

Allergen contents in *Alstroemeria* can be reduced by breeding

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Summary

The allergen content in *Alstroemeria* was determined by high-performance liquid chromatography (HPLC) in order to investigate breeding possibilities for reducing the risk of contact dermatitis caused by *Alstroemeria*. The potentials of both cross breeding and mutagenesis were studied. The allergens (6-tuliposide A, tuliposide D and tulipalin A) were determined in leaves, stems, and flowers. 6-Tuliposide A was found in the highest amounts in all plant parts and tulipalin A was mainly present in the flowers. Environmental effects (season and growth conditions) on the allergen content were significant. For the concentration of allergens coefficients of variation generally were above 40% within populations of single species. Positive correlations for 6-tuliposide A between plant parts and between 6-tuliposide A and tulipalin A within leaves and stems were found. Tuliposide D was not correlated with the other allergens. Significant narrow sense heritabilities were found for total allergen content as well as for 6-tuliposide A and tuliposide D, but not for tulipalin A. The heritability was higher for total allergen content and tuliposide D as compared to 6-tuliposide A. Mutagenesis was able to reduce the content of 6-tuliposide A. Allergen production in *Alstroemeria* seems multigenically controlled.

Abbreviations: CV – coefficient of variation; MW – molecular weight

Introduction

Alstroemeria is a popular cut flower available in many colors and with a long vase-life. The genus *Alstroemeria* (Alstroemiaceae) is widespread in South America, mainly in Chile and Brazil (Aker & Healy, 1990). The genus comprises 31 species in Chile (Bayer, 1987) and at least an equivalent number is expected in Brazil (F. Meyer, pers. comm.). Despite this large genetic potential only a very limited material has been included in commercial breeding (Healy & Wilkins, 1985).

Alstroemeria and *Tulipa* (Liliaceae) may cause severe allergic contact dermatitis for a large part of the personnel working in the cut flower industry (Rook, 1981; Rycroft & Calnan, 1981; Björkner, 1982; Hausen et al., 1983; Santucci et al., 1985; Hausen, 1988; Hausen & Oestmann, 1988; Thiboutot et al., 1990). For sensitized (allergic) persons *Alstroemeria* contact dermatitis normally develops on the hands and

finger tips ('tulip fingers') although severe eczematous reactions at the forearms, face, and neck have also been observed (Hausen, 1988). The causative agents of allergic contact dermatitis in *Alstroemeria* have been identified as 6-tuliposide A and tulipalin A (Verspyck Mijnsen, 1969; Slob et al., 1975; Santucci et al., 1985; Christensen & Kristiansen, 1995a). A methylene group in α position appears important for the allergenic activity (Santucci et al., 1985), even though tulipalin A and sesquiterpene lactones, that contain the same α -methylene- γ -butyrolactone moiety, do not cross react (Schlewer et al., 1980; Benezra & Ducombs, 1987). 6-Tuliposide A is degraded to D-glucose and tulipalin A, a process occurring both in the plant and on the human skin. Further, tuliposide D and E have recently been isolated from *Alstroemeria*, but whether they directly act as allergens or only after chemical breakdown to 6-tuliposide A and tulipalin A have to be determined (Christensen, 1995ab; Christensen & Kris-

tiansen, 1995ab). Tuliposides seem to be widely distributed in Liliaceae and especially the genera *Tulipa* and *Erythronium* contain relatively large amounts of tuliposide A and tuliposide B (β -hydroxy derivative of tuliposide A) (Tschesche et al., 1969; Slob et al., 1975). Tuliposide B seems not to be present in *Alstroemeria* (Slob et al., 1975; Christensen & Kristiansen, 1995c). Hausen (1988) states that tuliposide B is non-allergenic, although it has been found that tulipalin B, which is formed from tuliposide B, is able to sensitize guinea pigs and cross react with tulipalin A (Barbier & Benezra, 1986; Benezra & Ducombs, 1987).

Santucci et al. (1985) found that 6-tuliposide A and tulipalin A gave positive reactions after patch test at 0.01% in ethanol, indicating that sensitized people would only be able to work with allergen-free cultivars. The risk of sensitization increases with allergen concentration, but the recommended 6-tuliposide A patch test concentrations of 0.01–0.1% in petrolatum (Hausen, 1988) do not seem to cause active sensitization (E. Paulsen, pers. comm.). However, the allergen concentration in the plant that may cause active sensitization is at present not known as concentrations in petrolatum and in the plant may not directly be comparable with regard to allergenic activity and because the sensitizing effect of each of the allergens is unknown. Thus the total allergen content should be focussed on when discussing sensitization levels.

