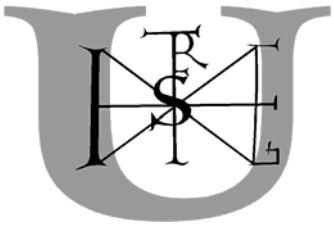

SZENT ISTVÁN EGYETEM

Two new tools of plant molecular breeding: the locust proteinase inhibitor and the pPROGMO transformation vector

PhD thesis

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BACKGROUND, AIMS

Genetic modification (GM) can generate crops with higher yield, pest and pathogen resistance, abiotic stress tolerance (e.g.: cold and drought), and increased nutrition values. In most cases, pest resistance of GM crops is based on expression of entomotoxic proteins of various origins. To increase the pest resistance of crops, genes derived from microorganisms, plants or animals can be integrated into the plant genome. One of the possibilities to develop pest resistant crops is the insertion and expression of protease inhibitor (PI) genes in the plant genome. PIs via their pesticid effect can provide a natural defence system for the plants against herbivores.

Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) is the most destructive pests of potato. The adult and all larval stages feed primarily on foliage and occasionally on stems. Extensive feeding at any time during the growing season can reduce yield, as a reduction in leaf surface area decreases the plant's ability to produce and store nutrients, which affects tuber size and number.

MALIK et al. (*Biochim. Biophys. Acta*, 1999, 1434: 143-150) have isolated and characterised two small serine protease inhibitor peptides, i.e. *Schistocerca gregaria* chymotrypsin inhibitor, SGCI, and *S. gregaria* trypsin inhibitor, SGTI from the haemolymph of locust *S. gregaria.* The primary structure of these peptides is very similar. They are composed of 35-36 amino acid residues and contain three homologous disulfide bridges. The characteristic feature of this structure is a compact globular fold that consist of a short three-stranded B-sheet. SGCI proved to be very good inhibitor of bovine chymotrypsin whereas SGTI was a reasonable inhibitor of bovine trypsin. The latter inhibited two arthropodal trypsins by five orders of magnitude more strongly than the mammalian one.

The major proteolityc activity in the gut of CPB is an aspartic protease with some serine, metallo-, and carboxy-peptidases also contributing to digestion. The SGTI and SGCI belong to serine PIs. The result of preliminary experiments suggested that expressing of SGTI and SGCI in "double headed" protein form (SGTI-Lys-Arg-SGCI) could be suitable to increase the resistance of potato against CPB.

One of the aims of our experiments was to generate transgenic potato lines expressing the desert locust trypsin (SGTI) and chymotrypsin (SGCI) inhibitor in double-headed protein form (locust inhibitor peptide, LIP) and examine their effect on development of CPB larvae.

Transformation of plant cells occurs at a very low frequency, while most of the cells remain non-transgenic. For identifying those cells that have integrated the DNA into their genome it is necessary to transfer a selectable marker gene together with the gene of interest. Usually, antibiotic- and herbicide-resistance genes are used for this purpose in plant transformation. The current selection methods have some potential pitfalls. On the medium containing the antibiotic or herbicide the non-transgenic cells can not grow and die. The dying non-transgenic cells inhibit the supply of the nutrients to the transgenic cells or excrete toxic compounds. These negative effects decrease the ability of transgenic cells to proliferate and differentiate into transgenic plants. The use of antibiotic resistance genes as markers for the selection of transgenic crops has resulted in the particular concern that these genes may be transferred into relative species or pathogenic bacteria. Technologically, in practice, the number of suitable marker genes is limited that can limit more desired genes integration by transformation. Transformation of plant cells using *Agrobacterium*-mediated transformation method often results in integration of extra backbone DNA originating from the binary plasmid in the plant genome. However, in the field, the presence of marker gene in the crop is unnecessary and unacceptable in

some respect except of herbicide resistance genes. Accordingly, such strategy and method must be developed and used which in the final cultivar can eliminate the unnecessary DNA sequence from the genome. So, the GM plants could be safe for the environment and acceptable for the public.

