JAUNU KARTUPEĻU ŠĶIRŅU PĒTĪJUMI LIETUVĀ

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Kartupeļu selekcija un sēklaudzēšana Lietuvā tiek veikta Lietuvas zemkopības institūta Vokes nodaļā. Kartupeļu selekcijas darbs aptver Lietuvas kartupeļu šķirnes, kolekcijas šķirnes un klonus. Kartupeļu krustošana tetraploīdā līmenī tiek veikta siltumnīcās un lauka kolekcijā. Vairāk kā divi miljoni hibrīdu (klonu) tiek izvērtēti izmēģinājumu laukā. Galvenais mērķis ir veidot jaunas kartupeļu šķirnes, kas ir izturīgas pret vēzi un nematodēm, kurām ir augsts izturības līmenis pret citām slimībām, izcilas agronomiskās un garšas īpašības, kā arī piemērotība pārstrādei. Selekcijas darba rezultātā izveidotas piecas jaunas kartupeļu šķirnes: Venta, VB Rasa, VB Liepa, Goda un VB Aista. Tās visas ir izturīgas pret bīstamāko kartupeļu slimību – kartupeļu vēzi (*Synchtrium endobioticum* Schilb), vairākas no tām ir izturīgas pret vietējo nematodes patotipu (*Globodera rostochiensis* Woll.). Citas pazīmes kā augsta raža, izcilas garšas īpašības, kā arī pievilcīga forma bija galvenie iemesli šo šķirņu atlasē.

Kartupeļu sēklaudzēšana ar meristēmu metodi tiek veikta Lietuvas zemkopības institūta Vokes nodaļas biotehnoloģiskajā laboratorijā. Tas ir kartupeļu sēklaudzēšanas centrs Lietuvā.

APPLYING COLCHICINE AND ORYZALIN IN LILIUM L. POLYPLOIDISATION

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Abstract

The genus Lilium (*Lilium* L.) is a vegetatively propagated bulbous plant – one of the economically most important of bulb flowers. To obtain new varieties with a wide range of colors and resistance to grey mold caused by fungi *Botrytis* Micheli ex Fr. a breeding program was carried out. The spreading of this fungus disease causes heavy losses as plants loose their general attractiveness. In lily breeding current activities are directed towards the development of disease resistant cultivars to avoid the use of chemicals to be economically sound and ecologically safe. The goal of this research was to investigate and to optimize polyploidy in the breeding of lilies. Several biotechnological methods were used to obtain new lily varieties. A crossing between different hybrid groups of lilies is not possible under natural conditions therefore embryo cultivation techniques are being developed to overcome incompatibility between plants and limiting factors after fertilisation. Mitotic and meiotic polyploidisations are applied and can result in fertile allopolyploids. The chromosome count of the varieties can be changed by treating bulb scales with a 0.1, 0.5 and 1 g kg^{-1} colchicine solution and 0.05, 0.1 and 0.5 g kg^{-1} oryzalin solution.

Key words: chromosomes, mitotic and meiotic polyploidisations

Introduction

The genus *Lilium* L. includes approximately 100 species, subspecies and varieties of species distributed throughout the cold and temperate parts of the Northern Hemisphere (McRae, 1998). The overall appearance of all plants is controlled largely by their genes that are packaged in chromosomes. Each species has a fixed number of chromosomes in their cells, but the number may differ between species. Each cell of *Lilium* species has 24 chromosomes, or 12 pairs of different chromosomes (2n = 2x = 24). These plants with their paired chromosomes are termed diploid, from the Greek word for ‘double’ (McRae, 1998). Polyploids have more chromosomes in every cell than others. The offspring of a tetraploid parent and a diploid parent is a triploid; this results from a failure of meiosis in one of the parents. With their 36 chromosomes, triploid lilies are difficult to cross with others. Tetraploid lilies have 48 chromosomes; this is double the normal number of diploids.