In order to quantify the allergen content and thereby to select genotypes with a low content a simple HPLC method was developed (Christensen & Kristiansen, 1995abc). A large number of plants was analysed and the amount of e.g. 6-tuliposide A in leaves ranged from not detectable to more than 1% of fresh weight, which is in accordance with previous findings by Slob et al. (1975). To reduce the risk of contact dermatitis in the cut flower industry in particular the leaves should have a low allergen content, as workers mostly come into contact with the plant sap during leaf removal after cut flower harvesting. In recent years an increasing interest to produce *Alstroemeria* as pot plants has arisen (Bridgen, 1993), which requires new compact cultivars. When breeding for compact cultivars, working environmental problems could be prevented by using low allergen content as selection parameter.

The aim of the work reported here was to investigate possibilities to breed *Alstroemeria* cultivars with low contents of allergens, including both hybridization and mutagenesis. Heritabilities of the allergen content in different plant parts, variation within species and populations, seasonal fluctuations and effects of muta-

genic treatments will be presented and the breeding possibilities and strategies discussed.

Material and methods

Plant material

The investigated plants consisted of 28 Chilean and 1 Brazilian species, a large number of interspecific hybrids presumably at the diploid level, 'Butterfly'-hybrids (*A. pelegrina* $\times$ *A. pulchella*) such as *A.* 'Rosy Wings' at the tetraploid level, and hybrids with *A.* 'Rosy Wings' presumably at the triploid level. The Chilean species were identified according to Bayer (1987) and produced from seeds collected in the wild. Hybrids were produced after controlled crossings in the greenhouse as described by Kristiansen (1995). The variation in allergen content within populations of some Chilean species was analysed.

One 'Butterfly'-hybrid clone was transplanted from *in vitro* conditions monthly to estimate environmental influences (season and growth conditions) on the allergen content. *In vitro* plants were flower induced by culturing at 10 °C for 7 weeks prior to transplantation as described by Pedersen et al. (1996). Further, other clones were analysed several times.

In preliminary experiments, shoots were harvested at the same time from single plants with coefficients of variation (CV) for leaves, stems, and flowers less than 6%, showing insignificant variations within the plant. Allergens were thus only investigated in one shoot per plant. Plant material for analyses were collected at flowering throughout the year; mostly from April to September. For analyses healthy flowering stems were used and they were divided into leaves, stems, and flowers and frozen at –20 °C until analysis.

Mutagenesis

In vitro plants from three unnamed tetraploid Butterfly-hybrids were radiated with 0, 5, or 10 Gy γ -rays. Plants consisted of a rhizome piece with approx. 2 aerial shoots and after radiation they were subcultured *in vitro* twice prior to root formation and flower induction as described by Pedersen et al. (1996). Plants were thereafter transplanted to greenhouse conditions and grown until flowering. Flowering stems were collected for chemical analyses as described above.

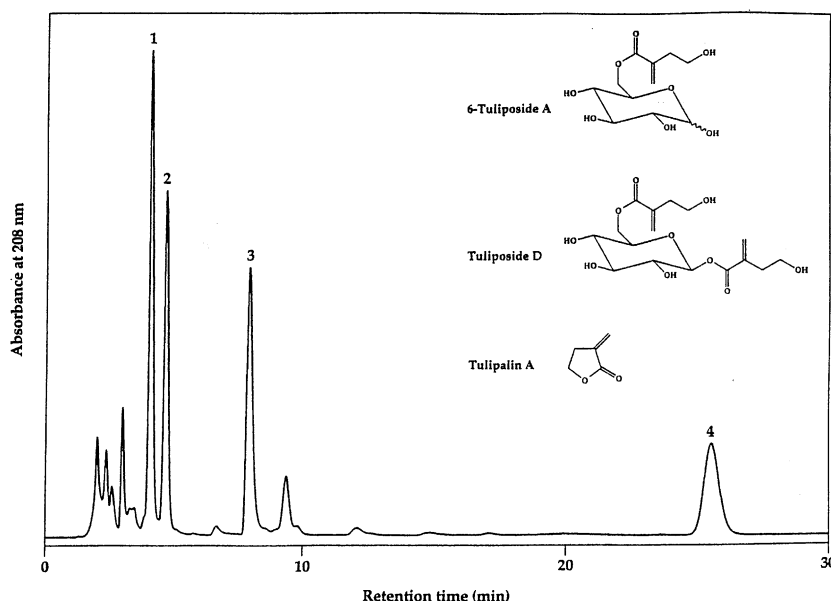


Figure 1. A HPLC chromatogram of an aqueous extract of *Alstroemeria aurea* flowers separated on a LiChrospher 100 RP-18 (5 μ m; 244 $\times$ 4 mm) column, showing peaks from 6-tuliposide A (β -anomer, 1; α -anomer, 2); tulipalin A (3); and tuliposide D (4).

