EFFECT OF A NEW COMPOUND ON THE MITOTIC POLYPLOIDIZATION OF *LILIUM LONGIFLORUM* AND ORIENTAL HYBRID LILIES

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Abstract

A new compound, Surflan™ (Surflan), for mitotic polyploidization *in vitro* in lily was tested and compared with the original compound 'oryzalin'. Surflan was effective for mitotic polyploidization of lilies. The optimal concentration of Surflan was 0.0075% for *in vitro* chromosome doubling. A low concentration (0.0025%) was still effective to produce polyploid plants, however, a high concentration (0.0125%) showed phytotoxicity for plant regeneration although the frequency of polyploid plants was high. In the 0.0075% Surflan treatment, the percentage of polyploids was 86, 68 and 100% in *L. longiflorum* 'White Fox', Oriental hybrid 'Le Rêve' and 'Sorbonne', respectively. These are similar effects to those in oryzalin treatments. Thus, *in vitro* chromosome doubling by using Surflan was successful in lilies, and may be considered as an alternative reagent of oryzalin.

1. Introduction

Mitotic polyploidization is one of the efficient breeding tools in ornamental plants enabling the combination of desirable characters. Since most interspecific F_1 hybrids are sterile, the recovering of their fertility by mitotic polyploidization is useful for further breeding programme. Even in fertile plants, some flower qualities are improved by polyploidization. For example, polyploids induced by mitotic chromosome doubling showed larger and deeper-colored flowers than diploids in carnation and cyclamen (Yamaguchi, 1989, Takamura and Miyajima, 1996). In lily, the large flower and sturdy stem in the tetraploids are useful as compared with those in the diploids (Van Tuyl, 1992).

The most popular chemical used for chromosome doubling in many crops has been colchicine, which has a spindle fiber inhibiting activity. However, colchicine is very harmful to human beings and in some cases shows undesirable mutagenetic activity on plants (Van Tuyl *et al*., 1992). Oryzalin also inhibits mitosis activity and is one of the chemicals used for chromosome doubling in lily (Van Tuyl *et al*., 1992), potato (Verhoeven *et al*., 1990), tobacco (Ramulu *et al*., 1991), and many others. In lily, Van Tuyl *et al*. (1992) reported that oryzalin was effective to induce polyploids and can be considered as an alternative for colchicine. However, oryzalin, which was originally developed as an herbicide, is not available for commercial use.

Surflan™ (Surflan) is one of the chemicals with the ability to induce mitotic polyploidization in plants. Surflan contains 40% oryzalin as active compound, and is cheep. Surflan is, therefore, expected to be an alternative for colchicine and oryzalin. However, Surflan contains besides oryzalin some other ingredients, and there is no information of the side effects of these additives in lily. In order to confirm the possibility that Surflan might be an alternative for chromosome doubling in lily, the effects of Surflan on the *in vitro* chromosome doubling were compared with those of oryzalin in the present study.

2. Materials and methods

In present study *Lilium longiflorum* 'White Fox', and the Oriental hybrids 'Le Rêve' and 'Sorbonne' were tested. The scales of these cultivars were resected from the bulbs grown *in vivo*, disinfected in 2% sodium hypochlorite solution containing a few drops of Tween 20® at room temperature for 20 minutes, and washed three times with sterile water.

In 'White Fox', the scales were soaked in 0, 0.001, 0.003% or 0.005% oryzalin, or 0.0025, 0.0075 or 0.0125% Surflan (Eli Lily ltd., USA) solution at room temperature for 3 hours. In the Oriental hybrid 'Le Rêve' and 'Sorbonne', only 0.003% oryzalin and 0.0075% Surflan were tested. The 0.0025, 0.0075 or 0.0125% Surflan solutions contained about 0.001, 0.003 and 0.005% oryzalin in itself, respectively. The stock solutions of oryzalin (20 mg oryzalin in 1 ml DMSO) and Surflan (25 mg Surflan in 1 ml DMSO) were prepared and then the stock solutions were diluted with sterile water for working solutions. After soaking in oryzalin or Surflan solutions, the explants were rinsed three times with sterile water and then plated on half strength solidified MS (Murashige and Skoog, 1962) media containing 5% sucrose and 0.4% gellan gum.

The explants were cultured at 25°C in 16-h day length of fluorescent light and then the regeneration was observed after 2 months. Ploidy level of the regenerated plantlets was determined by flow cytometry (Van Tuyl *et al*., 1989; Van Tuyl and Boon, 1997).

