

## Breeding primin-free *Primula obconica*

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### Abstract

Primin-free *Primula obconica* plants were obtained and the inheritance of this characteristic was studied. A range of *P. obconica* strains was screened on primin content of individual plants. Five primin-free plants were selected from a single strain. An analysis of the  $F_1$  and backcrosses indicated that the presence of primin is most likely inherited as a monogenic character. In the  $F_2$  generations, however, this could not be confirmed in all cases.

**Keywords:** *Primula obconica*, primin-free, breeding, Craventest

### Introduction

*Primula obconica* is a valuable, nearly year-round flowering potplant with a wide range of flower colours. However, consumer demand for *Primula obconica* remains relatively low due to the presence of an allergy-causing substance, which is also a problem for the grower. This substance, named primin, is secreted by the glandular hairs on the epidermis of the leaves and stems, calyx and peduncle of the plants. In sensitive people primin can cause a serious dermatitis. This allergy has been studied by many research workers as appears from the more than 420 articles published between 1888 and 1977 (Hausen, 1979).

In 1879, *Primula obconica* was imported in Britain from China, West Hupeh, by the Veitch Company, and in 1883 it was commercially introduced. In 1888 a dermatitis caused by *P. obconica* was first mentioned by White in America. Later this was confirmed by Nestler (1900a, b) who proved that a secretion from the topcells of the glandular hairs (with a length of 3-6 cells) is responsible for the primin dermatitis. Nestler (1908) developed a method for testing the presence or absence of this substance, which later was named 'primin' by Bloch & Karrer (1927), who analysed it as  $C_{14}H_{20}O_3$  or  $C_{14}H_{18}O_3$ .

A fast and accurate method of screening for primin presence was published in 1956 by Brachtendorf (1956). This method allows screening on primin content of *P. obconica* plants on a large scale.

The elucidation of the chemical structure of primin by Schildknecht, Bayer & Schmidt (1967) showed that primin is a benzoquinone. The presence of primin can

also be demonstrated by using tests developed for benzoquinones by Kesting (1929) and by Craven (1931). Both methods were used by Thomson (1971) with comparable results.

Klein & Tröthandl (1927) and Storck (1938) observed differences in primin content between the various parts of the plant. Those differences were related to the number of glandular hairs. The highest primin content was observed on calyx and peduncle. They also concluded that the production of primin is strongly influenced by external factors such as light, temperature and humidity, and varies with the season.

The breeding of *Primula obconica* started in 1887, as described by Maurer & Storck (1936). The German grower Arends developed over a period of 25 years many interesting large-flowered *P. obconica* cultivars. In order to obtain primin-free *P. obconica* hybrids, Arends crossed *P. obconica* with several primin-free *Primula* species. He apparently was successful in selecting primin-free plants after crossing and backcrossing *P. sinolisteri* with *P. obconica*. However, his primin-free genotypes were lost in 1943.

Storck (1944) suggested two ways to obtain primin-free *P. obconica* genotypes: — by selecting on low primin content in the wild *P. obconica* or its various cultivated strains, followed by selfing or hybridization and selection; — by crossing *P. obconica* with primin-free *Primula* species.

By crossing *P. obconica* × *P. sinolisteri*, Storck (1935) obtained hybrids, but apparently he was not successful in crossing or selfing these hybrid plants. After crossing the hybrids made by Arends of *P. (sinolisteri* × *obconica*) × *obconica* with *P. sinolisteri*, Storck did however obtain primin-free plants. About the ploidy level of these interspecific hybrids, no information was given.

Crosses between the primin-free plants resulted in only a few cases in primin-free progenies. So Storck concluded that there are probably several independent genes determining the level of the primin content. The same conclusion was recently drawn by Horn & Eltorky (1989), after analysing the inheritance of primin production.

In 1956 Brachtendorf (1956) considered the possibility that primin-free mutants of *P. obconica* could be present within a population, so he developed his quickest to detect such mutants. He investigated Mayer's *P. obconica* strains but did not find any primin-free plants. Mayer (1971) reported that his so called primin-free strain never resulted in completely primin-free progenies, and Fregert & Hjorth (1977) demonstrated the presence of primin in the primin-free strain of Mayer.

In 1982, the Institute for Horticultural Plant Breeding started a program to try to detect primin-free *P. obconica* mutants as suggested by Brachtendorf, and to investigate the possibilities for breeding primin-free strains of different flower colours. In the same period, Horn & Eltorky (1989) investigated the genetic background of the flower colours and the primin content of *P. obconica*.