Chemical analyses

Freshly frozen leaves, stems, and flowers (fresh weight 1 to 2 g) were ground in distilled water (20 ml) with an Ultra-Turrax T25 and stored in the dark at 4 °C for 24 h. After filtering, extracts were analysed by reversed phase HPLC. Samples (2 ml) were pressure-filtered through cellulose acetate membranes (0.2 μ m) before analysis. Tuliposides and tulipalin A were separated at room temperature on an analytical LiChrospher 100 RP-18 (5 μ m; 119 $\times$ 4 mm) column as described previously (Christensen & Kristiansen, 1995ab) or on a LiChrospher 100 RP-18 (5 μ m; 244 $\times$ 4 mm) column with a flow rate of 0.6 ml min⁻¹ and a mobile phase consisting of distilled water:methanol (80:20). The injection volume was 25 μ l. Tuliposides and tulipalin A were detected at 208 nm with a Shimadzu SPD-10AV spectrophotometer. Retention and quantification calculations of tuliposides and tulipalin A were obtained with Shimadzu chromatopac (C-R7A). Pure tuliposides and tulipalin A were used for calibration. The compounds were isolated by column chromatography and identified by UV, TLC, FAB-MS, ¹H- and ¹³C-NMR as described previously (Christensen, 1995ab).

Statistics

For determination of total allergen content fresh weight percentages of 6-tuliposide A (MW 278) and tuliposide

D (MW 376) were converted to tulipalin A equivalents, defined as the concentration of tulipalin A (MW 98) in percent fresh weight that would result after total hydrolysis of the tuliposides. One percent fresh weight of 6-tuliposide A and tuliposide D corresponds thus to 0.36 and 0.52 tulipalin A equivalents, respectively. Pearson coefficients of correlations (R^2) between concentration of allergens in different plant parts and between different allergens were determined. Narrow sense heritability of the different allergens was estimated by linear regression of the hybrid value on the mid-parental value. Mean of plants from each species and locality were used to calculate mid-parental value, and not the exact parental plants. Further, means were calculated from plants where more than one shoot had been analysed. Concentrations of allergens in γ -radiated plants were compared with control plants by analysis of variance.

Results

Distribution of allergens

Among plants, the total allergen content in leaves, stems, and flowers varied between 0.0–1.4, 0.1–1.2, and 0.1–3.5 tulipalin A equivalents, respectively. One tulipalin A equivalent corresponds to 2.8% 6-tuliposide A and 1.9% tuliposide D. Allergen concentrations gen-

Table 1. 6-Tuliposide A, tuliposide D, and tulipalin A concentrations (% fresh weight) and coefficients of variation (CV) in some Chilean *Alstroemeria* species

Species and locality	N ¹	Plant part	6-Tulipo- side A	Tulipo- side D	Tulipo- lin A
<i>A. aurea</i> Graham					
Conguillio National Park, Region Araucaria	4	Leaves	0.67 ± 48 ²	0.00	0.11 ± 75
		Stems	0.53 ± 26	0.04 ± 75	0.04 ± 75
		Flowers	0.86 ± 48	0.07 ± 43	0.12 ± 58
West of Laguna de la Laja, Region Bio Bio	5	Leaves	1.05 ± 65	0.02 ± 100	0.11 ± 45
		Stems	0.73 ± 85	0.09 ± 67	0.06 ± 50
		Flowers	1.13 ± 30	0.18 ± 67	0.21 ± 57
Between Temuco and Cunco, Region Araucaria	7	Leaves	1.36 ± 35	0.03 ± 67	0.01 ± 75
		Stems	1.04 ± 48	0.04 ± 50	0.07 ± 71
		Flowers	1.05 ± 31	0.19 ± 42	0.45 ± 47
<i>A. ligtu</i> L. ssp. <i>ligtu</i>					
Between Constitucion and Cauquenes, Region del Maule	6	Leaves	0.12 ± 142	0.00	0.02 ± 200
		Stems	0.56 ± 77	0.04 ± 100	0.04 ± 150
		Flowers	0.96 ± 18	0.04 ± 50	0.06 ± 67
South of Coronel, Region Bio Bio	9	Leaves	0.38 ± 61	0.00	0.03 ± 67
		Stems	0.60 ± 27	0.02 ± 50	0.05 ± 60
		Flowers	1.05 ± 14	0.12 ± 50	0.11 ± 73
South of Valparaiso, Region Valparaiso	4	Leaves	0.00	0.00	0.00
		Stems	0.56 ± 70	0.04 ± 100	0.01 ± 100
		Flowers	1.05 ± 31	0.10 ± 20	0.07 ± 43
<i>A. pallida</i> L.					
West of Portillo Region Valparaiso	8	Leaves	1.34 ± 37	0.09 ± 44	0.06 ± 33
		Stems	0.97 ± 36	0.15 ± 60	0.03 ± 67
		Flowers	1.61 ± 12	0.29 ± 10	0.28 ± 39
<i>A. versicolor</i> Ruiz et Pavon					
Termas de Cauquenes, Region General B. O'Higgins	5	Leaves	0.08 ± 63	0.00	0.01 ± 100
		Stems	1.73 ± 33	0.45 ± 7	0.07 ± 43
		Flowers	1.80 ± 9	0.35 ± 9	0.18 ± 33