3. Results and discussion

Van Tuyl *et al*., (1992) considered below 0.005% as the optimal concentration of oryzalin for the in vitro chromosome doubling in lily. For both the oryzalin and Surflan, the high concentrations obviously obstructed the bulblet formation from explants in 'White Fox' (Table 1). On the other hand, higher frequency of polyploid formation was seen in 0.003 or 0.005% oryzalin, and 0.0075 or 0.0125% Surflan compared to the lowest concentration. From the results of bulblet formation and the percentage of polyploids the optimal concentration of oryzalin and Surflan should be about 0.003% and 0.0075%, respectively. Lower concentrations than those are not efficient for polyploidization and higher concentrations may be too harmful for plant regeneration. Table 1 also showed that there was no significant difference in the percentage of explants forming bulblets between oryzalin and Surflan treated plants when 0.0025, 0.0075 and 0.0125% Surflan treatment are compared with 0.001, 0.003 and 0.005% oryzalin, respectively. The number of regenerated bulblets from the oryzalin treatments was significantly larger compared to Surflan. However, the percentage of polyploids in the Surflan treatment was little higher than those in the oryzalin treatment for each concentration.

In the Oriental hybrid lilies 'Le Rêve' and 'Sorbonne', Surflan was also effective for mitotic polyploidization. Bulblet formation after Surflan treatment was compatible to oryzalin in both cultivars (Fig.1). Respectively 66.7 and 100% of regenerated plants were polyploids or mixoploids of 2x and 4x in 'Le Rêve' and 'Sorbonne' in 0.0075% Surflan treatment (Fig. 2). These values were similar in 'Le Rêve' or even higher in 'Sorbonne' when compared to those in 0.003% oryzalin treatment. These results confirm the possibility that Surflan is also effective for the polyploidization of Oriental lily. However, genotypes related differences in the polyploidization of lily by using Surflan are to be expected because of the different efficiency of the polyploidization by using Surflan between *L. longiflorum* 'White Fox' and the Oriental hybrid cultivars. Surflan treatment should be, therefore, examined for the polyploidization of various genotypes in lily except *L. longiflorum* and Oriental hybrids.

In 'White Fox' and 'Sorbonne', the frequencies of chimeras in polyploids (Table 1 and Fig. 2) in each concentration of Surflan treatment were higher than those in oryzalin treatment. Tosca *et al*. (1995) reported that treating plants longer with oryzalin could reduce the problem of chimera in gerbera. Therefore, the effect of a longer Surflan

treatment on the percentage of ploidy chimera should be examined in lily.

Our results suggest that Surflan has the same effect as oryzalin on mitotic polyploidization and that Surflan has no or few side effects, such as inhibiting the plant regeneration and polyploidization, on the in vitro chromosome doubling of lily. In addition, Surflan has the advantage to be much cheaper than oryzalin. Since oryzalin was also considered as the most reliable compound for the mitotic polyploidization in kiwifruit, onion, gerbera, apple, maize, potato and tobacco (Chalak and Legave, 1996; Geoffriau *et al*., 1997; Tosca *et al.*, 1995; Bouvier *et al.*, 1994; Wan *et al*., 1991; Ramulu *et al*., 1991), it is expected that Surflan might have the same possibilities for the polyploidization of these plants. As a conclusion, Surflan might be an alternative for oryzalin in the chromosome doubling of lily and many other plant species because of the effectiveness and cheap price, though further tests in various genotypes and of treatment duration are desired.

References

Bouvier L., Fillon F.R., and Lespinasse Y., 1994. Oryzalin as an efficient agent for chromosome doubling of haploid apple shoots in vitro. Plant Breeding 113: 343-346.

Chalak L., and Legave M., 1996. Oryzalin combined with adventitious regeneration for an efficient chromosome doubling of trihaploid kiwifruit. Plant Cell reports 16: 97-100.

Geoffriau E., Kahane R., Bellamy C., and Rancillac M., 1997. Ploidy stability and in vitro chromosome doubling in gynogenic clones of onion (*Allium cepa* L.) Plant Science 122: 201-208.

Murashige T., and Skoog F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Ramulu K.S., Verhoeven H.A., and Dijkhuis P., 1991. Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. Protoplasma 160: 65-71.

Takamura T., and Miyajima I., 1996. Colchicine induced tetraploids in yellow-flowered cyclamens and their characteristics. Scientia Horticulturae 65: 305-312.

Tosca A., Pandolfi R., Citterio S., Fasoli A., and Sgorbati S., 1995. Determination by flow cytometry of the chromosome doubling capacity of colchicine and oryzalin in gynogenetic haploid of *Gerbera*. Plant Cell Reports 14: 455-458.

Van Tuyl J.M., 1992. Survey research on mitotic and meiotic polyploidization at CPRO-DLO. The Lily Yearbook of North American Lily Society 43: 11-18.

Van Tuyl J.M., De Vries J.N., Bino R.J., and Kwakkenbos T.A.M., 1989. Identification of 2n-pollen producing interspecific hybrids of *Lilium* using flow cytometry. Cytologia 54: 737-745.