There are several strategies that are based on co-transformation of genes of interest with selectable marker genes followed by the segregation of the separate genes through sexual crosses or transformation followed by a recombination. Disadvantage of these methods is that most of them are not suitable for the crops propagated vegetatively and/or don't prevent the integration of backbone gene(s) of the transformation vector.

The other aim of our experiment was to develop a new transformation vector, which can be used for the potato as a crop propagated vegetatively to generate of marker- and backbone-free transgenic plants.

MATERIAL AND METHODS

Living materials

Escherichia coli DH5α, BL21 or JM109 strains were used for molecular cloning. *Agrobacterium tumefaciens* C58C1 with pGV2260 or AGL0 strains were used for plant transformations. The leaves of *Solanum tuberosum* cv. Désirée were used for genetic modifications. *In vitro* plant materials were propagated vegetatively from cuttings on RM and MS media with 16/8 h light/dark periods and at 24°C temperatures. Plants were cultivated in pots either in greenhouse.

Molecular biology methods

Isolation and manipulation of nucleic acids (nucleic acid preparation, restriction endonuclease cleavage, electrophoretic separation, cloning into

vectors, polymerase chain reaction, hybridisation techniques, sequencing, plant transformation, detection of β-glucoronidase activity) were carried out following techniques commonly used in molecular biology as well as by following guidelines provided by the suppliers.

Growth of Colorado potato beetle larvae

Experiments to study the effect of LIP expressing transgenic potato plants on the growth of larvae were performed with a modified method of SÁRINGER (*Acta Agr. Acad. Scient. Hung*. 1967, 16: 113-120).

RESULTS

Generation of transgenic potato lines expressing two protease inhibitors originated from *Schistocerca gregaria* in double-headed form and studying their effect on Colorado potato beetle larvae

Cloning of Shistocerca gregaria double headed protease inhibitor gene LIP into a binary vector

For the expression of *LIP* in transgenic potato, the cDNA of *LIP* was cloned into the binary vector pCP60 between the constitutive CaMV35S promoter and *nos* terminator. The resulted recombinant plasmid was transferred into *A. tumefaciens* by triparental mating.

Transformation of Solanum tuberosum cv. Désirée with pCP60::LIP transformation vector

105, 6 week-old potato leaves were transformed with *A. tumefaciens* carrying the pCP60::LIP vector. From 80 leaves, 417 regenerated shoots were

obtained, and 226 independent, kanamycin resistant potato plants were isolated, out of which 23 lines were used for further experiments.

Isolation of transgenic potato lines expressing the LIP

In the transgenic lines, the expression of LIP was analyzed by both northern blot and chymotrypsin inhibition displayed in the rapid screening assay. The rapid assay was repeated with plants of the northern positive lines. Three lines, LIP 7, 9, and 13, were chosen for further analysis based on their significantly increased inhibitory activity compared to the untransformed control plants in both tiers.

The N-terminal sequence and the inhibitory constants against chymotrypsin and trypsin of LIP proteins purified from the three transgenic lines were practically identical with that determined with the individual peptides of desert locust. The quantity of LIP of transgenic lines was 0,04% of the total soluble protein.

Feeding trials with transgenic lines LIP7, 9 and 13

Feeding of potato beetle larvae with leaves of transgenic plants was slightly but significantly inhibited by LIP. The average daily weights of control larvae fed with untransformed potato leaves were higher than the weight of larvae fed with transgenic potato leaves from the first day till the day of pupation. Significant differences were observed between the weight of control and LIP consuming larvae on the 2^{nd} and 4^{th} days. Three larvae fed on LIP transgenic plants died during the bioassay.

During the bioassays the weight of consumed leaves (dry matter) was also measured. These data showed that feeding increased proportionally with larval growth. This also conveys the suggestion that LIP has a physiologically toxic effect.