## Materials and methods

The investigation of *Primula obconica* genotypes started with eight different diploid

strains with different flower colours: the 'Donkerrose' (mauve pink), 'Donkerrood' (dark red), 'Stadt Zürich' (dark mauve pink), 'Koperrood' (copper red), 'Appelbloesem' (apple blossom), 'Lents rood' (dark mauve pink), 'Blauw' (blue) and 'Donkerblauw' (dark blue) genotypes from J. C. Maarse Company at Aalsmeer. The seeds obtained were sown in 1981. Since heterostyly is one of the characteristics of *P. obconica*, from each strain one longstyled and one shortstyled plant were selected on plant type. Reciprocal crosses were made in 1982, between the long- and shortstyled plants of the same strain. From the eight reciprocal crosses only 5 long-styled  $\times$  shortstyled, and 5 shortstyled  $\times$  longstyled crosses succeeded, while from two strains (the dark red and the dark blue) both the reciprocal crosses failed. After selfing the selected plants, only the selected longstyled plant of the dark blue strain produced seeds.

All the seeds obtained were sown in 1983, and, to screen the offspring of the different genotypes for primin content, the seedlings (25 plants per progeny) were subjected to the 'Craventest' (Heyting & Toxopeus, 1987). The stems of the third, fully expanded leaves of the young seedlings were tested on their primin content six weeks after planting out and, according to the blue colour reaction, divided into primin-free ( $p^-$ ) plants, showing no colour reaction, and primin-producing ( $p^+$ ) plants, showing a blue coloration.

In 1983, crosses between the different primin-free plants were made, as well as crosses between the primin-free plants and primin-producing plants descending from the original strains. The  $F_1$  plants obtained were tested ( $\pm 250$  plants per progeny).

Backcrosses of three  $F_1$  plants (copper-red and apple-blossom hybrids) with the primin-free parents were made in 1985.

In 1984 and 1985, full sib crosses were made between longstyled plants ( $\varnothing$ ) and shortstyled plants ( $\sigma$ ) in several  $F_1$  progenies to obtain  $F_2$  generations for a study of the segregations.

In 1986 and 1987 crosses were made between the selected primin-free  $F_2$  plants, with the aim to obtain primin-free  $F_3$  progenies.

## Results

After screening the seedlings of the two reciprocal progenies (long-  $\times$  shortstyled and short-  $\times$  longstyled) of the different strains on their primin content, it appeared that, with the exception of the 'Donkerrose' strain, they all were producing primin ( $p^+$ ). In the two reciprocal progenies of the 'Donkerrose' strain, five plants did not show any colour reaction in the Craventest. Of the five plants, one was longstyled (82270-20) and four were shortstyled (82261-6, 82261-8, 82270-11 and 82270-22). After intercrossing the primin-free ( $p^-$ ) plants, only primin-free progenies were obtained.

Twenty crosses between the original  $p^-$  plants and  $p^+$  plants from different strains yielded only  $p^+$  plants (2473  $F_1$  plants tested altogether) indicating dominance of  $p^+$ . However, in one other progeny, involving 82270-20 and a  $p^+$  plant of the variety 'Stadt Zürich', 33 out of 108 plants were primin-free. Backcross-

Table 1. Segregation of the F<sub>2</sub> populations, arranged according to the primin-free parents 82261-8, 82270-11, 82270-22 shortstyled, 82270-20 longstyled. p<sup>-</sup> = primin-free; p<sup>+</sup> = primin progeny.

Source of p <sup>+</sup> parents of F <sub>1</sub>	Crossings	Progenies		
		p <sup>+</sup>	p <sup>-</sup>	χ <sup>2</sup>
<i>p<sup>-</sup> parent: 82261-8</i>				
Copper red	83348- 56 × 83348- 84	171	56	0.013
	83348- 56 × 83348- 85	195	58	0.581
	83348- 80 × 83348- 85	183	70	0.960
	83350- 1 × 83350- 2	191	64	0.001
Blue	83375- 1 × 83375- 2	196	58	0.635
	83375- 1 × 83375- 16	155	65	2.424
	83375- 3 × 83375- 2	201	52	2.668
	83375- 3 × 83375- 16	194	49	3.030
	83375- 97 × 83375- 16	122	41	0.002
Apple blossom	83382- 3 × 83382- 1	134	84	21.291*
	83382- 63 × 83382-102	187	65	0.085
	83382-179 × 83382- 54	191	60	0.161
	83385- 1 × 83385- 4	198	56	1.181
<i>Total</i>		<i>2318</i>	<i>778</i>	<i>0.028</i>
<i>p<sup>-</sup> parent: 82270-11</i>				
Copper red	83349- 7 × 83349- 3	203	52	2.888
	83349- 7 × 83349- 2	188	67	0.221
	83349- 4 × 83349- 2	181	69	0.901
	83351- 6 × 83351- 5	185	38	7.535*
	83351- 1 × 83351- 3	184	66	0.261
Blue	83358- 1 × 83358- 6	213	56	2.509
	83358- 4 × 83358- 6	257	35	26.374*
	83376- 3 × 83376- 2	212	43	9.005*
Apple blossom	83383- 1 × 83383- 2	196	58	0.635
	83383- 6 × 83383- 3	214	31	19.920*
	83383- 5 × 83383- 3	200	51	2.934
	83386- 5 × 83386- 1	182	73	1.790
	83386- 3 × 83386- 2	174	58	0.000
	83386- 3 × 83386- 1	186	61	0.012
<i>Total</i>		<i>2775</i>	<i>758</i>	<i>23.680*</i>

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Table 1 continued.

