INHIBITION OF ETHYLENE BIOSYNTHESIS IN APPLE AND TAXONOMICAL COMPARISON OF DIFFERENT *FESTUCA* SPECIES USING MOLECULAR METHODS

Ph.D. thesis

Zsolt Galli

Gödöllő
2004

Ph.D. School: Plant Sciences

Scientific Code: 4. Agricultural Sciences
4.1. Agronomy and Horticulture

Ph.D. Program: Plant breeding, genetics and biotechnology

Director: Prof. Dr. Ferenc Virányi, Ph.D., DSc
SZIU Department of Plant Protection

Scientific Secretary: Dr. Gábor Gyulai, Ph.D., CSc
SZIU Department of Genetics and Plant Breeding

Program Head: Prof. Dr. László Heszky, Ph.D., DSc, mHAS
SZIU Department of Genetics and Plant Breeding

Advisors: Prof. Dr. Erzsébet Kiss, Ph.D., CSc
SZIU Department of Genetics and Plant Breeding

Prof. Dr. László Heszky, Ph.D., DSc, mHAS
SZIU Department of Genetics and Plant Breeding

……
Dr. Erzsébet Kiss
advisor

……
Dr. László Heszky
program head and advisor

……
Dr. Ferenc Virányi
director

1. PRIORITIES, OBJECTIVES

Biotechnology is one of the determining fields of the development of knowledge-based societies in the 21st century already and supposedly its significance will be growing in the future. Using more and more sophisticated molecular techniques as a routine allows developing our cultivated plants without changing the favourable traits generated by conventional breeding. Nevertheless using molecular tools in basic research help us recognize the surrounding living world deeper than phenotypic manifestation.

This dissertation summarizes results which were gained both in basic and applied research fields.

1.1. Molecular inhibition of ethylene biosynthesis in apples

Physiological processes in plants can be deliberately manipulated by inhibition of known biosynthetic pathways. The clarification of steps of ethylene biosynthesis and the possibility of molecular inhibition of gene expression paved the way for the manipulation of ripening processes, as well. In so-called climacteric fruits such as apples, a sharp increase in ethylene production is observed which is considered to be the root cause of the ripening process.

The first successful inhibition of autocatalytic ethylene biosynthesis was achieved in tomato at the beginning of 1990's. This made it possible to introduce the first transgenic tomato on the market, which can be stored for a longer time even at room temperature. Based on the results with tomatoes, research was initiated with apples, *in order to produce transgenic apple lines with extended storage life of fruits and to diminish the quality loss during storage.* Significant storage cost could be saved using this molecular approach because there is no need to establish the expensive controlled atmospheric storage rooms.

In this field, our results mean the first data to address the post harvest of transgenic fruits in which the ethylene biosynthesis was inhibited by an antisense transgene. Beside their scientific value, these results can help to study, understand and realize the biochemistry and physiology of fruit ripening.

1.2. Molecular taxonomy of different *Festuca* species

Systematization of the sorrounding living world was first based on morphology, then histology and cytology. The identification and classification of some species is still not unambiguous, due to the strong environmental dependence of the taxonomic traits. Since environmental factors can be eliminated using molecular genetic methods, the most reliable method for the separation of different species is probably using DNA based molecular markers. These markers could represent random sections of the whole genom (RFLP, RAPD, AFLP …) or a specific portion (SSR, EST, ITS …). The comparison based on DNA sequence and functional analysis has an unquestioned value, however the whole genome sequence is currently available only for a few species. The identification and classification of the species using morphological traits should be reassessed in the future since in many cases it is not going to be the same as the separation created by molecular methods. The differences of gene regulation in the same genetic background, which are the results of the inherited methylation patterns of the genomic DNA, can occasionally cause bigger phenotypic differences (taxonomy based on morphology) than the change of DNA itself (taxonomy based on molecular methods).

Classification of some species from genus *Festuca* is still a controversial question. *The goal of our experiments was the molecular comparison of the most questionable Festuca species to supply new data for the conventional taxonomy.* There was no available molecular analysis regarding the most examined *Festuca* species before our experiments.

Examinations were executed at molecular marker and sequence levels, as well.

