkio universiteto Agrobiotechnologijos laboratorijoje pradėti sėmeninių linų tyrimai taikant įvairius biotechnologinius metodus. Tyrimų tikslas – ištirti įvairius veiksnius, lemiančius sėmeninių linų morfogenezę *in vitro*.

Siekiant indukuoti somatinių audinių regeneraciją, tirti skirtingi maitinamųjų terpių deriniai. Atlikti tyrimai rodo, kad sėmeninių linų hipokotilių ir stiebo segmentų audiniai geba formuoti pridėtinius ūglius.

Izoliuotų dulkinių ir neapvaisintų mezginių kultūrose tirta genotipo, augimo reguliatorių ir augalo-donoro auginimo sąlygų įtaka kaliaus indukcijai. Analizuojant tyrimų duomenis nustatyta, kad generatyvinių eksplantų kaliaus formavimuisi svarbią reikšmę turi genotipas bei maitinamosios terpės sudėtis, kuri turi būti parenkama kiekvienam genotipui individualiai.

Optimizuotos sėmeninių linų somatinių ir generatyvinių audinių kultūros naudojamos pradinei selekcinei medžiagai kurti.

Reikšminiai žodžiai: morfogenezė, sėmeniniai linai, somatiniai ir generatyviniai audiniai.