¹ N: Number of plants investigated within one locality

² Mean ± CV,%

erally were highest in flowers and lowest in leaves, although the content in leaves exceeded that in stems in some plants (Table 1, Figure 2). 6-Tuliposide A, tuliposide D and E as well as tulipalin A were found, whereas tuliposide B was not detected. In general, the amount of 6-tuliposide A exceeded that of the other allergens in leaves and stems (Table 1), accounting in average for 62 and 66% of the allergen content based on tulipalin A equivalents, respectively. Tulipalin A was mainly found in the flowers (Table 1, Figure 2A), accounting in average for 48% of the total allergen content. Tuliposide D was only in few plants present in high concentrations and then mainly in the flowers

(Table 1, Figure 2C). Tuliposide E was only found in few species and always in very low amounts (data not shown).

Environmental variation

The environmental influence on the content of 6-tuliposide A was estimated by CV within individual clones. For most clones and plant parts CV was less than 30% (Table 2). For one clone of the 'Butterfly' type transplanted monthly from *in vitro* cultures and brought into flower from April to September CV for 6-tuliposide A was found to be 11, 20, and 29% in leaves, stems, and flowers, respectively (Table 2).

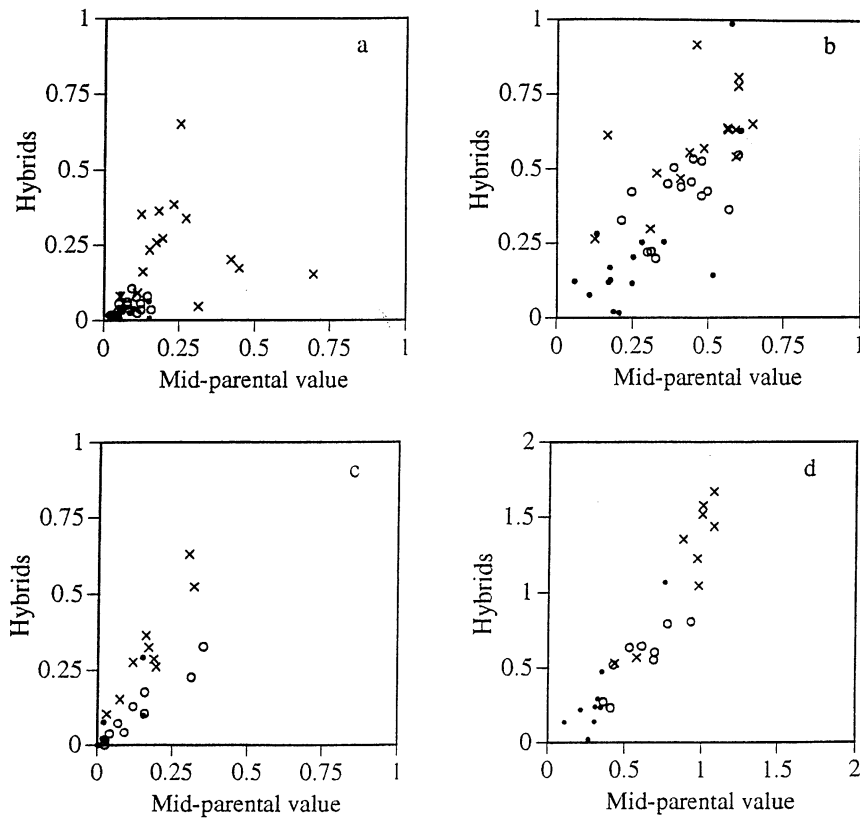


Figure 2. Concentrations (tulipalin A equivalents) of tulipalin A (a), 6-tuliposide A (b), tuliposide D (c), and total allergen content (d) in hybrids as a function of the mid-parental concentration in leaves (●), stems (○), and flowers (×). One tulipalin A equivalent corresponds to 2.8% 6-tuliposide A (fresh weight) and 1.9% tuliposide D (fresh weight). Estimates of slopes of regression lines are shown in Table 3.