2. MATERIALS AND METHODS

M26 rootstock and Royal Gala scion cultivars were transformed with the gene *MdACS*2 in antisense orientation. This gene codes the key enzyme (ACC-synthase) in the ethylene biosynthesis. To accomplish this, methods were established to initiate, maintain and propagate the *in vitro* shoot cultures, regeneration from leaves tissues and rooting and acclimatization of the regenerated shoots. The successful transformations were proved by PCR using transgene specific primers and Southern hybridization.

Transgenic fruits were examined in the USA at the research center of the Cornell University (New York State Agricultural Experiment Station, Department of Food Science and Technology, Geneva). More than 150 fruit bearing transgenic trees were available (98 Royal Gala and 57 McIntosh). After harvesting, the fruits were stored at room temperature and cold room (5 °C), respectively and at different intervals, ethylene production was measured by gas chromatography, firmness by hand penetrometer and soluble solids by refractometer. Total protein was extracted to measure the activity of ACC synthase enzyme. Expression of *MdACS*2 gene was followed from early stage of fruit development to the second month after harvesting by Northern hybridization. Copy number of transgene(s) were determined using Southern hybridization in the best transgenic lines, where the inhibition of ethylene biosynthesis was significant.

10 taxa (*F. dalmatica, F. javorkae, F. pallens, F. pseudodalmatica, F. pseudovina, F. rupicola, F. stricta, F. vaginata, F. valesiaca, F. wagneri*) were investigated in the molecular taxonomy of *Festuca* species whose classifications are the most problematic and are present in the Carpathian Basin.

Altogether 129 specimens were examined in these experiments which were collected from different species and habitats. In case of doubtfully identifiable species, samples from the *locus classicus* or from herbarium material were also examined. 47 RAPD and 19 AP-PCR primers were tested on the defined 14 pools specified by different habitats. Among these primers 14 showed polymorphic patterns, respectively. For the statistical analysis (SPSS 8.0) the binary codes of 111 fragments of these 14 polymorphic primers were used. Genetic distances were calculated then cluster and multidimensional analysis (MDS) were performed.

The amplification of the whole ITS region (ITS1-5.8S-ITS2) was achieved and then sequenced by an ABI Prism 310 device in 27 specimens of 10 species within the *F. Ovinae* group. Our own sequences were completed with other available sequences collected from the NCBI GenBank for the phylogenetic analysis: *Secale cereale* (AF303400) and *Poa pratensis* (AF171182) were used as outgroups. The sequences were first aligned by the ClustalW program and were further adjusted manually. The aligned sequences were analysed by maximum parsimony using MEGA (Molecular Evolutionary Genetics Analysis) program version 2.1. To test the reliability of the consensus tree bootstrap analysis was performed with 250 resampling.

The precise ploidy level of the studied taxa was determined by PARTEC I type flow cytometer.

3. RESULTS

This thesis contains results from two research fields that might seem far from each other at first sight. However, at molecular level there is a very close relationship, a single molecule (DNA) was investigated in both cases. In the first case it was modified by genetic transformation while in the second case the same segments of different DNA molecules were compared in molecular taxonomy.

In the first field: to start the transformation experiments in Hungary we had to establish a single, rapid and efficient regeneration system from leaves tissues, and a quick method of rooting of the gained shoots and acclimatization to the field. After that M26 rootstock and Royal Gala scion cultivars were transformed with the antisense gene and the transgenic trees were placed in a greenhouse. The examination of transgenic fruits took place in the USA at the research center of the Cornell University (New York State Agricultural Experiment Station, Department of Food Science and Technology, Geneva NY). More than 150 fruit bearing transgenic trees were available for the investigation which were originated from different transgenic events. In this field our results mean the first data to address the transgenic fruits in which the ethylene biosynthesis was inhibited by genetic engineering.

Great difference was observed in ethylene production of each transgenic tree. Most of the lines showed no significant difference compared to controls while in some lines higher ethylene production was observed. In those transgenic lines where the inhibition of ethylene production worked well (2-3 %), the delay of onset of ethylene synthesis was 1-4 weeks. After delay, the autocatalytic ethylene synthesis started up in these fruits as well, but the peak of ethylene level remained lower. These fruits barely differed from the control´s regarding sugar content, while the firmness of their fruit-flesh kept up for a longer time. Our results proved that the introduction of an antisense ACC-synthase gene into the apple genome inhibited the softening of fruits, also.