The bioassays ended when the largest larvae wanted to enter the soil for pupation. All larvae entered the soil within three days. After pupation adult beetles emerged from the soil and were reared in laboratory for two weeks. Every larvae reached adulthood and no morphological deformation could be observed on them.

Developing a transformation vector suitable for generation of marker- and backbone-free transgenic plants and testing it in potato

For generation of marker- and backbone-free transgenic plants a new vector, designated pPROGMO36 was designed. This vector contains the plant adapted *R* recombinase and *Rs* sites originated from *Zygosaccharomycaes rouxii,* a strongly in size reduced binary vector containing only one border (*RB*) sequence, positive/negative marker genes and the *GUS* reporter gene. The idea was that after integration of the whole binary vector, the active recombinase enzyme recombines the recombinase gene, the markers and backbone sequence out of the plant genome resulting in marker- and backbone-free transgenic potato plants, while only the *GUS* reporter gene can stay as a new genetical element in the potato genome.

Construction of the binary vector pPROGMO36

The binary vector, pPROGMO36 was based on the plant adapted version of the *R* recombinase (SCHAART et al. *Plant Biotechnol. J.*, 2004, 2:233-240) and the bifunctional hybrid marker gene *codA-nptII*. To avoid the synthesis of recombinase in *Agrobacterium*, the intron *ST-LS1* was placed into the *R* coding region. Then *35Spr-SYNREC-ST-LS1int-nosT* and *35S-codA-nptII-nost* was integrated into pPROGMO02 plasmid. In contrast to normal T-DNA transformation vectors, pPROGMO carries only the *RB*. Furthermore, the backbone vector DNA has been reduced to the limited amount of DNA

sequences which is still necessary for proper maintenance, propagation and plant transformation. Thus the whole plasmid is practically a T-DNA when inserted into the plant genome. The recombinase recognition sites (*R*s) are in direct repeat and surround the multicloning site (*MCS*)-*RB* fragment. The *R* gene, and the other part of the vector including the *nptIII* gene that is used for selection in bacteria, and the *codA*-*nptII* that is used for selection in plants are outside of the *R*s-flanked fragment.

To test the pPROGMO vector, the *GUS* reporter gene controlled by the *Rubisco* promoter was inserted into the *MCS*.

Detection of R recombinase activity in potato

pPROGMO-GUS, designed pPROGMO36, was used for *Agrobacterium*-mediated transformation of leaf explants. Callus induction, regeneration, and rooting were all carried out in the presence of kanamycin (positive selection by *nptII*). GUS activities of 91 kanamycin resistant lines were tested by histochemical staining. Blue leaves were detected in 68 lines, albeit with very different staining intensity. Copy number of insertions was investigated by Southern hybridisation. Using *nptII* as a probe 8 out of 11 tested lines carried a single insertion. Leaves of these lines were collected and transferred to callus induction-, and later to regeneration medium both supplemented with 5-FC (negative selection by *codA*) to obtain marker- and backbone-free derivatives. 242 regenerated shoots were isolated, however, only 18 were sensitive to kanamycin when tested for rooting, probably due to leakiness of 5-FC selection in potato. PCR analysis demonstrated that 4 lines were generated by recombinase-mediated bona fide recombination that left only the *GUS* gene in the potato genome. These plants derived from two parental lines. The recombination was supported in two lines by Southern hybridisation.

Generation of marker- and backbone-free potato lines with temporary selection

Subsequently linking a positive selection for temporary marker gene expression to negative selection against marker gene integration increases the frequency of marker-free lines. Based on this, after transformation of Désirée leaf explants with pPROGMO36 kanamycin selection was applied only during callus induction (7days) while the negative selection by 5-FC was employed during the entire shoot regeneration period. Histochemical staining of 150 regenerants resulted in detection of GUS activity in the leaves of 9 lines. In agreement with the histochemical staining very high activities were detected by fluorometric assay in these lines. This result indicates that a large number of non-transgenic shoots could start regeneration after finishing the callus induction phase with positive selection.