Van Tuyl J.M., Meijer B., and Van Dien M.P., 1992. The use of oryzalin as an alternative for colchicine in *in-vitro* chromosome doubling of *Lilium*. The Lily Yearbook of North American Lily Society 43: 19-22.

Van Tuyl J.M., and Boon E., 1997. Variation in DNA-content in the genus *Lilium*. Acta Hortic 430: 829-835.

Verhoeven H.A., Ramulu K.S., and Dijkhuis P., 1990. A comparison of the effects of various spindle toxins on metaphase arrest and formation of micronuclei in cell-suspension culture of *Nicotiana plumbaginifolia*. Planta 182: 408-414.

Wan Y., Duncan D.R., Rayburn A. L., Petolino J.F., and Widholm J.M., 1991. The use of antimicrotubule herbicides for the production of doubled haploid plants from anther-derived maize callus. Theor. App. Gen. 81: 205-211.

Yamaguchi M., 1989. Basic studies on the flower color breeding of carnations (*Dianthus caryophyllus* L.). Bulletin of the Faculty of Horticulture Minami Kyushu University 19: 1-78 (In Japanese with English and German summary).

Table 1. Effects of oryzalin and Surflan on in vitro chromosome doubling in *L. longiflorum* 'White Fox'.

Treatment [z]	No. of explants cultured bulblets	% (no.) of explants forming bulblets	No. of bulblets per explant	No. of plantlets tested	Percentage of plantlets with indicated the ploidy					Percent poly-ploids
					2x	2x -4x	4x	4x -8x	8x	
First experiment										
Control	10	100(10)	5.0±0.7	-						
0.001% ory	18	89(16)	2.5±0.3	35	91.4	0	8.6	0	0	8.6
0.003% ory	16	56(9)	0.7±0.2	6	33.3	0	50.0	0	16.7	66.7
0.005% ory	18	39(7)	0.5±0.2	7	28.6	0	42.9	0	28.6	71.4
0.0025% sur	18	83(15)	1.8±0.3	12	83.3	0	16.7	0	0	16.7
0.0075% sur	16	44(7)	0.6±0.2	10	20.0	0	40.0	20.0	20.0	80.0
0.0125% sur	17	28(5)	0.4±0.2	4	0	0	25.0	0	75.0	100
Second experiment										
Control	8	100(8)	4.8±0.6	-						
0.001% ory	15	93(14)	2.2±0.3	35	88.6	2.9	8.6	0	0	8.6
0.003% ory	15	47(7)	0.7±0.2	8	37.5	0	25.0	0	37.5	62.5
0.005% ory	15	13(2)	0.2±0.1	3	0	0	33.3	0	66.7	100
0.0025% sur	19	15(79)	1.9±0.3	14	71.4	14.3	7.1	7.1	0	28.6
0.0075% sur	16	7(44)	0.7±0.2	11	9.1	18.1	36.4	9.1	27.3	90.9
0.0125% sur	17	3(18)	0.2±0.1	2	0	50.0	50.0	0	0	100
Total										
Control	18	100(18)	4.9±0.5	-						
0.001% ory	33	91(91)	2.4±0.2	70	90.0	1.4	8.6	0	0	10.0
0.003% ory	31	52(16)	0.7±0.1	14	35.7	0	35.7	0	28.6	65.3
0.005% ory	33	27(9)	0.4±0.1	10	20.0	0	40.0	0	40.0	80.0
0.0025% sur	37	81(30)	1.9±0.2	26	76.9	7.7	11.5	3.8	0	23.1
0.0075% sur	32	44(14)	0.7±0.2	21	14.3	9.5	38.1	14.3	23.8	85.7
0.0125% sur	34	24(8)	0.3±0.1	6	0	16.7	33.3	0	50.0	100
Significance [y]										
Substances (S)		NS	*							*
Concentrations (C)		***	***							***
S × C		NS	NS							NS