Expression of *MdACS*2 gene was followed from early stage of fruit development to the second month after harvesting by Northern hybridization. The gene activity was detectable only after the harvesting time, so the *MdACS*2 gene has a ripening specific expression. A strong correlation was perceived between the measured ethylene level and mRNA accumulation during storage.

In cold storage the fruits of the best transgenic lines reached their peak of ethylene production after a delay of 3-4 months.

According to our detailed examinations, this molecular approach based on antisense technology can provide an alternative way to replace the commonly used and costly atmospheric storage of fruits.

In the second field our aim was the molecular comparison of the most disputed *Festuca* species belonging to the *Ovinae* section. Among the tested 47 RAPD and 19 AP-PCR primers, eight and six showed polymorphic patterns, respectively. For the determination of genetic distance between the examined species, the binary codes of 111 fragments of these 14 polymorphic primers were used. The examined *Festuca* species were classified into three well-separable groups on the basis of these results.

It has been proved that the separate *Festuca rupicola* groups independently of their separation based on the shape and size of their leaves, compose uniform species at molecular level.

It has been proved that the sub-Mediterranean tetraploid *F. pallens* (4x) differs genetically from the subalpine diploid *F. pallens* (2x), which may modify the present classification.

The species *F. javorkae* and *F. rupicola* can be differentiated from each other at the molecular level based on a 800 bp fragment amplified by the PAL1 primer.

It is worthy of note that the species *F. wagneri* based on our molecular classification was placed in a different group than its supposed parent species (♀: *F. vaginata*, ♂: *F. valesiaca* or *F. pseudovina* or *F. rupicola*).

After the above experiments based on PCR techology, comparison of internal transcribed region (ITS) have been executed at sequence level in the studied species. The amplification and sequencing of the ITS region was achieved in 27 specimens of 10 species.

The determined sequences were placed into the NCBI Genbank where among the 10 species in 8 cases this meant the first entry. Sequencing resulted in a fragment of 596 bp in each species. Intraspecific ITS variation was detected only in *F. rupicola.* In the other 9 species there was no detectable difference between their individuals even if they originated from different locations. In these species polymorphism was manifested only in intragenomic differences which can be explained by the high number of copies of rDNA and the possible differences between them.

For the phylogenetic analysis of the whole *Festuca* genus, our isolated sequences were completed with all available ITS sequences from the Genbank which were isolated from other *Festuca* species. The found 1609 parsimonuos Maximum Parsimony trees were 629 steps long and the strict consensus tree was tested by bootstrap analysis. According to our results, the early divergence of broad-leaved and fine-leaved fescues was confirmed while the existence of a third and basal *Festuca* clade was not supported. The phylogeny of broad-leaved *Festuca* species is almost completely resolved, while in the case of fine-leaved *Festuca* species more detailed experiments are required, although the monophyletic and basal origin of the *Festuca rubra* aggregate was confirmed.

3.1. New scientific achievements

Molecular inhibition of ethylene biosynthesis in apples

1. The successful inhibition – and the differences of the rate of inhibition in different transgenic lines – was proved in transgenic apples, where the ethylene biosynthesis was inhibited by the introduction of an antisense ACC-synthase gene.
2. It was proved that the expression of the *MdACS*2 gene is ripening-specific and the mRNA accumulation of the gene is in close correlation with the ethylene production of the transgenic fruits.
3. It was proved that the fruits of the transgenic lines where the strongest inhibition of ethylene production was achieved could be stored for 1-2 months longer at room temperature without spoilage, compared to non-transformed control fruits.
4. It was proved that for the long storage of transgenic fruits where the ethylene synthesis was blocked there is no need to use controlled O_2, CO_2 and humidity atmosphere storage.
5. It was proved that – with the antisense inhibition of ethylene biosynthesis – the softening of fruits was also inhibited.