The temporary-selected 9 lines were further investigated by PCR. The results show that 6 lines lack all the genes introduced by pPROGMO36 except for *GUS,* and that recombination has taken place in these lines. Probably, because of the partial insertion of pPROGMO36 the recombination could not eventuate in 3 residual lines.

The correlation between the loss of marker- and backbone-genes and the copy number of *GUS* transgene was analysed by Southern hybridisation. The hybridisation pattern demonstrated low copy *GUS* insertion in all the 9 lines obtained. Using this method, in one regeneration event, six marker- and backbone-free transgenic plants were isolated from 150 regenerants, which means a frequency of 4%. All the six lines had a high level of transgene expression and low copy number transgene insertion.

Novel results

1. For the expression of LIP in transgenic potato, the cDNA of *LIP* was cloned into the binary vector pCP60 and transferred into *A. tumefaciens. S. tuberosum* cv. Désirée was transformed with this *A. tumefaciens*

strain. In the transgenic lines the expression of LIP was analyzed by both northern blot and chymotrypsin inhibition displayed in the rapid screening assay. Three lines were chosen for further analysis based on their significantly increased inhibitory activity compared to the untransformed control plants. Both the sequence and the inhibitory constants against chymotrypsin and trypsin of LIP of transgenic lines were practically identical with that determined with the individual peptides of desert locust.

2. The effect of LIP expressing transgenic lines was examined in feeding trials. The result supported that the feeding of potato beetle larvae was slightly but significantly inhibited by LIP. The average daily weights of control larvae fed with untransformed potato leaves were higher than the weight of larvae fed with transgenic potato leaves from the first day till the day of pupation.
3. We developed a transformation vector suitable for generation of marker- and backbone-free transgenic plants. The whole plasmid is practically a T-DNA. The transformation vector based on a plant adapted *R* recombinase and a *codA-nptII* bifunctional selectable marker gene and was termed pPROGMO.
4. The *GUS* reporter gene was inserted into the pPROGMO. Transgenic potato plants were generated with pPORGMO36. Expression of GUS was tested by histochemical staining. Transgenic potato plants with single copy insertion were chosen by Southern hybridisation and expression of recombinase gene was investigated by northern hybridisation.
5. The activity of recombinase in potato was tested in transgenic potato plants with single copy insertions. Leaves were cut, and after callus induction regenerated shoots were isolated in the presence of 5-FC that selected for the plants in which recombination occurred and the codA

was lost. Molecular methods demonstrated that in 4 lines only the *GUS* gene remained in the potato genome.

6. Marker- and backbone-free transgenic plants were generated also by temporary positive selection followed by negative selection. Using this method, six marker- and backbone-free transgenic plants were isolated with efficiency as high as 4%. All the six lines had a high transgene expression and low copy number transgene insertion.

CONCLUSIONS AND SUGGESTIONS

Conclusions from studying the effect of expression of a locust proteinase inhibitor in potato on the development of Colorado potato beetle larvae

Colorado potato beetle (CPB) is the major pest of potato in many areas of the world. Commercial GM potato varieties resistant to CPB all produce the *Bacillus thuringiensis* toxin. In laboratory experiments, introduction of heterologous protease inhibitors into crops has also increased their resistance towards pests. However, effective CPB resistance couldn't be generated by the cysteine protease activity. This might be explained by the ability of CPB to synthesise insensitive enzymes or inactivate not only prtotease inhibitors of potato's self defence but also several heterologous protease inhibitors introduced into potato by cleavage.

