THE USE OF ORYZALIN AS AN ALTERNATIVE FOR COLCHICINE IN IN-VITRO CHROMOSOME DOUBLING OF *LILIUM* AND *NERINE*

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Abstract

Colchicine has been used for doubling the number of chromosomes of many crop plants over a period of more than 50 years. This natural alkaloid with an antimitotic activity, obtained from the wild *Colchicum autumnale*, however, is very toxic for human beings and also shows undesirable mutagenic activity on plants. An other compound with mitosis inhibiting activity is oryzalin (4-(dipropylamino)-3,5-dinitro-benzene-sulfonamide) which is developed as a herbicide. Oryzalin is tested as an alternative for colchicine in experiments with *Lilium* and *Nerine* conducted for mitotic polyploidization of sterile interspecific F1-hybrids in order to restore fertility. In vitro treatment with oryzalin in concentrations varying from 0.001%-0.01% appeared to be less inhibiting for the regeneration and resulted in a higher number of polyploid plants than treatments with colchicine of which a tenfold higher concentration was needed. Therefore oryzalin can be considered as a preferable alternative for the very toxic colchicine.

1. Introduction

Colchicine has been used for doubling the number of chromosomes of many crop plants over a period of more than 50 years (Blakeslee & Avery, 1937). The reasons for using polyploidy in lily breeding are the larger flowers, the stronger stems (especially important for forcing under low light conditions during the winter period) and in interspecific hybridization the restoration of F1-sterility at the tetraploid level. In research on overcoming interspecific crossing barriers artificial chromosome doubling (mitotic polyploidization) is used to restore fertility of sterile interspecific F1-hybrids in *Lilium* (Van Tuyl et al., 1989). For this reason colchicine was used successfully to produce tetraploids in lily (Van Tuyl, 1990). Colchicine is a natural alkaloid with an antimitotic activity (Fig. 1: chemical structure), obtained from the wild *Colchicum autumnale*, is very toxic for human beings and also shows undesirable mutagenic activity on plants. A suitable procedure for in vivo colchicine treatment was developed consisting of plunging scales in an aqueous solution of 0.05 - 0.1 % colchicine for 2-8 hours. In research on intergeneric hybridization of *Nerine* using in vitro methods hybrids are obtained between *Nerine bowdenii* and *Amaryllis belladonna* and *Lycoris aurea* (Van Tuyl et al., 1990; Van Tuyl et al. 1992). Sterility of the F1-hybrids can be expected as well. Therefore it is necessary to develop a method for chromosome doubling in *Nerine*. Up till

now artificial chromosome doubling in *Nerine* has not been successful.

An other compound with mitosis inhibiting activity is oryzalin (Fig. 1) (4-(dipropylamino)-3,5-dinitro- benzenesulfonamide) which is developed as a herbicide and used in the USA to control weeds in several ornamental grasses (Neal and Senesac, 1991). In cell-suspension cultures of *Nicotiana plumbaginifolia* and potato clear evidence was provided for the antimitotic activity (Sree Ramulu et al., 1991; Verhoeven et al., 1990).

To investigate the potential of oryzalin for chromosome doubling in in vitro experiments a comparison was made between colchicine and oryzalin.

2. Plant material

Interspecific hybrids of *Lilium longiflorum* x Whilito' and *L. henryi* x *L. candidum* developed at CPRO-DLO (Van Tuyl et al., 1991) and maintained in vitro culture after embryo culture.

Nerine bowdenii 'Albivetta' originated from CPRO-DLO breeding research was propagated in vitro by Carant Vitro BV, Wateringen, The Netherlands and in vitro cultures were used for the experiments.

3. Methods

The media used for the in vitro propagation of lily are the Murashige and Skoog medium (MS) (Murashige and Skoog, 1963) supplemented with 0.2 mg/l NAA and 4% saccharose. *Nerine* was cultured on a modified MS supplemented with vitamins, BA and IBA and contained 4% saccharose and 0.85% Daichin agar.

Colchicine was purchased by Sigma Ltd and oryzalin (4-(dipropylamino)-3,5-dinitro- benzenesulfonamide) by Duchefa Biochemicals. Colchicine was prepared as an aqueous solution in water and prepared freshly for each experiment. For oryzalin a stock solution was prepared of 20 mg/ml in water-free DMSO.

Treatments of the lily scales and *Nerine* explants with colchicine and oryzalin were carried out in aqueous solutions at 20°C for 4 hours, after which the chemicals were washed three times in sterile water. After the treatments the scales and explants were cultured on the media described.

Tetraploids were detected by using flow cytometry. Flow cytometric procedures are performed as described by Van Tuyl et al., (1989). Root or scale pieces were used as samples. The *Nerine* cultures were propagated after the treatment at least two times to prevent a high percentage of chimaera.

4. Results and discussion

4.1. *Lilium*

Thirty one lily plants of 92 regenerated oryzalin and colchicine treated plants appeared to be polyploid (Table 1). A high percentage is chimeric (di_tetra, di_octa, tetra_octa) probably because of juvenility

of the material. Most of these probably mericlinal chimeras went back to one level of ploidy, which was dominating in the determination. Oryzalin appeared to be less inhibiting for regeneration than colchicine and also the number of polyploids after oryzalin treatment was higher.

Using this method the first tetraploid *L. henryi* x *L. candidum* obtained from the oryzalin treatment came into flower and showed a fully restored fertility (Fig. 2). This breeding material was released to the lily breeding firms in 1990.