Molecular taxonomy of different *Festuca* species

6. A problematic group of ten *Festuca* taxa belonging to the *Ovinae* group was separated into three groups using RAPD and AP-PCR primers. The genetic distance between the studied taxa was estimated by cluster and multidimensional analysis. It has been proved that the separate *Festuca rupicola* groups – independently of their separation based on the shape and size of their leaves – compose uniform species at molecular level. The species *F. javorkae* and *F. rupicola* can be differentiated from each other at the molecular level based on a 800 bp fragment amplified by the PAL1 primer.
7. It was proved that *Festuca pallens* located in the Budai mountains is tetraploid and differs genetically from the subalpine diploid *F. pallens*, which may modify the present classification.

8. Among the 10 studied species in 8 cases their ITS (internal transcribed spacer) sequences were the first entry in the NCBI Genbank. Based on the results of the ITS sequences the examined species were indistinguishable, supposedly they should be considered as taxa under species level.

9. A phylogenetic tree was made of the whole *Festuca* genus *in silico*, based on their ITS sequences. In the fined-leaved fescues, the monophyletic and basal origin of the *Festuca rubra* aggregate was confirmed.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Molecular inhibition of ethylene biosynthesis in apples

In this field our results supply the first data in the matter of post-harvest feature of ethylene inhibited transgenic apple fruits.

It has been assessed according to our results that the molecular inhibition of ethylene biosynthesis can be achieved by genetic transformation of *MdACS*2 gene in antisense orientation. Among numerous transgenic lines originated from different transformation events it is possible to choose the ones, whose fruits show sufficient decline in their ethylene production. Since the activity of cell wall degradable enzymes is under direct ethylene control as well, the softening of transgenic fruits was also inhibited.

The fruits could be stored for 1-2 months longer at room temperature without spoilage, consequently those can be stored longer at the shelves of markets, as well. The initiation and the peak of autocatalytic ethylene synthesis can be delayed for 4-5 months storing at cold temperature (5°C). It was proved that for the long storage of transgenic fruits where the ethylene synthesis was blocked there is no need to use controlled O_2, CO_2 and humidity atmosphere storage, so significant cost can be saved using this technique.

Following examinations of TG508 transgenic line where supposedly due to a good positional effect the inition of ethylene synthesis was blocked may provide additional information of physiology and biology of fruit ripening to clarify the molecular mechanisms.

Economically, it should be important in the future to replace the widely used constitutive viral promoter in the vector used for plant transformation with a ripening and tissue/organ specific promoter.

4.2. Molecular taxonomy of different *Festuca* species

Molecular analysis of the 10 studied *Festuca* species present in the Carpathian Basin had not been conducted until our examinations.

The used RAPD and AP-PCR molecular methods was proved to be appropriate to determine the identity or to distinguish the studied taxa of the *Festuca* genus. The further investigation at sequence (internal transcribed spacer) level was not successful to demonstrate the differences between the species. Presumably these species are in very close relationship at evolutionary level.

Since their basic ITS sequences were the same in all cases and ITS sequences deriving from other genomes were not found, supposedly in case of species with higher ploidy level we face autopolyploidy.

Further on, other sequence level comparisons are planned, namely genes, introns and/or intergenic spacers of chloroplast and mitochondria DNA because of the possible cross fertilization. Other molecular markers are planned to be introduced into the clarification of taxonomic problems. Specific microsatellite markers for the *Festuca* genome have not been available yet and *Lolium* specific markers do not work well. Thus, AFLP technique is planned to be included in our further investigations.

5. PUBLICATIONS RELATED TO THE TOPICS OF THE THESIS

Scientific articles:

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** N. Nagy, L.E. Heszky, E. Tóth, G. Hrazdina (2000): Production of transgenic carnation with antisense ACS (1-aminocyclopropane-1-carboxylate synthase) gene. International Journal of Horticultural Science pp. 103-107

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** N. Nagy, L.E. Heszky, E. Tóth, G. Hrazdina (2000): Transformation of Carnation: Agrobacterium-mediated Transformation of Carnation with Antisense 1-aminocyclopropane-1-carboxylate Synthase (ACS) Gene. In: Hrazdina G. (ed.): Use of Agriculturally Important Genes in Biotechnology, 91-97. IOS Press (NATO Science Series) Amsterdam, Berlin, Oxford, Tokyo, Washington D.C.