Variability

Variation within populations of *A. aurea*, *A. ligtu* ssp. *ligtu*, *A. pallida*, and *A. versicolor*, were determined with CV above 40% in most cases (Table 1). One *A. ligtu* ssp. *ligtu* population (South of Valparaiso) did not contain allergens in the leaves. One of the *A. aurea* populations (Conguillio National Park) had relatively low amounts of allergens in all plant parts (Table 1).

Individual plants with a low content of either 6-tuliposide A, tuliposide D, or tulipalin A in the different plant parts were found within many species. Plants with a 6-tuliposide A concentration below 0.05% fresh weight in the leaves were found in e.g. *A. ligtu* ssp. *ligtu*, *A. pelegrina*, *A. magenta*, and *A. pulchra* ssp. *maxima*, whereas this low concentration was not detected in stems and flowers, except for the flowers in one *A. polyphylla* plant. Plants within many species contained low amounts of tuliposide D and/or tulipalin A (data not shown).

Heritability

Significant narrow sense heritabilities were found for the concentration of 6-tuliposide A, tuliposide D, as well as the total allergen content based on tulipalin A equivalents (Figure 2BCD, Table 3). The heritability of 6-tuliposide A was less than that of tuliposide D and total allergen content, whereas no clear trend in the magnitude of the heritability in the different plant parts was observed (Table 3). The heritability of tulipalin A was not significant, neither based on the concentration of tulipalin A nor the percentage of tulipalin A of the total allergen content (Figure 2A, Table 3).

Correlations

The distribution of allergens in different plant parts varied among individuals (Figure 3C) and the magnitude and significance of correlations depended on whether they were determined within *A. aurea*, *A. ligtu* ssp. *ligtu*, or the total plant population (Table 4). In general, however, significant positive correlations for the

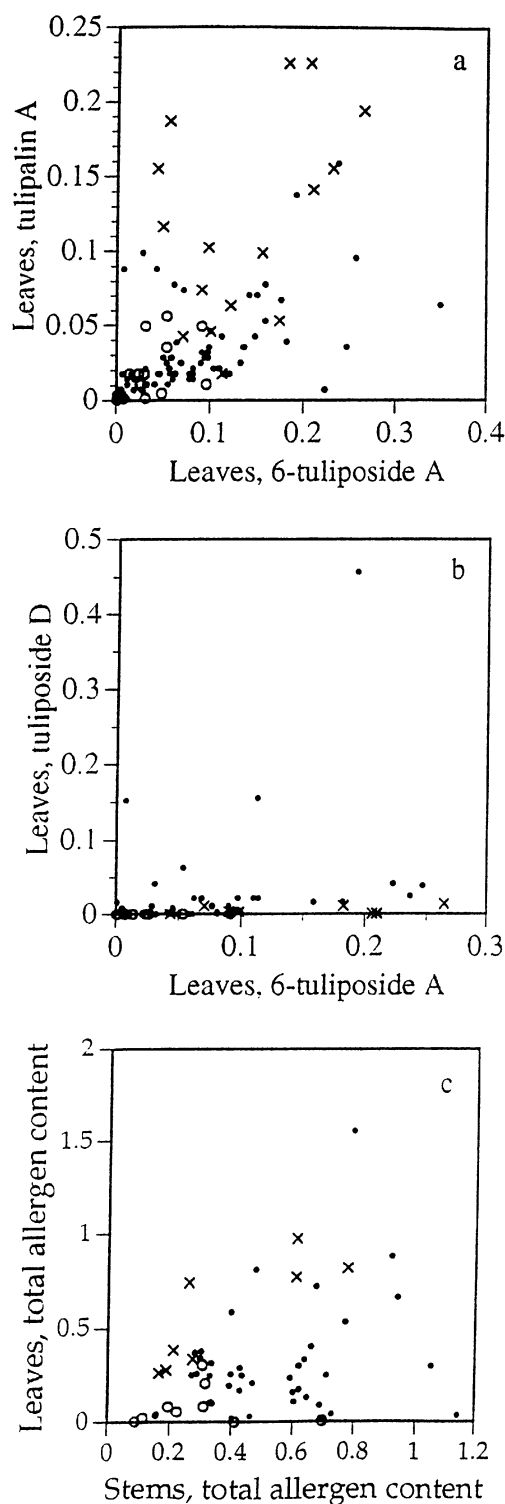


Figure 3. Relationship in leaves between (a): 6-tuliposide A and tulipalin A, and (b) 6-tuliposide A and tuliposide D. (c) total allergen content in stems and leaves. *A. ligtu* ssp. *ligtu* (×), *A. aurea* (○), and other species and hybrids (●). Concentrations are expressed as tulipalin A equivalents (see legend for Figure 2).