The reasons for using polyploidy in lily breeding are the larger flowers, the stronger stems and in interspecific hybridization the restoration of F1-sterility at the tetraploid level (Van Holsteijn,

1994; Van Tuyl *et al.*, 1990). Interspecific hybridisation and polyploidy are recognized as the most important sources of evolution and domestication of flowering plants. To overcome F1-sterility, mitotic and meiotic polyploidisations are applied and can result in fertile allopolyploids. A distinction was made between mitotic polyploidisation and meiotic polyploidisation. Mitotic polyploidisations possess one homologous chromosome set. Mitotic polyploidisation comprises all techniques in which artificial chromosome number doubling was accomplished by treating bulb material with colchicine. Meiotic polyploidisation often shows irregular chromosome division that results in two unreduced chromosome numbers (Van Tuyl *et al.*, 1990; Van Tuyl and Ki-Byung, 2003).

Polyploids are obtained through artificial chromosome doubling by treatment of vegetative tissue with spindle inhibitors such as colchicine (Blakeslee and Avery, 1937; Emsweller, 1988) or oryzalin. Colchicine has been used for doubling the number of chromosomes of many crop plants over a period of more than 50 years (Blakeslee and Avery, 1937). Colchicine is a natural alkaloid with an antimitotic activity, obtained from the plant *Colchicum autumnale* L. (Emsweller, 1988; Van Tuyl *et al.*, 1990). When colchicine is present in a cell that is undergoing mitotic division, the chromosomes split at all points except the centromere. The main action of the colchicine is to prevent the formation of a spindle so the anaphase movement of the chromosomes does not take place and the cell fails to divide. When the daughter chromosomes finally divide, they are all included in one cell and the chromosome number is doubled. To be effective colchicine must be present in the cell when the chromosomes divide. Colchicine is very harmful to humans and in some cases shows undesirable mutagenetic activity on plants (Van Tuyl *et al.*, 1990). In addition to colchicines, several other chemicals are also effective in doubling the chromosome number. One of the chemicals that also inhibits mitosis activity and is used for doubling the chromosome number in lilies is oryzalin. For doubling the chromosome number, oryzalin is used for other plants as well – such as: potatoes (Van Tuyl *et al.*, 1992; Verhoeven *et al.*, 1990), tobacco (Scree Ramulu *et al.*, 1991). The goal of this research was to investigate and to optimize polyploidy in the breeding of lilies.

Materials and Methods

The polyploid forms were produced utilizing bulb scales of diploid lilies (2n = 2x = 24). In the present study, scales from diploidal lily bulbs from 13 different genotypes were tested: Asiatic hybrids – 'Arabeska', 'Baltais Lācis', 'Brushstroke', 'Evrika', 'Lastočka', 'Lolly', 'Miss Alice', 'Nakts Tango', 'Saules Meita', 'Višenka'; Trumpet hybrid 'Zemgale' and the species *L. kesselringianum* Miscz. and *L. monadelphum* Bieb. The chemicals colchicine and oryzalin were used in chromosome doubling. Bulb scales were treated with a 0.1, 0.5 and 1 g kg^{-1} colchicine solution and a 0.05, 0.1 and 0.5 g kg^{-1} oryzalin solution.

For chromosome number determination, the bulbs were kept in a washed river sand medium at 25 ºC for 3 weeks – until clean and white root tips had developed. Prior to chromosome counting, these bulbs were kept for 24 hours at 4 ºC. After this treatment, undamaged healthy root tips were cut off – about 5 to 7 mm long, and washed under running water. Because the process of cell division can be stopped by colchicine before chromosomes multiply, all dividing cells are allowed to proceed up to this stage. The cut root tips were put into 50 ml beakers filled with 0.7 g kg^{-1} colchicine solution and kept for 2 hours; then washed three times in running water and transferred to a modified Clarke's Fluid (750 g kg^{-1} ethyl alcohol, 250 g kg^{-1} ethanol glacial acetic acid) for 30 min at 20 ºC. The acetic acid effect was neutralized by keeping the tips of the rootlets for 45 minutes in distilled water and 24 hours in 700 g kg^{-1} ethyl alcohol. For chromosome counting, the root tips were left for 48 hours in a colouring solution of 5 g kg^{-1} carmine in 450 g kg^{-1} propionic acid. The root tips were cut to about 1-2 mm and, in a drop of stain, mashed with a steel needle. The preparation was then covered. With a microscope, the chromosomes were counted and cells of five rootlets inspected for each genotype.

