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CYTOGENETIC ANALYSIS OF PROGENY DERIVED FROM ALLOTRIPLOID AND ALLOTETRAPLOID LILIES *LILIUM*

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Abstract

Cytogenetic analysis of progeny, performed after crossing four allotriploid and one allotetraploid cultivars of *Lilium* with fertile pollen producing tetraploids, showed that fertile female gametes of allopolyploids can possess a balanced haploid (n = x = 12) or diploid (2n = 2 x = 24) number of chromosomes as well as can contain an unbalanced aneuploid sets with additional number of chromosomes. Some offspring of allopolyploid mothers were apomictic and inherited diploid number of chromosomes. Allotriploid lilies were male sterile, however allotetraploid cultivar 'Lesly Woodrif' , which possessed 3 sets of chromosomes inherited from Trumpet hybrid and one inherited from Oriental hybrid lilies, was male fertile and produced haploid pollen.

Key words: *Lilium*, interspecific hybridization.

INTRODUCTION

Reproductive isolation barriers may act in pre- and post- zygotic stage and are one of the main criteria determining species and limiting exploration of interspecific hybrids in breeding programs. Different methods allowing to overcome incongruity at pre- and post- zygotic stage were employed and used for breeding of lilies (Van Tuyl et al., 1991; Chi, 2000). It resulted in appearance of *Lilium* hybrids with traits inherited from phylogenetically distant species or cultivars. New groups of lilies (OA, LA, OL) combining integrated traits of Asiatic hybrids (A), Oriental hybrids (O) and *L. longiflorum* were resulted and

are used in cut flower trade (Van Tuyl et al., 2000). However, if crosses between distantly related species are possible, in almost all cases the interspecific hybrids are highly sterile. The sterility of interspecific hybrids is determined by the disturbed separation of chromosomes during meiosis. It leads to the formation of gametes with unbalanced chromosome set. Doubling of the chromosome set in the interspecific hybrids of the first generation allows restoration of their fertility. Opposite to animals, plants tolerate polyploidy, and interspecific hybridization and polyploidy are important factors in plant evolution and breeding (Harlan end De Wit, 1975). 70% species of angiosperms are contributed by polyploidization at least once. It is assumed that unreduced 2n gametes mainly contributed for the origin of natural polyploidy in plants (Harlan and De Wit, 1975). Some interspecific hybrids tend to produce relatively high frequencies of unreduced gametes (Lim et al., 2004). If such gametes of F1 interspecific hybrids are involved in formation of a second generation, it gives rise to fertile allotetraploids possessing two pairs of different genomes. Experimental doubling of chromosome set in interspecific hybrids allows to overcome sterility if spontaneous formation of unreduced gametes is prevented. The traditional method of restoring fertility in such cases is treatment of meristematic cells of F1 hybrids by factors that prevent separation of chromatids during mitosis or application of tetraploid paternal forms for hybridization (Lim et al., 2000; Lim et al., 2005). Such allopolyploids are appropriately called „permanent“ hybrids, because their progeny never segregate from parental characters due to the strict autosyndetic pairing of different genomes in allopolyploids (Lim, 2000). However, selection of combinations with desirable traits inherited from different species and separation from undesirable ones is the main goal of breeding. New combinations of traits may arise in progeny derived from interspecific hybrids only in cases when recombination happens between alien genomes. It was recently shown that different mechanisms, e.g. genomic rearrangement and epigenetic factors, are involved to coordinate action of two different genomes in allopolyploid hybrids (Comai, 2000; Molinier, 2004). It was shown that in cases when F1 interspecific hybrids produced unreduced diploid gametes, some of them possessed recombinant chromosomes resulted by crossing over between alien

homeologous chromosomes as well as some of them possessed hybrid genome in which single or several pairs of chromosomes were replaced by chromosomes of other species (Barbara-Gonzalez, 2004; Lim et al., 2004). Recently, it has been recognised that allotriploids resulted after backcrossing 2n forming F1 interspecific hybrids with primary diploid forms can be successfully used for introgression in breeding programs of lilies, since the recombinant chromosomes in variable numbers can be transmitted to backcross progeny (Lim et al., 2004; Proscevičius et al., 2007).

The new cultivars of hybrid lilies, resulted by interspecific hybridization of phylogeneticaly distant species, are either diploid F1 interspecific hybrids or allotriploids derived after backrossing 2n gametes forming F1 hybrids with diploid primary forms, or allotetraploids derived from backcrossing it with tetraploid forms (van Tuyl et al., 2000). Such allopolyploids possess one set of chromosomes inherited from one species and two (in alotriploids) or three (in allotetraploids) genomes inherited from other species. Such allopolyploids are usually sterile or can possess low female or male fertility (Proscevičius et al., 2007). In this research, fertility of allopolyploid cultivars of diverse origin was evaluated and backcrossings to tetraploid and diploid forms were performed to receive next generation.