Table 2. Concentrations (% fresh weight) and coefficients of variation (CV) for 6-tuliposide A in different clones

Clone	N ¹	Plant part	6-Tuli- poside A	Range ³
<i>A. pelegrina</i> ×	3	Leaves	0.35 ± 31 ²	0.23–0.45
<i>A. polyphylla</i>		Stems	1.20 ± 27	0.84–1.46
		Flowers	1.74 ± 12	1.50–1.88
<i>A. polyphylla</i> ×	3	Leaves	0.05 ± 80	0.01–0.09
<i>A. 'Rosy Wings'</i>		Stems	0.56 ± 59	0.37–0.94
		Flowers	0.75 ± 29	0.55–1.00
<i>A. 'Rosy Wings'</i> ×	4	Leaves	0.79 ± 35	0.46–1.06
<i>A. pallida</i>		Stems	0.56 ± 25	0.40–0.73
		Flowers	1.00 ± 38	0.63–1.45
<i>A. pulchella</i>	3	Leaves	0.40 ± 63	0.16–0.65
		Stems	0.78 ± 33	0.49–1.00
		Flowers	0.10 ± 20	0.09–0.12
<i>A. 'Butterfly'</i> no 1	5	Leaves	0.20 ± 65	0.12–0.44
		Stems	0.76 ± 19	0.57–0.96
		Flowers	0.75 ± 25	0.55–0.90
<i>A. 'Butterfly'</i> no 2 ⁴	6	Leaves	0.33 ± 11	0.27–0.37
		Stems	0.94 ± 20	0.68–1.21
		Flowers	0.98 ± 29	0.57–1.34

¹ Number of shoots investigated

² Mean ± CV, %

³ Lowest - highest measured concentration

⁴ Plant material is collected monthly from April to September, thus including seasonal fluctuations.

content of 6-tuliposide A in leaves, stems, and flowers were found as well as between 6-tuliposide A and tulipalin A in leaves ($R^2 = 0.58$, Figure 3A) and stems ($R^2 = 0.43$) (Table 4). Tulipalin A and 6-tuliposide A were not correlated in the flowers ($R^2 = -0.03$). Significant correlations with tuliposide D were not found (Table 4), except within the total plant population, however, it was due to one plant with a high tuliposide D concentration (data not shown). It should be noticed that differences between *Alstroemeria* species in allergen distribution may be present, as for *A. ligtu* ssp. *ligtu* only correlations including tulipalin A were significant (Table 4).

Mutagenesis

After γ -radiation of three tetraploid clones, plants with a reduced content of 6-tuliposide A in leaves and/or flowers were found in one of the clones (Table 5). The variation of the content of 6-tuliposide A in the stems was too large to allow detection of changes caused by the γ -radiation. Further, the tulipalin A content was not

Table 3. Heritabilities of different allergens estimated as slope of regression between hybrid-value and mid-parent value. Plots of values are presented in Figure 2

	Heritability	P-value	R ²
6-Tuliposide A			
Leaves	1.11 ± 0.28	0.002	0.6
Stems	0.58 ± 0.24	0.03	0.3
Flowers	0.66 ± 0.23	0.01	0.4
Tuliposide D			
Leaves	1.29 ± 0.34	0.007	0.7
Stems	0.87 ± 0.10	0.000	0.9
Flowers	1.62 ± 0.24	0.000	0.9
Tulipalin A			
Leaves	0.17 ± 0.15	0.3	0.10
Stems	0.17 ± 0.17	0.3	0.07
Flowers	-0.09 ± 0.26	0.7	0.01
Per cent tulipalin A			
Leaves	0.16 ± 0.16	0.4	0.1
Stems	-0.06 ± 0.19	0.9	0.001
Flowers	0.07 ± 0.18	0.7	0.02
Total allergen content			
Leaves	1.60 ± 0.27	0.001	0.8
Stems	0.91 ± 0.21	0.003	0.7
Flowers	1.91 ± 0.32	0.001	0.8

affected after γ -radiation in neither of the clones (data not shown). Four months after the first determination, plants with a low content of allergens were still found to have less than the control plants.