The natural peptide from a locust haemolymph inhibiting both trypsin and chymotrypsin with its twin heads has been successfully cloned and introduced into potato. Three LIP-producing transgenic potato lines were chosen for further analysis by northern hybridisation and rapid screening protease inhibitor assay. In all three lines the LIP expression was high and the inhibition of chymotrypsin was increased significantly compared to the untransformed

control plants. The presence of the active LIP protein in the leaf extracts albeit in low amounts (0,04 %) was demonstrated. Others showed that 0,5-1% PI concentration of soluble protein content should be reached to inactivate gut protease activity fully. To enhance the effect of LIP against CPB it would be necessary to increase the level of LIP in transgenic potato plants. This can be achieved by enhancing LIP expression or protecting the protein from degradation in the plant. The amount of LIP could be increased by an N-terminal signal that would target and accumulate the LIP in the endoplasmatic reticulum or chloroplast. Other possibility is the reduction of LIP degradation. Replacement of the amino acids presently cleaved by functionally equivalent amino acids, which preserve the biological activity of the protein but prevent cleavage, may allow the design of stable molecules for expression in plants. Alternatively, the co-expression of protease inhibitors that prevent protein degradation in vitro may also stabilize the LIP in plant.

Despite of the low level of LIP the growth of the CPB larvae reared on the transgenic plants grew slightly slowly (16-25 %) than those on non-transformed control plants, and some of them even died. The inhibition of the larval growth might be due to the inhibition of the chymotrypsin-like gut proteases of potato beetle larvae. Serine proteases are known to process pro-proteins including many proteases synthesised in a proenzyme form. Such an activating protease, present probably at a very low concentration, can also be inactivated by LIP. It is also possible that LIP, as a heterodimeric serine PI might be involved in physiological processes such as immune response, larval growth and ovarian development.

The results support the notion that expression of multifunctional protease inhibitors of insect origin might be a good strategy to improve insect resistance in plants. As the proteins of other insect resistance genes, except of the *Bt.* toxin, the LIP doesn't cause full lethality of the target insect. LIP might have higher effect to other target insects than to CPB that have serine-type protease e.g.

Lepidopthera and Dipthera, and could be applicable in pest resistance for other crops. Combining LIP with other pest resistance proteins, e.g. *Bt* toxin or lectins, the pest resistance could be more effective and stable.

Conclusions from generation of marker- and backbone-free transgenic potatoes by site-specific recombination and a bi-functional marker gene

We developed a transformation system suitable for generation of marker- and backbone-free transgenic plants. Our system differs from other *Agrobacterium*–mediated transformation approaches in utilising only the *RB* for T-DNA transfer. T-DNA border sequences are imperfect direct repeats. The *LB*s occasionally function as *RB*s to initiate T-strand synthesis. Thus the transfer of backbone sequences reported for many plant species was possibly due to the initiation of T-strand production at the left border. In other events, this could be simply the result of failure to terminate a *RB*-initiated T-strand at *LB*. This could results in integration of extra backbone DNA originating from the binary plasmid in the plant genome. By using only the *RB* and reducing the binary pPROGMO vector backbone DNA, the whole vector will be integrated in the plant genome as one large T-DNA and hopefully this will solve these problems.

During transformation of plant cells all the interested-, marker-, and backbone genes of the plasmid integrate into the genome. After integration, the *R*s sites are orientated as such that he whole vector DNA (backbone DNA and selection marker genes) is recombined out of the genome. In the pPROGMO transformation vector the *R* recombinase gene, selectable marker- and backbone-genes are flanked by the *R*s sites and will be recombined out of the genome when the recombinase enzyme is active. It results in generating marker- and backbone-free transgenic plants.