MATERIALS AND METHODS

Plants were grown in greenhouse in standard conditions for lily growth. Flowers were emasculated 1 day before anthesis. For pollination, the pollen was applied on the stigma smeared by water solution of 10% sucrose and 0.02% boric acid. 30 days after pollination, the embryos were isolated from ovules of swelled ovary and cultured *in vitro* on solid MS medium (Murasige and Skoog, 1962) supplemented with 3% sucrose at 25°C in dark. After the germination, plantlets were grown in light and later transplanted in pot with peat under greenhouse conditions.

To evaluate viability of pollen, mature fresh pollen was suspended in drop of solution with 10% sucrose and 0.02% boric acid and

incubated at 25°C in wet camera to perform test of germination. After 10-12 hours, the germinated pollen was counted.

The root tips, collected from *in vitro* grown plants, were treated with 0,01% colchicine solution at +4°C for 17 hours and then fixed with an acetic acid and ethanol (1:3) mixture. The fixed root tips were stored in 70% ethanol solution in a freezer until use. Dried root tips were immersed in 4% $(Fe(NH_4)(SO_4))\times12H_2O$ solution for 2 minutes, and latter stained for 7 min in hot solution of acetocarmine. After cooling, root tips were softened by chloralhydrate. The metaphase chromosomes were observed on temporary preparations using a microscope (Nicon eclipse) (Proscevičius et al., 2012).

RESULTS AND DISSCUSION

Since meiosis in distant interspecific hybrids is complicated, they are usually sterile or can possess only very low fertility, and usualy produce empty, wrincled seeds with aborted embryos. To receive progeny from interspecific alopoliploid hybrids, embryos were isolated 30 days after pollination and later cultured *in vitro*. In this research, the progeny were received from cultivars which were interspecific hybrids belonging to differrent phylogenetic groups of horticular lilies. Allotriploid cultivars: 'Roter Kalif' (TTA) containing two genomes inherited from Trumpet lilies (T) and one inherited from Aziatic hybrid (A); 'Cocopa' (OAA) containing one genome of Oriental hybrid lilies (O) and two inherited from Aziatic hybrids; 'Carbonaro' (TOO) possessing one T and two O genomes; 'Silk Roud' (TTO) containing two T and one O genomes and allotetraploid 'Lesly Woodrif' (TTTO) possessing one O and three T genomes.

Pollen germinating test showed that only allotetraploid cultivar 'Lesly Woodrif' (OTTT) produced 10% viable pollen and was used to perform reciprocal crosses. Allotriploid cultivars 'Roter Kalif', 'Cocopa', 'Carbonaro' and 'Silk Roud' were totally male sterile and were used as female partners in crossings. All allopolyploid lilies used in this research were pollinated by fertile tetraploid cultivars producing

diploid pollen ('Longistar Tetra', 'Greesbach Tetra Pink' and 'Greesbach Tetra Gold') (Table 1).

Genomes of all species of genus *Lilium* consist of 12 chromosomes (n = x = 12). Allotriploid cultivars used in this research were combined of three genomes (2n = 3x = 36): two identical (TT, AA or OO) and one different (A, O or T) genome per set. Allotetraploid cultivar 'Lesly Woodrif' (2n = 4x = 48) contains three genomes of Trumpet lilies and one genome of Oriental hybrid lilies. Determination of chromosome number in progeny derived from allopolyploids after crossing with standard fertile cultivars in which meiosis is unaffected allowed to evaluate the number of chromosomes inherited from fertile gametes of allopoliploids. Crossing allotriploids and allotetraploid 'Lesly Woodrif' with tetraploid male cultivars resulted in diploid (2n = 2x = 24), triploid (2n = 3x = 36), tetraploid (2n = 4x = 48) and aneuploid progeny with 32, 34, 38, 49, 52 chromosomes (Table 1). It means that despite different origin and polyploidy level, allopoliploids possessing low female fertility can produce some viable haploid, diploid and aneuploid egg cells. Viable pollen of allotetraploid cultivar 'Lesly Woodrif' was used to pollinate tetraploid Trumpet hybrids 'Greesbach Tetra Pink' and 'Greesbach Tetra Gold', and diploid Trumpet hybrid 'Pink Perfection'. However, progeny were resulted only from diploid 'Pink Perfection' and all offspring in this crossing were diploid. It allows to predict that fertile pollen of allotetraploid cultivar 'Lesly Woodrif' are haploid. The failure to obtain progeny from tetraploid Trumpet hybrids pollinated by 'Lesly Woodrif' can be explained by disturbing of optimal ratio between chromosome number in embryo and endosperm. Since the embryo sac of the *Lilium* species is tetrasporic, or eight-nucleated type, and the secondary nucleus in the embryo sac of diploid lilies possesses a set of four chromosomes, the ratio of chromosome number in the embryo and endosperm is 2.5. This ratio should be optimal for embryo development. When a female possesses a lower chromosome set than a male, this ratio is lower and prevents abortion of embryo, but it dramatically increases if the chromosome set of the female is higher than that of male (Lim et al., 2003).