Results and Discussion

The bulb scales were treated with a 0.1, 0.5 and 1 g kg^{-1} colchicine solutions. The treatment with 0.1 g kg^{-1} colchicine solution resulted in the production of 1.1 bulblets per scale on average (min - 0.1; max - 2.8) (Fig 1).

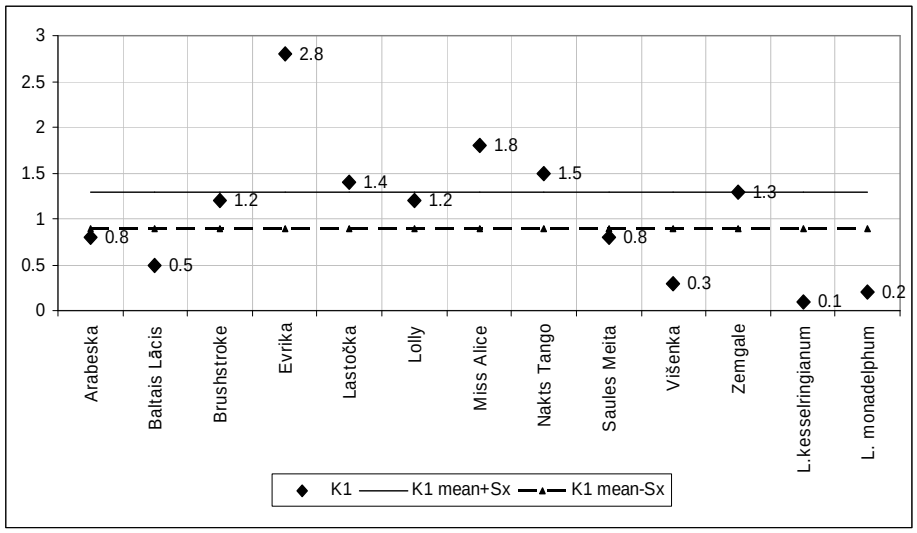

Figure 1. The results of the production of bulblets by treated bulb scales with 0.1 g kg^{-1} colchicine
Abbreviations: K1 – 0.1 g kg^{-1} colchicine; Sx – Standard error

The treatment with a 0.5 g kg^{-1} colchicine solution resulted in 0.97 bulblet on average (min - 0.1; max - 1.9) (Fig 2).

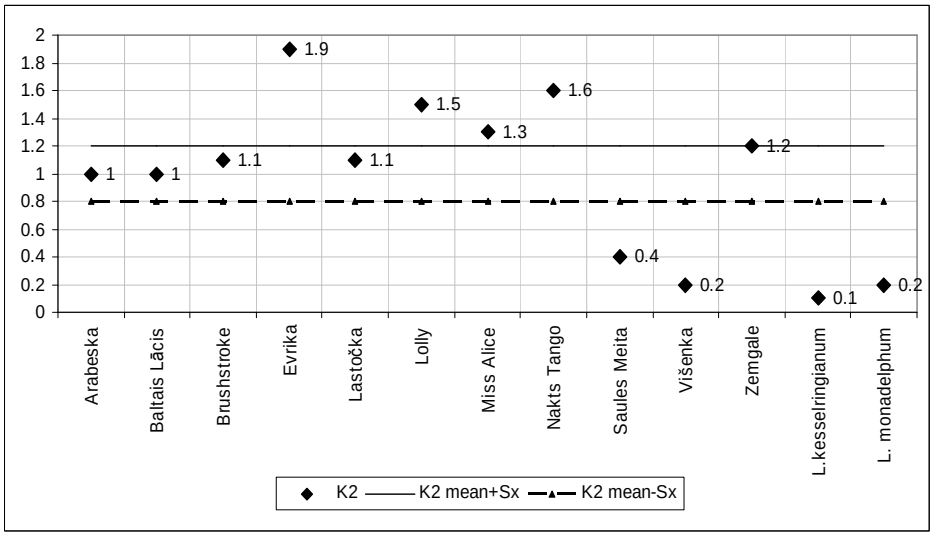

Figure 2. The results of the production of bulblets by treated bulb scales with 0.5 g kg^{-1} colchicine
Abbreviations: K2 – 0.5 g kg^{-1} colchicine; Sx – Standard error