Discussion

Function and biosynthesis of the allergens

Tuliposides and tulipalin A have fungitoxic effects (Bergman et al., 1967; Tschesche et al., 1969) and may thus be involved in resistance against pests. However, the high concentration of 6-tuliposide A (above 2.8% fresh weight) found in some plants indicate that tuliposides may be primary metabolites which function as storage compounds for D-glucose or regulators of osmolarity. Although the biosynthesis of tuliposides has not been investigated their chemical structure indicate that it is rather simple. Tuliposides are most likely biosynthesized from β -D-glucose and 4-hydroxy-2-methylenbutanoic acid, the latter being formed by a condensation between pyruvate and acetate (Hutchinson & Leete, 1970). However, as 4-methyleneglutamic acid is a predominant amino acid in *Tulipa* and many

other Liliaceae (Fowden & Steward, 1957; Kasai et al., 1984), it cannot be excluded that tuliposides are biosynthesized from this amino acid or its decarboxylation product (2-methylene-4-aminobutanoic acid) and β -D-glucose.

It has been suggested that the unsaturated lactonized aglycone tulipalin A is not naturally occurring but is formed after excision of the stems (Hausen et al., 1983; Hausen, 1988). However, we have found that the concentrations of tuliposides and tulipalin A immediately after homogenization of the fresh plants are the same as after several days, clearly indicating that tulipalin A is present in the plants before excision. This is also in accordance with the large differences in concentration of tulipalin A found in the different plant parts (Table 1). It is more likely that the content of tulipalin A in the plants is controlled by the environment (e.g. developmental stage, pest attacks, drought), also explaining the lack of a significant heritability for tulipalin A. Since tulipalin A is formed by hydrolysis of tuliposides (Hutchinson & Leete, 1970; Christensen & Kristiansen, 1995ab), this also explains why the total allergen content has a higher heritability than either 6-tuliposide A or tuliposide D.

Variability and heritability

The difference among species and the high coefficients of variation within populations of single species show a large genetic variation in the genus (Table 1). The high amounts of allergens, the variability among plants, the significant heritabilities, and the biogenetic considerations indicate that the biosynthesis of tuliposides is multigenically controlled. However, also environmental influences were significant and must be taken into account during selection (Table 2). Environmental effects on plant chemical composition are well documented and with respect to allergens season, irradiance, temperature, and plant age affect primin concentrations in *Primula obconica* (Hjorth, 1966; Horn & Eltorky, 1989). Due to the environmental influences potential parents for crossings should be screened for their allergen content at e.g. different times of the year.

Mutagenesis

Even though only few plants were analysed after γ -radiation, plants with a reduced allergen content were detected, indicating the potentials of mutagenesis in breeding of low allergen cultivars. Also in polyploids such as potato, sweet potato, and sugar cane the potentials of changing characteristics probably of multigenic

Table 4. Pearson coefficients of correlation (R^2) between 6-tuliposide A, tuliposide D, tulipalin A and total allergen content in leaves, stems and flowers within the total population (110 plants), *A. aurea* (16 plants), or *A. ligtu* ssp. *ligtu* (19 plants). For correlations including total allergen content based on tulipalin A equivalents from 6-tuliposide A, tuliposide D, and tulipalin A, 57 plants in the total population, 8 in *A. aurea*, and 9 in *A. ligtu* ssp. *ligtu* were included. Only correlations between allergens within one plant part and for each allergen between the different plant parts are shown

	Leaves, 6-tuliposide A			Stems, 6-tuliposide A			Flowers, tulipalin A			Flowers, tuliposide D		
	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>
Stems, 6-tuliposide A	0.31 ²	0.77 ³	0.19 ^{n.s.}									
Flowers, 6-tuliposide A	0.27 ²	0.68 ²	0.31 ^{n.s.}	0.52 ³	0.39 ^{n.s.}	- 0.26 ^{n.s.}	- 0.03 ^{n.s.}	- 0.04 ^{n.s.}	- 0.00 ^{n.s.}	0.41 ²	0.65 ^{n.s.}	0.42 ^{n.s.}
Leaves, tulipalin A	0.58 ³	0.42 ^{n.s.}	0.61 ²				0.40 ³	0.52 ¹	0.72 ³			
Stems, tulipalin A				0.43 ³	0.58 ¹	0.61 ²	0.48 ³	0.20 ^{n.s.}	0.61 ²			
Flowers, total allergen							0.65 ³	0.79 ¹	0.75 ¹	0.62 ³	0.54 ^{n.s.}	0.56 ^{n.s.}
Leaves, tuliposide D	0.25 ^{n.s.}	0.31 ^{n.s.}	.							0.33 ¹	0.27 ^{n.s.}	.
Stems, tuliposide D				0.36 ²	- 0.28 ^{n.s.}	0.83 ²				0.73 ³	- 0.27 ^{n.s.}	0.03 ^{n.s.}
Flowers, tuliposide D							0.03 ^{n.s.}	0.06 ^{n.s.}	0.24 ^{n.s.}			