Galli Zs., Debreceni D., Kiss E., Heszky L.E. (2001): Az alma sikeres transzformációját befolyásoló tényezők. Kertgazdaság 33: 23-32

Galli Zs., Penksza K., Kiss E., Bucherna N., Heszky L.E. (2001): *Festuca* fajok molekuláris taxonómiai vizsgálata: a *Festuca ovina* csoport RAPD és AP-PCR analízise. Növénytermelés 50 (4): 375-384

A. Veres, E. Kiss, **Z. Galli**, N. Nagy, E. Tóth, Á. Varga, G. Hrazdina, L.E. Heszky (2002): Transgenic carnation harbouring antisense ACS (1-aminocyclopropane-1-carboxylate synthase) gene. Bulletin of the Szent István University 2001-2002 pp. 49-56

Z. Galli, E. Kiss, G. Hrazdina, L.E. Heszky (2003): The effects of *ACS* (1-aminocyclopropane-1-carboxylate synthase) gene down regulation on ethylene production and fruit softening in transgenic apple. International Journal of Horticultural Science 9 (2): 65-70

Proceedings:

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** L.E. Heszky, E. Tóth, G. Hrazdina (1999): Genetic transformation of carnation to downregulate ethylene biosynthesis. In Vitro Cellular and Developmental Biology - Plant 35: 174-175

Penksza K., **Galli Zs.,** Bauer L., Kiss E. (2001): Morfológiai és molekuláris biológiai vizsgálatok Kárpát-medencei *Festuca* fajokon. II. Kárpát-medencei Biológiai Szimpózium. 33-37. o

Galli Zs., Kiss E., G. Hrazdina, Heszky L.E. (2003): Az ACS (1-aminociklopropán-1-karboxilát szintáz) gén antiszensz gátlásának hatása transzgénikus alma tárolási paramétereire. EU Konform – Mezőgazdaság és Élelmiszerbiztonság. Nemzetközi Tudományos Konferenia SZIE, Gödöllő június 5-6. 85-91. oldal

Oral presentations:

Kiss E., **Galli Zs.,** Veres A., Varga Á., Tóth E., Hrazdina G., Heszky L.E. (1999): Szegfű és alma transzformációja az etilénbioszintézis módosítása céljából. IV. Magyar Genetikai Kongresszus Siófok, 1999 ápr. 11-14 Összefoglalók 88-89. oldal

E. Kiss, A Veres, Á. Varga, **Z. Galli,** N. Nagy, L.E. Heszky, E. Tóth, G. Hrazdina (1999): Transformation of carnation to modify ethylene production. Use of Agriculturally important genes in agricultural biotechnology. NATO Advanced Research Workshop, Szeged 1999 okt. 17-21 Abstracts book p. 7

E. Kiss, **Z. Galli,** E. Halász, K. Varga, G. Hrazdina, L.E. Heszky (2001): Apple transformation experiments with antisense ACC (1-aminocyclopropane-1-carboxylate synthase) gene. Meeting of International Federation of Fruit Juice Producers (IFU) Workshop on Food Safety, Budapest. Összefoglalók 4-5. o

Galli Zs. Debreceni D. Halász E. Varga K. Kiss E. (2001): Az alma in-vitro szaporítása és genetikai transzformációja. "Tavaszi Szél" 2000, 2001. Fiatal magyar tudományos kutatók

és doktoranduszok IV. és V. világtalálkozója. Utókiadvány 3-4. o

Galli Zs., Penksza K., Kiss E., Heszky L., Heszky L.E. (2001): A Kárpát-medencei *Festuca rupicola* alakkör fajainak molekuláris szintű genetikai vizsgálata. Magyar Biológiai Társaság: Botanikai szakosztályülés márc.19

Penksza K., **Galli Zs.,** Bauer L., Kiss E. (2001) Morfológiai és molekuláris biológiai vizsgálatok Kárpát-medencei *Festuca* fajokon. II. Kárpát-medencei Biológiai Szimpózium nov. 20-22 Botanika szekció