Bulb scales treated with 1 g kg^{-1} colchicine solution produced 0.2 bulblet per scale on average (Fig 3). This concentration turned out to be toxic. The use of this concentration resulted in the production of 10 polyploid plants.

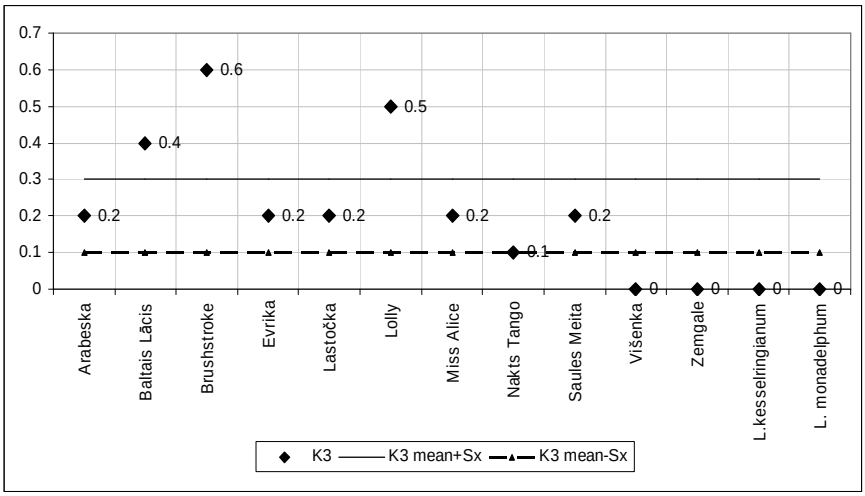

Figure 3. The results of the production of bulblets by treated bulb scales with 1 g kg^{-1} colchicine
Abbreviations: K3 – 1 g kg^{-1} colchicine; Sx – Standard error

The cultivars that excelled with a greater amount of min and max bulblets were: 'Evrika' (1.9 and 2.8), 'Miss Alice' (1.3 and 1.8), 'Nakts Tango' (1.5 and 1.6), 'Lolly' (1.2 and 1.5). On average, less than 1 bulblet was obtained with 'Višenka' (0.2 and 0.3) and 'Saules Meita' (0.4 and 0.8). The species *L. monadelphum* produced 0.2 and 0.2 bulblets on average, and *L. kesselringianum* P. Miscz. - 0.1 and 0.1 bulblets on average. These results may be traced to the sensitivity of the species to concentrations of colchicine solution. Polyploid plants were not produced.
Bulb scales were treated with oryzalin solution in concentrations of 0.05, 0.1 and 0.5 g kg^{-1} (Fig 4; Fig 5 and Fig 6).