Our experiments showed that the R/Rs system of *Zygosaccharomyces rouxii* was active in potato. In the first transformation experiment we generated

stable transformants with positive selection like the other marker-free transformation method for vegetatively propagated crops. Starting from eight transgenic lines with single copy PROGMO GUS insertions, new regeneration was induced on leaf explants under selection of 5-FC. Only plant cells in which the recombinase has become active and as consequence the *nptII-codA* marker genes have been recombined out of the genome, can grow and regenerate on 5-FC. After second regeneration under selection of 5-FC, only 18 of insensitive to 5-FC shoots were sensitive to kanamycin when tested for rooting, probably due to the insufficient (150mg/l) 5-FC concentration or the regenerated shoots were mosaics. Four plants were obtained in which, due to R recombinase-mediated recombination, only the *GUS* gene remained in the potato genome. The four plants were derived from two lines. From the other six lines no marker-free derivatives were obtained or the plants lacked the *nptII-codA* gene but still, retained the *R* gene or contained the *R* gene together with *nptIII*. The northern hybridisation showed that the recombinase mRNA of that transgenic line from which marker-free plants was not regenerated was greater in size than we expected. Probably, during the integration a rearrangement occurred that inactivated the recombinase. In the other lines, in which only the marker gene was eliminated a full and a partial insertion might occurred. The full insertion could be recombined by recombinase while the partial insertion remained in the genome because the second *Rs* site was missed and the recombination event could not occur.

In the next experiment, marker- and backbone-free transgenic plants were generated with temporary positive selection followed by negative selection. Six marker- and backbone-free *GUS* transgenic lines were isolated. All six lines had high GUS activities and low copy transgene insertions. It is fairly likely that the transformation system itself selects for such kind of transgenic lines. The earliest detection of gene expression from T-DNA encoded genes was reported 18h after infection that peaked at 36h and declined over 4-10

days as the cells which only transiently expressed T-DNA encoded genes fail to become stably transformed. We incubated the leaf explants together with the *Agrobacterium* for two days and then applied kanamycin selection for 7 days. Thus, by this time, the recombinase has already been integrated into the plant genome. Our results indicated that a large number of non-transgenic shoots could start regeneration after finishing the callus induction with kanamycin selection. The 5-FC selected for loss of *nptII-codA* that probably needed high recombinase activity that could be achieved only from certain chromosomal positions. After recombination the *GUS* remained at the same chromosomal position that may explain the high GUS activities of the marker- and backbone free transgenic lines. The low copy number of insertions detected in the same lines is due to the higher probability of removing *codA* in low than in high copies.

The pPROGMO system can be developed further. There are several possibilities to increase the frequency of marker- and backbone-free transgenic lines:

- Prolongation of the time of positive selection can inhibit further the growth of non-transformed cells while it can favour the development of transgenic cells and thereby the marker- and backbone free transgenic plants will appear with higher frequency.
- Using an inducible promoter the expression of recombinase can be regulated temporally. In this case, two regeneration steps will be applied. In the first regeneration, stable transformants with non-expressing recombinase gene will be generated with positive selection. Then in the second regeneration, by the induction of the promoter of recombinase gene the recombinase will be activated to eliminate the marker- and vector backbone genes from the genome. Under negative selection only the marker- and backbone free shoots will regenerate. Unfortunately, however, no inducible promoter was found until now which could have

been used in potato. The recombinase was expressed constitutively in our vector. The constitutively expressed recombinase can work during the positive selection and eliminate the positive marker gene decreasing the efficiency of transformation system. Inducible promoter regulated recombinase can solve this problem.

- By cloning the bi-functional marker gene in the pPROGMO in front of the recombinase related to *RB* partial integrations into the plant genome can be excluded. Since in the pPROGMO36, the negative marker gene is at the end of the construct during the partial insertion it does not integrate into the plant genome and can not inhibit the regeneration of lines with partial insertion. If the marker gene would be placed behind the gene of interest, it could express in the case of partial integration and inhibit the regeneration of these lines.

Our experiments provided evidence that the pPROGMO vector is suitable for transformation to generate marker- and backbone-free transgenic plants. The pPROGMO vector approach is simple, consists of only one transformation and one or two regeneration steps, and efficient as it generates marker- and backbone-free transgenic plants with high frequency.