[z] ory; oryzaline, sur; Surflan

[y] NS; not significant, *; *P<0.05, **; P<0.01, ***; P<0.001.*

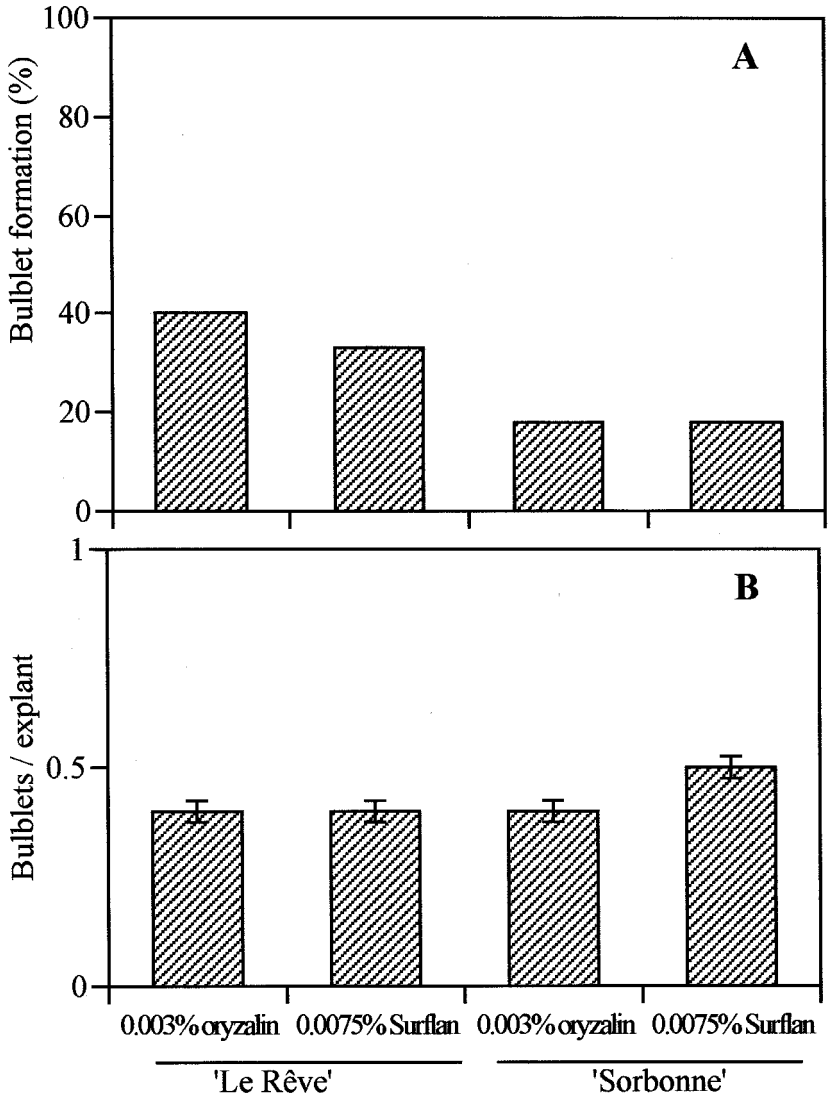

Figure 1. Effects of oryzalin and Surflan on bulbet formation (A) and number of bulbets per explant (B) in Oriental hybrid lily ‘Le Rêve’ and ‘Sorbonne’.

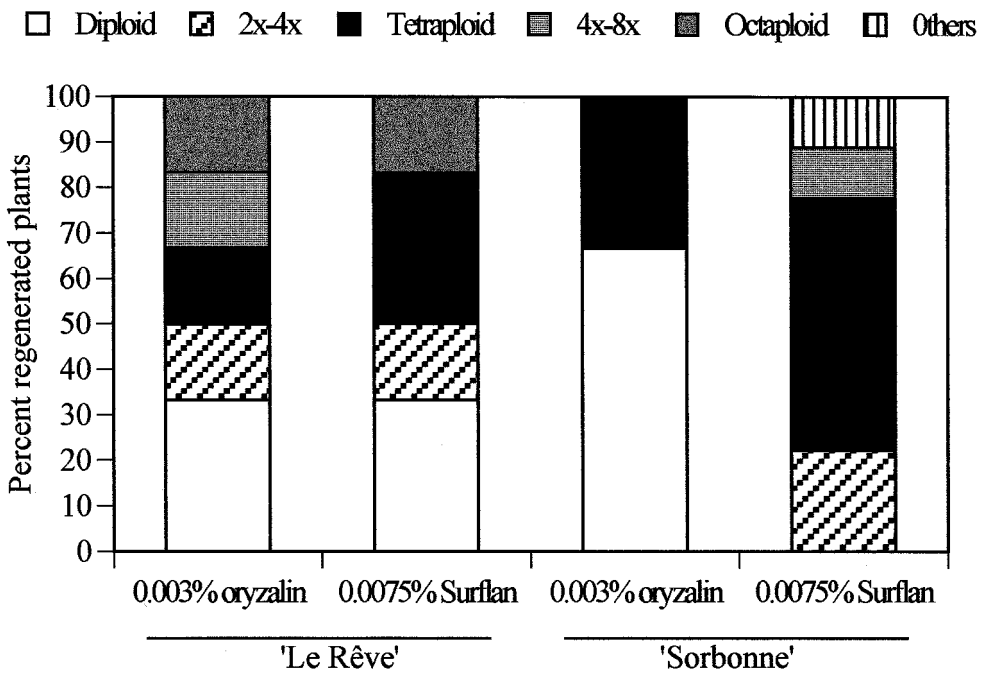

Figure 2. Effects of oryzalin and Surflan on the polyploidization of Oriental hybrid lily 'Le Rêve' and 'Sorbonne'.