	Leaves, 6-tuliposide A			Stems, 6-tuliposide A			Flowers, tulipalin A			Flowers, tuliposide D		
	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>	Total	<i>aurea</i>	<i>ligtu</i>
Stems, total allergen	0.32 ¹	0.83 ¹	- 0.04 ^{n.s.}							0.73 ³	- 0.14 ^{n.s.}	0.83 ²
Flowers, total allergen	0.24 ^{n.s.}	0.75 ¹	0.51 ^{n.s.}	0.58 ³	0.66 ^{n.s.}	0.01 ^{n.s.}						
Stems, tulipalin A				0.65 ³	0.85 ²	0.81 ²	0.42 ³	0.40 ^{n.s.}	0.48 ¹	0.45 ³	0.10 ^{n.s.}	0.51 ^{n.s.}
Leaves, tulipalin A	0.71 ³	0.79 ¹	0.94 ³									
Leaves, tuliposide D	0.63 ³	0.30 ^{n.s.}	.				0.21 ^{n.s.}	0.05 ^{n.s.}	.	0.61 ³	- 0.20 ^{n.s.}	.

^{n.s.}, ¹, ², ³ Not significant different from 0, significant at 5, 1, or 0.1% level, respectively

Table 5. 6-Tuliposide A concentrations (% fresh weight) in plants of *A. 'Butterfly'* no 1 after γ -radiation as compared to control plants. Only γ -radiated plants with a significantly lower concentration in one of the plant parts are shown. In further two 'Butterfly'-clones no plants with a reduced concentration of 6-tuliposide A were found

	6-Tuliposide A		
	Leaves	Stems	Flowers
Control	0.13 $\pm$ 0.01 ^a	0.75 $\pm$ 0.10	0.89 $\pm$ 0.01
Plant 1	0.02 ^{*b}	0.66 ^{n.s.}	0.54 ^{**}
Plant 2	0.03 [*]	0.51 ^{n.s.}	0.84 ^{n.s.}
Plant 3	0.06 ^{n.s.}	0.15 ^{n.s.}	0.51 ^{**}
Plant 4	0.03 [*]	0.48 ^{n.s.}	0.99 [*]
Plant 5	0.03 [*]	0.33 ^{n.s.}	0.57 ^{**}

^a Mean $\pm$ standard error of the mean

^b ^{n.s.}, ^{*}, ^{**} Not significant different from control plants, significant different at 5 and 1% level, respectively.

control by mutagenesis have been indicated (Broertjes & van Harten, 1988).

Breeding potentials and strategies

The content of all the allergens can be determined in the same HPLC analysis with 6-tuliposide A as the predominantly allergen in most plants and plant parts. If many samples are to be analysed speed can be increased by omitting determination of tuliposide D as it has a much longer retention time than 6-tuliposide A and tulipalin A (Figure 1). This can be justified as tuliposide D most often is present in only small amounts. The significant heritability of 6-tuliposide A, the positive correlation between the content of 6-tuliposide A and tulipalin A in both leaves and stems as well as the positive correlations between the total allergen content in different plant parts show that selection for low 6-tuliposide A and tulipalin A content in one plant part is likely to reduce the total content of allergens in the plant. Thus 6-tuliposide A and tulipalin A should be focused on during selection.

Plants with low amounts of allergens in the leaves are of special interest as workers mostly come into contact with the plant sap by removal of leaves during cut flower harvesting. High concentrations in the flowers

and stems are more likely to be tolerated as they are left undamaged during plant handling. Also when growing *Alstroemeria* as pot plants a low allergen content in the leaves is most important.

The present results show that the allergen content can be reduced both by hybridizations and mutagenesis. However, due to high allergen contents in most plants and multigenic control of the allergen production several breeding generations will be necessary before cultivars that will reduce the risk of sensitization can be introduced to the market. To produce *Alstroemeria* cultivars totally without allergens must be considered very difficult or even impossible.

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