Although, we have tested the LIP and pPROGMO only in potato, our results indicate that both biotechnological plant breeding methods may be applicable for many crop species.

PUBLICATIONS

Articles:

KONDRÁK M., KUTAS J., SZENTHE B., PATTHY A., BÁNFALVI Z., NÁDASY M., GRÁF L. ASBÓTH B. (2005): Inhibition of Colorado potato beetle larvae by a locust proteinase inhibitor peptide expressed in potato. *Biotechnology Letters*, 27: 829-834. p.

KONDRÁK M., ROUWENDAL G.J.A., VAN DER MEER I., BÁNFALVI Z. Generation of marker- and backbone-free transgenic potatoes by site-specific recombination and a bifunctional marker gene in a non-regular one-border *Agrobacterium* transformation vector. *Transgenic Research* közlésre beküldve)

SÓS-HEGEDŰS A., LOVAS Á., **KONDRÁK M.**, KOVÁCS G., BÁNFALVI Z. (2005): Active RNA silencing at low temperature indicates distinct pathways for antisense-mediated gene-silencing in potato. *Plant Molecular Biology*, 59: 595-602 p.

KUTAS J., **KONDRÁK M.**, SZENTHE B., PATTHY A., BÁNFALVI Z., NÁDASY M., GRÁF L., ASBÓTH B. (2004): Colorado potato beetle larvae on potato plants expressing a locust proteinase inhibitor. *Communications in Agricultural and Applied Biological Sciences*, 69(3): 281-287. p.

DÓCZI R., **KONDRÁK M.**, KOVÁCS G., BECZNER F., BÁNFALVI Z. (2005): Conservation of the drought-inducible *DS2* genes and divergences from their *ASR* paralogues in solanaceous species. *Plant Physiology and Biochemistry*, 43(3): 269-276. p.

KISS J., **KONDRÁK M.**, TÖRJÉK O., KISS E., GYULAI G., MÁZIK-TÖKEI K., HESZKY L.E. (2001): Morphological and RAPD analysis of poplar trees of anther culture origin. *Euphytica*, 118(2): 213-221. p.

TÖRJÉK O., KISS E., KISS J., **KONDRÁK M.**, GYULAI G., HESZKY L.E. (2001): Evaluation of genetic diversity of poplar genotypes by RAPD and AP-PCR analysis. *Acta Biologica Hungarica,* 52(2-3): 345-354. p.

KISS J., **KONDRÁK M.**, GERGÁCZ J., HESZKY L.E. (1997): A protoplaszt-növény rendszer kidolgozása hazai nyárfaklónokra. *Erdészeti Kutatások*, 86-87: 143-153. p.

BÁNFALVI ZS., **KONDRÁK M.** (2005): Hosszan tárolható paradicsom. *Zöld Biotechnológia*, 2: 2-5. p.

KISS J., **KONDRÁK M.**, GERGÁCZ J., HESZKY L.E. (1997): Towards a heterosis hybrid poplar**.** *Hungarian Agricultural Research*, 3: 18-21. p.

Conferences:

KONDRÁK M., BÁNFALVI ZS. (2005): Rekombinázon alapuló marker- és vektor-mentes növényi transzformációs rendszer. VI. Magyar Genetikai Kongresszus, XIII. Sejt- és Fejlődésbiológiai Napok, Eger, április 10-12. Összefoglaló:161. p. (PO51)

SÓS-HEGEDŰS A., LOVAS Á., **KONDRÁK M.**, KOVÁCS G., BÁNFALVI ZS. (2005): A géncsendesítés hőmérséklet-függőségének vizsgálata antiszensz burgonya növényekben. VI. Magyar Genetikai Kongresszus, XIII. Sejt- és Fejlődésbiológiai Napok, Eger, április 10-12. Összefoglaló:193. p. (PO86)

SÓS-HEGEDŰS A., LOVAS Á., **KONDRÁK M.**, KOVÁCS G., BÁNFALVI Z. (2005): Antisense-mediated gene-silencing is manifested in different forms in potato. EMBO/HHMI Central European Scientists Meeting, Budapest, Abstract: 79. p.