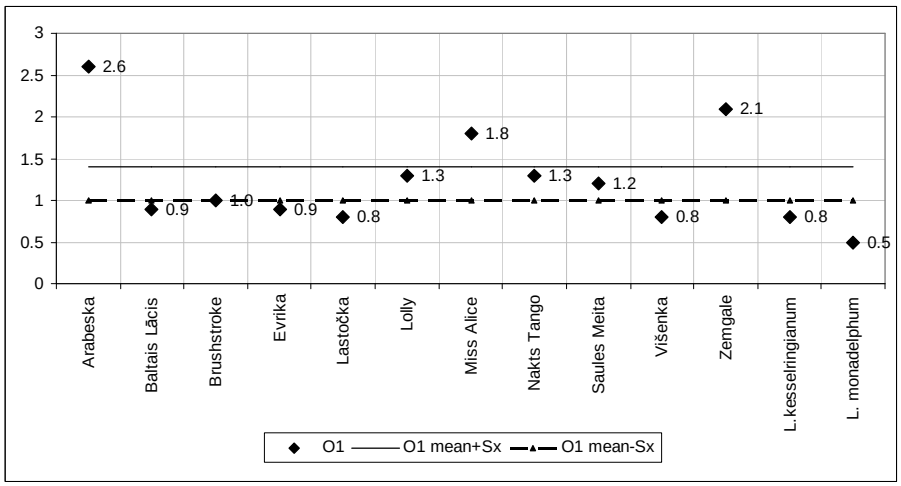

Figure 4. The results of the production of bulblets by treated bulb scales with 0.05 g kg^{-1} oryzalin
Abbreviations: O1 – 0.05 g kg^{-1} oryzalin; Sx – Standard error

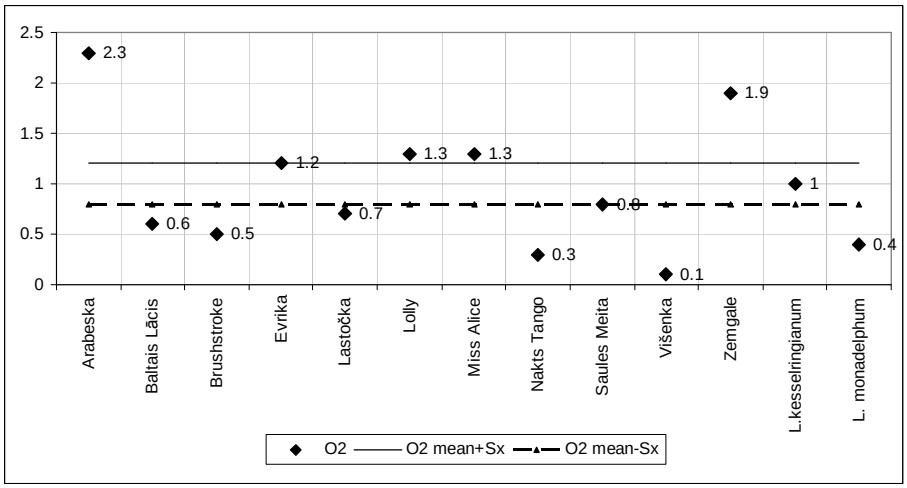

Figure 5. The results of the production of bulblets by treated bulb scales with 0.1 g kg^{-1} oryzalin
Abbreviations: O2 – 0.1 g kg^{-1} oryzalin; Sx – Standard error

The obtained average yield of bulblets per scale was, respectively, 1.23 (min - 0.5, max - 2.6), 0.95 (min - 0.1, max - 2.3), and 0.42 (min 0.0, max 0.9). The use of 0.05 g kg^{-1} oryzalin solution resulted in 2 polyploid plants, 0.1 g kg^{-1}- in 13 polyploid plants, but with 0.5 g kg^{-1} oryzalin solution no polyploids were produced. Bulblets more in count and greater in size were developed from outer scales of bulbs when compared to inner thinner scales.