Penksza K., Bauer L., **Galli Zs.,** Engloner A., Kiss E., Bucherna N., Heszky L.E. (2002): Morfológiai és molekuláris taxonómiai vizsgálatok Kárpát-medencei *Festuca* fajokon. Aktuális flóra- és vegetációkutatások a Kárpát-medencében V. Pécs, márc. 8-10

Galli Zs., Kiss E., G. Hrazdina, Heszky L.E. (2003) Post-harvest érés késleltetése az etiléntermelés gátlásával almában. IX. Növénynemesítési Tudományos Napok MTA Bp. márc 5-6. Összefoglalók 53. o

Penksza K., **Galli Zs.,** Bauer L., Kiss E., Bucherna N., Illyés Z., Rudnóy Sz.,Bratek Z., Heszky L.E. (2003): A Kárpát-medence *Festuca ovina* csoportjának újabb taxonómiai eredményei. Magyar Biológiai Társaság: Botanikai szakosztályülés márc. 10

J. Dobránszky, I. Hudák, K. Magyar-Tábori, E. Jámbor-Benczúr, **Z. Galli,** E. Kiss (2003): Role of different cytokinins in the shoot regeneration of apple leaves. 5th. International Symposium in the series recent Advances in Plant Biotechnology. Book of abstracts: p. 7

Posters displayed:

Galli Zs., Á. Varga, A. Veres, E. Kiss (1997): Constructing vectors for plant transformation harbouring the aminocyclopropane carboxylate (ACC) synthase gene. VIII. European Congress on Biotechnology, Budapest August 18-22. Abstracts book p.: 48

Galli Zs., Boldizsár A., Varga Á., Veres A., Lendvai A., Hrazdina G., Kiss E., Heszky L.E. (1998): Növénytranszformációs vektorok előállítása az ACC-szintáz-gén felhasználásával. IV. Növénynemesítési Tudományos Napok MTA, 1998 jan. 28-29 Poszter szám: 14.

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** L.E. Heszky, E. Tóth, G. Hrazdina (1998): Agrobacterium-mediated transformation of carnation with antisense 1-aminocyclopropane-1-carboxylate synthase (ACS) gene. IX. International Congress on Plant Tissue and Cell Culture, Israel June 14-19, Abstracts book p.: 152

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** L.E. Heszky, E. Tóth, G. Hrazdina (1998): Production of transgenic carnation to modify ethylene biosynthesis. XVII. International Congress of Genetics, China August 10-15. Abstracts book p.: 169

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** L.E. Heszky, E.Tóth, G. Hrazdina (1998): Genetic transformation of carnation to down-regulate ethylene biosynthesis. Symposium zum Gedenken an die 100. Wiederkehr der Begründung der Gewebekultur durch Gottlieb Haberlandt, Wien Oktober 8-9. Abstracts book p.: 36

E. Kiss, A. Veres, Á. Varga, **Z. Galli,** N. Nagy, L.E. Heszky, E. Tóth, G. Hrazdina (1998): Transgenic carnation harbouring antisense *ACS* (1-aminocyclopropane-1-carboxylate synthase) gene. First European Symposium on Applied Genome Research, Belgium, Brussels, Nov. 26-27, Abstracts book p.: B13

Debreceni D., Petus M., **Galli Zs.,** Kiss E., Heszky L.E. (1999): Alma mikroszaporítása és molekuláris transzformációja. V. Növénynemesítési Tudományos Napok MTA, 1999 márc. 9. Összefoglalók: 63. o

Galli Zs. Debreceni D. Halász E. Varga K. Kiss E. Heszky L.E. (2001): Az alma in-vitro szaporítása és genetikai transzformációja. VII. Növénynemesítési Tudományos Napok MTA Bp. Összefoglalók 87. o

Galli Zs., Penksza K., Kiss E., Heszky L.E. (2001): *Festuca* fajok molekuláris taxonómiai vizsgálata. VII. Növénynemesítési Tudományos Napok MTA Bp. Összefoglalók 88. o