4.2. *Nerine*

In an experiment with *Nerine bowdenii* 'Albivetta' per treatment 110 divided bulblets were treated with colchicine 0.1%, oryzalin 0.01 and 0.001% and a control. The colchicine treatment appeared to be lethal: no bulblet did survive the treatment. After two propagation cycles the bulblets were observed visually and the largest and probable polyploids were tested using flow cytometric analysis. Sixteen of 41 tested bulblets appeared to polyploid of which 3 were chimeric.

The non tested bulblets and the diploid ones are planted into soil, while the polyploid bulblets are propagated further in vitro. From the results it is clear that oryzalin is effective in doubling the number of chromosomes of *Nerine*. The most suitable concentration is 0.001%. In cell cultures of potato and tobacco (Verhoeven et al., 1991; Sree Ramulu et al., 1990) and in maize callus (Wan et al., 1991) oryzalin proved also to be the most efficient chromosome-doubling agent as compared to APM (Tokunol M, a phosphoric amide herbicide) and colchicine. For further experiments oryzalin 0.001% is used.

4.3. Conclusions

- Restoration of F1-sterility by doubling the chromosome number was performed successfully in the *Lilium henryi* x *L. candidum* hybrid using oryzalin.
- Oryzalin inhibited plant cell division much more effective than colchicine, which is more active in animal tissues. Oryzalin is applied successfully in doubling the number of chromosomes of lily and *Nerine* in lower concentrations (0.01-0.001%) than colchicine (0.1%).
- Tetraploids are produced for the first time in *Nerine* using oryzalin.
- Disadvantageous effects like growth abnormalities caused by mutation induction possibly do not occur by using oryzalin.
- Oryzalin can be considered as an alternative for the very toxic colchicine in doubling the number of chromosomes of plants.

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Table 1 - In vitro chromosome doubling of sterile interspecific hybrids of lily using colchicine (col 0.1%) and oryzalin (ory 0.005 en 0.01 %). Determination of the level of ploidy with flow cytometry. Tetra=tetraploid, di=diploid, octa = octaploid, poly=polyploid. Given are the total number of plants investigated, the total number of polyploids (Total poly) distributed over the various levels of ploidy.

Level of polyploidy	Treatment				
	col 0.1	ory 0.01	ory 0.005	control	total
Tetra	3		4		7
Di_tetra	3	6	9		18
Di_octa			1		1
Tetra_octa	3				3
Octa			2		2
Total poly	9	6	16		31
Total	13	41	38	15	107

Table 2 - Number of tetraploid and chimeric *Nerine* bulblets after oryzalin treatment after testing 41 bulblets using flow cytometric analysis.

Level of polyploidy	Treatment	
	ory 0.001	ory 0.01
Tetraploid	7	6
Chimeric	2	1

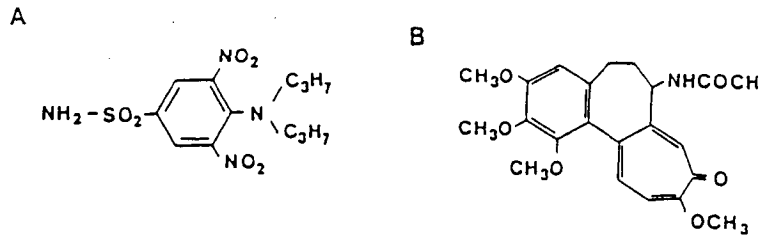

Fig. 1 - The chemical structures of oryzalin (A) and colchicine (B).

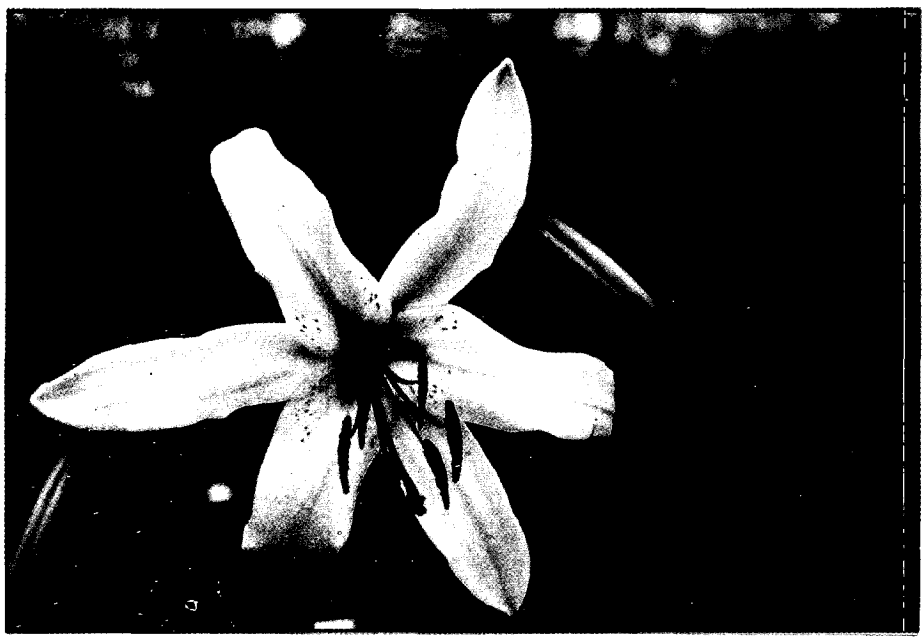

Fig. 2 - An interspecific hybrid between *L. henryi* x *L. candidum* of which by in vitro treatment of oryzalin a tetraploid form was obtained with restored fertility.