It is interesting phenomenon that in all combinations of crossings among progeny derived from allopolyploid females, diploid (2n = 2x = 24) offspring were found. Such progeny must be interpreted as apomicts and can appear among progeny of allopolyploid hybrids of lilies despite what species were involved in interspecific hybridization. Previously we detected an apomictic diploid progeny among progeny derived from interspecific hybrids of *L. longiflorum* × AH (Aziatic hybrids) and *L. lankongense* × AH (Proscevičius al., 2007). It is known that formation of functional gametes in interspecific hybrids is associated with recombinant events in homologous and homeologous chromosomes during meiosis (Barbara-Gonzalez, 2004; Lim et al., 2005). If such recombinant chromosomes were transmitted to diploid progeny of allopolyploids, such offspring would be valuable in the evaluation of interspecific hybrids for introgression and breeding at the diploid level.

CONCLUSIONS

1. Allopolyploid interspecific hybrids of lilies possessing odd number of alien genomes can produce fertile haploid, diploid and aneuploid egg cells.
2. The fertile pollen of allotetraploid cultivar 'Lesly Woodrif' possessing odd number of alien genomes are haploid.
3. Some progeny derived from allopolyploid lilies are apomictic.

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Table 1. *Chromosome number in progeny derived from allopolyploid hybrids*

Crossing combination ☐		Number of progeny			
Female partner	Male partner	Diploid (2n = 24)	Triploid (2n = 36)	Tetraploid (2n = 48)	Aneuploid 2n = 32–52
'Roter Kalif' (TTA)	'Longistar Tetra' (LLAA)	3	5	2	2 (2n = 32) 1 (2n = 52)
'Cocopa' (OAA)	'Longistar Tetra' (LLAA)	3	3	1	1 (2n = 32) 1 (2n = 49)
'Carbonaro' (TOO)	'Greesbach Tetra Pink' (TTTT)	2	5	3	1(2n = 32) 1(2n = 38) 1(2n = 49)
'Silk Roud' (TTO)	'Greesbach Tetra Pink' (TTTT)	1	1	0	0
'Lesly Woodrif' (TTTO)	'Greesbach Tetra Gold' (TTTT)	4	6	5	1(2n = 34)
'Lesly Woodrif' (TTTO)	'Greesbach Tetra Pink' (TTTT)	1	7	6	0
'Pink Perfection' (TT)	'Lesly Woodrif' (TTTO)	14	0	0	0

☐ A – genome of Aziatic hybrid, O – genome of Oriental hybrid, T – genome of Trumpet hybrid, L – genome of *L. longiflorum*.

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ALOTRIPLOIDINIŲ IR ALOTETRAPLOIDINIŲ LELIJŲ *LILIUM* TARPRŪŠINIŲ HIBRIDŲ PALIKUONIŲ CITOGENETINĖ ANALIZĖ

Santrauka

Atlikus palikuonių, kilusių iš keturių skirtingos kilmės alotriploidinių ir vienos alotertaploidinės lelijų veislės, citogenetinę analizę paaiškėjo, kad ir alotriploidų, ir alopotetraploidų gyvybingos kiaušialąstės gali turėti tiek subalansuotus haploidinius (n = x = 12) arba diploidinius (2n = 2x = 24) chromosomų rinkinius, tiek tokius, kuriuose yra daugiau negu viena papildoma chromosoma. Dalis alopoliploidinių motinų palikuonių buvo diploidiniai apomiktai, atsiradę iš neapvaisintų kiaušialąsčių. Tik alotetraploidinė veislė 'Lesly Woodrif', turinti tris Vamzdinių (T) ir vieną Rytinių hibridų (O) genomus (TTTO), formavo vaisingas haploidines žiedadulkes.

Reikšminiai žodžiai: *Lilium*, tarprūšinė hibridizacija, alpolopolploidai.