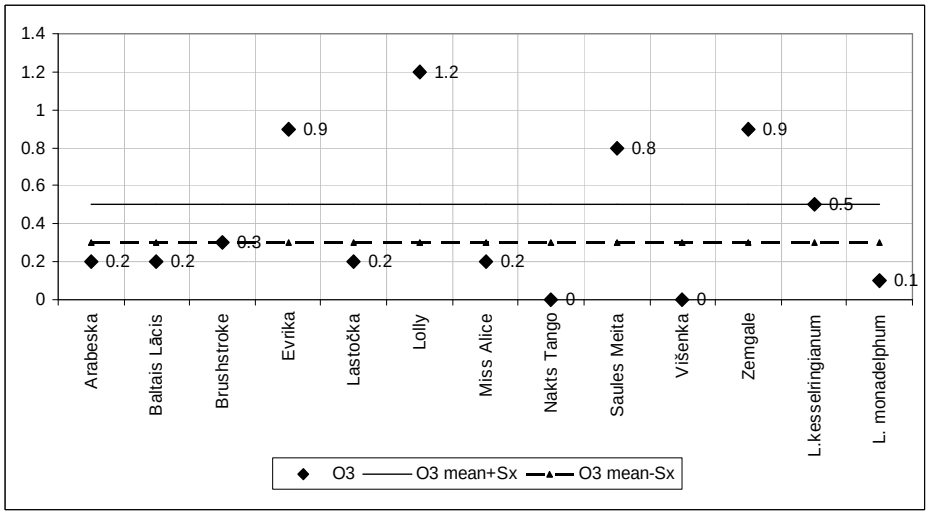

Figure 6. The results of the production of bulblets by treated bulb scales with 0.5 g kg^{-1} oryzalin
Abbreviations: O3 – 0.5 g kg^{-1} oryzalin; Sx – Standard error

According to their morphological traits, selections of polyploidy plants were made and the degree of polyploidy was determined cytologically. Morphological differences between the diploid and tetraploid forms of the same clone have been studied in Asiatic hybrid lilies. Lengths of stems, lengths and widths of leaves, lengths of petals, numbers of leaves and flowers, data on flowering, degrees of leaf scorch were all recorded at flowering time (Okazaki and Hane, 2005). According to the findings, most tetraploid forms came into bloom later than diploids; in conclusion – higher ploidity correlates with delayed flowering.
In our study we found that, in comparison with diploids, the polyploid plants we produced had a larger flower diameter (+2 up to 2.5 cm), more extended plant height (+10 up to 20 cm), increased

flower count (+2), and bloomed 4-10 days later than diploids of the same variety. A visual estimation of the bulbs also indicated differences. When compared to diploids, the roots of polyploids were shorter, rather stout, stumpy, smaller in numbers, and bulb scales were wider, more swollen with the outer scales and curved in a 90 degree angle.

The polyploid forms raise genotypic variability in diploid genotypes; improve their general attractiveness; increase plasticity and resistance against diseases and unfavorable biotic and abiotic conditions. Okazaki and Hane, 2005 suggested that the production of true tetraploid Asiatic hybrid lilies via colchicine treatment is necessary in polyploid breeding. A full understanding of the agronomic traits of polyploid lilies requires the evaluation of the morphological and physiological differences among diploids, triploids and tetraploids.

Conclusions

The scales of bulbs treated with 1 g kg^{-1} colchicine and 0.1 g kg^{-1} oryzaline solutions have successfully produced polyploid forms. The duration of exposure might be for 2, 4, 6 and 24 hours. Oryzalin inhibited plant cell division much more effectively than colchicine, and is applied successfully in doubling the number of chromosomes in lower concentrations (0.05, 0.1 and 0.5 g kg^{-1}) than colchicine (1 g kg^{-1}). In our research, 5.5 % polyploids were obtained from the total number of bulblets. The application of molecular, genomic and cytogenetic techniques can be of great help for fastening interspecific hybridisation programmes. Obtained polyploid forms were used in interspecific crossings to restore fertility.

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KOLHICĪNA UN ORIZALĪNA PIELIETOŠANA LILIJU (LILIUM L.) POLIPLOIDIZĀCIJĀ

Balode A.

Liliju ģints (*Lilium* L.) ir viena no ekonomiski nozīmīgākajām veģetatīvi pavairojamo sīpolaugu ģintīm. Lai iegūtu jaunas šķirnes ar vēlamām saimnieciskām īpašībām - plašu ziedu krāsu spektru, izturīgas pret pelēko puvi, kuru ierosina sēnes no *Botrytis* Micheli ex Fr. ģints, tiek veikta selekcija. Pelēkās puves infekcijas dēļ zaudējumus cieš liliju audzētāji, jo slimības rezultātā lilijas zaudē dekorativitāti. Mūsdienās selekcijas darbs ir vērsts uz to, lai veidotu pret slimībām izturīgas šķirnes, kuru audzēšanā nebūtu jāpielieto ķīmiskie augu aizsardzības līdzekļi, tā būtu ekonomiski izdevīga un ekoloģiski nekaitīga. Tā kā dažādu grupu liliju sugas savā starpā nekrustojas un, lai pārvarētu nesaderību, pēc apaugļošanās barjeru, izstrādā embriju kultivēšanas metodes. Poliploīdijas