KONDRÁK M., ASBÓTH B, SZENTHE B, GRÁF L, BÁNFALVI ZS (2002): Sáska eredetű proteináz inhibitor peptid transzgénikus burgonyában. A Magyar Biokémiai Egyesület Molekuláris Biológiai Szakosztálya 7. Munkaértekezlete, Keszthely, május 14-17, Összefoglaló: 59. p.

DÓCZI R., CSANAKI CS., **KONDRÁK M.**, BÁNFALVI Z. (2001): Expression and promoter activity studies on the dessication-specific *DS2* gene in *Solanaceous* species, 4th International Symposium in the Series Recent Advances in Plant Biotechnology, Trebon, Czech Republic, September 17-21, Book of Abstracts :.111. p.

KISS J., **KONDRÁK M.**, TÖRJÉK O., KISS E., MÁZIKNÉ TŐKEI K., HESZKY L.E. (2000): Portok-kultúrából regenerált *Populus nigra* növények morfológiai és molekuláris vizsgálata. VI. Növénynemesítési Tudományos Napok, MTA, Budapest, március 8-9. Összefoglaló: 82.p.

KISS J., **KONDRÁK M.**, TÖRJÉK O., KISS E., MÁZIK-TŐKEI K., HESZKY L.E. (2000): Portokkultúrából regenerált *Populus nigra* növények morfológiai és

molekuláris vizsgálata, Az Agrobiodiverzitás megőrzése és hasznosítása, Budapest, május 4-5.

KONDRÁK M., KISS J., GERGÁCZ J., MÁZIKNÉ TŐKEI K., KISS E., TÖRJÉK O., HESZKY L. (1999): Pollen eredetű nyárfák molekuláris és morfológiai analízise. IV. Magyar Genetikai Kongresszus, Siófok, április. 11-14. p.148. Összefoglaló: 180. p.

STILLER I., **KONDRÁK M.**, BÁNFALVI Z. (2004): Drought tolerance mediated by trehalose-6-phosphate synthase in transgenic potato plants. 14th FESPB Congress, Cracow, Poland, August 23-27, Abstract published in Acta Physiologiae Plantarum 26 (suppl.)

*Lectures***:**

SÓS-HEGEDŰS A., LOVAS A., **KONDRÁK M**., KOVÁCS G., BÁNFALVI ZS. (2003): A géncsendesítés hőmérsékletfüggőségének vizsgálata antiszensz burgonya növényekben. MBK napok, Gödöllő

KONDRÁK M., BÁNFALVI ZS., ROUWENDAL G, VAN DER MEER I. (2003): Rekombinázon alapuló marker- és vektormentes transzformációs rendszer létrehozása és tesztelése burgonyában. MBK napok, Gödöllő

KONDRÁK M., BÁNFALVI ZS. (2002): A Kecskeméti 262 paradicsomfajta fertőzés-ellenállóságának és tárolhatóságának javítása poligalakturonáz gátlással. MBK napok, Gödöllő

Patents:

BÁNFALVI ZS., DÓCZI R., CSANAKI CS., **KONDRÁK M.**, SILHAVY D.: A *DS2* gén szárazság-specifikus expressziót biztosító promoter szakasza. P0103697 ügyszámú bejelentés a Magyar Szabadalmi Hivatalhoz (2001. szeptember 13.)

BÁNFALVI Z., DÓCZI R., CSANAKI C, SILHAVY D., **KONDRÁK M.**: Isolation and use of the desiccation-specific promoter from *DS2* gene. Internatonal Patent Application No. PCT/HU02/00090. International filling date: 03/09/2002, priority date 13/09/2001