Galli Zs. Debreceni D. Halász E. Varga K. Kiss E. (2001): Az alma in-vitro szaporítása és genetikai transzformációja. "Tavaszi Szél" 2000, 2001 Fiatal magyar tudományos kutatók és doktoranduszok IV. és V. világtalálkozója. Utókiadvány 36-37. o

Z. Galli, G. Hrazdina, E. Kiss, C. Rosenfield, H.S. Aldwinckle, J.L. Norelli (2002): Down regulation of ethylene production in apples. Fulbright Challenges and Responses 10th Anniversary Conference MTA Bp. ápr. 24-25 Abstracts book p. 18

Galli Zs., Kiss E., G. Hrazdina, Heszky L.E. (2003): Az *ACS* (1-aminociklopropán-1-karboxil-szintáz) gén antiszensz gátlásának hatása az etiléntermelésre és a gyümölcsök puhulására almában. V. Magyar Genetikai Kongresszus Siófok ápr. 13-15 Összefoglalók 105. o

Z. Galli, K. Penksza, E. Kiss, N. Bucherna, L.E. Heszky (2003): Phylogenetic analysis in *Ovinae* section of *Festuca* genus (*Poaceae*) based on ITS rDNA sequences. VII. International Congress of Plant Molecular Biology, Barcelona June 23-28. Book of abstracts 253. o

5.1. Publications not related to the topics of the thesis

Proceedings:

E. Kiss, A. Balogh, P. Kozma, T. Koncz, **Z. Galli,** L.E. Heszky (2003): Molecular analysis of Grapevine Cultivars Indigenous in the Carpathian Basin. Proceedings of the Eight International Conference on Grape genetics and Breeding. Acta Horticulturae 603: 95-102

P. Kozma, A. Balogh, E. Kiss, **Z. Galli,** T. Koncz, L.E. Heszky (2003): Study of Origin of Cultivar „Csaba Gyöngye" (Pearl of Csaba). Proceedings of the Eight International Conference on Grape genetics and Breeding. Acta Horticulturae 603: 585-592

Oral presentations:

E. Kiss, A. Balogh, P. Kozma, T. Koncz, **Z. Galli,** L.E. Heszky (2002): Molecular analysis of grape vine cultivars indigenous in the Carpathian Basin. VIII. International Conference on Grape Genetics and Breeding. Kecskemét 26-31 August. Abstracts book E10

Posters displayed:

Galli Zs. Halász E. Varga K. Kiss E. Heszky L.E. (2001): Erwinia amylovora elleni rezisztencia kialakítása almában géntechnológiai módszerrel 47. Növényvédelmi Tudományos Napok MTA Bp. febr 27-28. Összefoglalók 146. o

P. Kozma, A. Balogh, E. Kiss, T. Koncz, **Z. Galli,** L.E. Heszky (2002): Study on origin of cultivar „Csabagyöngye". VIII. International Conference on Grape Genetics and Breeding. Kecskemét August 26-31. Abstracts book P38

Balogh A., Kozma P., Kiss E., **Galli Zs.,** Koncz T., Kocsis M., Veres A., Heszky L.E. (2003): Kárpát-medencei szőlőfajták jellemzése mikroszatellit analízissel. IX. Növénynemesítési Tudományos Napok MTA Bp. Összefoglalók 76. o

Füle L., Bucherna N., **Galli Zs.,** Hódosné Kotvics G., Heszky L.E. (2003): A Perenne interspecifikus hibrid rozs molekuláris elemzése. V. Magyar Genetikai Kongresszus Siófok ápr. 13-15 Összefoglalók 85. o

Veres A., Balogh A., Kozma P., Kiss E., **Galli Zs.,** Koncz T., Kocsis M., Heszky L.E. (2003): SSR analízis alkalmazása Kárpát-medencei szőlőfajták jellemzésére. V. Magyar Genetikai Kongresszus Siófok ápr. 13-15 Összefoglalók 99. o

Kiss E., Balogh A., Veres A., **Galli Zs.,** Koncz T., Kocsis M., Heszky L.E., Kozma P. (2003): SSR analysis of grapevine varieties autochthonous in the Carpathian Basin. VII. International Congress of Plant Molecular Biology, Barcelona June 23-28. Book of abstracts 408. o