pielietošana selekcijā arī paplašina mainības ietvarus, paaugstina augu plastiskumu un izturību pret nelabvēlīgiem biotiskiem un abiotiskiem apstākļiem. Pētījuma mērķis ir izpētīt un optimizēt poliploīdijas pielietošanas iespējas liliju selekcijā, apstrādājot liliju sīpolu zvīņlapas ar 0.1, 0.5 un 1 g kg^{-1} kolhicīna šķīdumu un 0.05, 0.1 un 0.5 g kg^{-1} orizalīna šķīdumu.

GENETIC FINGERPRINTING OF LATVIAN RED CLOVER (*TRIFOLIUM PRATENSE* L.) VARIETIES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS: COMPARISONS OVER TIME AND SPACE

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Abstract
We have established Simple Sequence Repeat (SSR) marker genetic fingerprinting protocols for red clover (*Trifolium pratense* L.) varieties found in the Latvian Gene Bank (LGB). As red clover is an obligate outcrosser, and the varieties are grown and renewed in the field without any particular isolation techniques, a high degree of intra-varietal heterogeneity is to be expected.
We analysed 7 diploid varieties, which were developed in three different breeding stations. We tested seeds from these varieties that were placed into the LGB in 1999 and 2000. In addition, we analysed a range of source material for one variety ('Priekuļi 66'). For this variety, we tested seeds that were repatriated from the VIR institute (placed into the VIR collection in 1982, seeds reproduced in 2005), the samples from the LGB (seed reproduction - 1997), and also plant material grown in the field this year (2007).
By analysing samples from various sources, we can examine the effect of space (varieties developed at different breeding stations), as well as time (repatriated seeds, LGB seeds, and current crop), in an obligate outcrossing crop species, where intra-varietal heterogeneity is high.

Key words: red clover, *Trifolium pratense L.*, genetic fingerprinting, Simple Sequence Repeat, Latvian Gene Bank, plant genetic resources

Introduction
Red clover is an important forage legume, widely grown in temperate regions and used in crop rotations. It is an obligate outcrosser, with a gametophytic self-incompatibility system (Taylor and Quesenberry 1996). Red clover is a diploid ($2n = 2x = 14$) species, however artificial tetraploid varieties have been created in breeding programs. Generally breeding programs are based on mass selection, and therefore the varieties produced are heterogeneous with highly heterozygous individuals. Initial molecular analyses of this species were undertaken using dominant marker systems such as RAPDs and AFLPs (Ulloa *et al*, 2003; Herrmann *et al*, 2005). The development of Simple Sequence Repeat (SSR) markers for red clover has allowed the analysis of these highly heterozygous varieties using highly informative co-dominant markers (Kolliker *et al*, 2006). At the Latvian Gene Bank, we are in the process of establishing protocols for genetic fingerprinting of all species in our collection. We have focused our efforts on the use of SSR markers for as many species as possible, due to their high information content (alleles per marker), co-dominant nature (which allows more sensitive detection of heterozygosity and variation within cultivars and lines), and their ease of use (provided appropriate SSR marker primers have been developed).
Our aims were to establish SSR fingerprinting protocols for the red clover varieties placed in the Latvian Gene Bank and to examine the inter- and intravarietal variation of Latvian clover varieties. Clover breeding has been undertaken in at least three breeding institutes in Latvia, and the Latvian Gene Bank holds seeds of accessions developed at all of these institutes. Currently, the main institute involved with clover breeding and maintaining the genetic resources is Skriveri Breeding Institute, but varieties have also been developed at the Priekuli Breeding Institute and Stende. Prior to the establishment of the Latvian Gene Bank, some of these cultivars developed at these breeding