Source of p <sup>+</sup> parents of F <sub>1</sub>	Crossings	Progenies		
		p <sup>+</sup>	p <sup>-</sup>	χ <sup>2</sup>
	<i>p<sup>-</sup> parent: 82270-22</i>			
Copper red	83352- 4 × 83352- 14	179	75	2.777
	83352- 5 × 83352- 7	196	57	0.823
	83352- 10 × 83352- 7	204	51	3.400
	83352- 2 × 83352- 3	197	55	1.354
	83328- 2 × 83328- 3	231	55	5.077*
Dark blue	83329- 1 × 83329- 2	139	61	3.227
	83329- 1 × 83329- 3	195	62	0.105
Blue	83359- 3 × 83359- 4	179	43	3.754
	83359- 3 × 83359- 11	191	59	0.261
	83359- 5 × 83359- 4	61	13	2.180
	83359- 5 × 83359- 11	79	23	0.327
	83377- 3 × 83377- 7	49	4	8.610*
	83377- 3 × 83377- 15	110	49	2.870
	83377- 4 × 83377- 7	217	29	22.900*
	83377- 4 × 83377- 15	215	25	27.222*
Apple blossom	83384- 1 × 83384- 9	170	80	6.533*
	83384- 7 × 83384- 9	202	50	3.577
	83384- 7 × 83384- 5	191	63	0.005
	83389- 1 × 83389- 3	182	70	1.037
	83389- 11 × 83389- 10	191	58	0.387
	83389- 1 × 83389- 10	181	71	1.354
<i>Total</i>		<i>3559</i>	<i>1053</i>	<i>11.560*</i>
	<i>p<sup>-</sup> parent: 82270-20</i>			
Blue	83378- 2 × 83378- 7	150	22	13.674*
	83378- 8 × 83378- 7	211	40	10.997*
Stadt Zürich	83392- 14 × 83392- 6	179	70	1.286
	83392- 14 × 83392- 12	97	44	2.896
Apple blossom	83390- 1 × 83390- 5	218	33	18.806*
	83390- 1 × 83390- 3	195	51	2.390
	83390- 4 × 83390- 5	231	24	33.047*
<i>Total</i>		<i>1281</i>	<i>284</i>	<i>39.190*</i>

\* Significant deviation from 3:1 segregation.

ings of copper-red and apple-blossom  $F_1$  hybrids (supposedly heterozygous for primin production) with the primin-free parents 82270-20, 82270-22 and 82261-8, did not give a significant deviation from a 1:1 segregation. No backcrossings were made with the primin-free 82270-11 or 82261-6.

The results of the segregations in  $p^+$  and  $p^-$  plants in the  $F_2$ 's, are listed in Table 1 and Table 2. The data in Table 2 are summarized according to the strain they descend from and according to the collective  $p^-$  parent (82261-8, 82270-11, 82270-22 and 82270-20) of the  $F_1$  progenies. Assuming that the characteristic primin-free ( $p^-$ ) is a monogenic recessive factor, a segregation into  $p^+ : p^- = 3:1$  would be expected. In the majority of the  $F_2$  progenies no significant deviation from 3:1 segregation was found; in 14 out of 55 progenies a deviation from 3:1 segregation was however observed. When the data were summarized according to the collective  $p^-$  parent, only the  $F_2$  populations with the collective 82261-8 parent showed no deviation from a 3:1 segregation.

The primin-free  $F_3$  progenies, obtained after crossing different primin-free selected plants from the  $F_2$ , were released by the Institute for Horticultural Plant Breeding (IVT) in 1987.

Table 2. Segregation of  $F_2$  populations. The data are summarized according to the strains they descend from and according to the collective  $p^-$  parent.  $p^-$  = primin-free,  $p^+$  = primin progenies.

Strain	$p^-$ parent	Progenies			
		$p^+$	$p^-$	$\chi^2$	$\chi^2$ per strain
Copper red	82261- 8	740	248	0.005	3.382
	82270-11	941	292	1.142	
	82270-22	1007	293	4.201*	
Blue	82261- 8	868	265	1.568	71.655*
	82270-11	682	134	32.026*	
	82270-22	1101	245	33.174*	
	82270-20	361	62	24.133*	
Dark blue	82270-22	334	123	0.894	0.894
Apple blossom	82261- 8	710	265	2.470	7.784*
	82270-11	1152	332	5.466*	
	82270-22	1117	392	0.769	
	82270-20	644	108	45.390*	
Stadt Zürich	82270-20	276	114	3.723	3.723

\* Significant deviation from 3:1 segregation.

## Discussion

Primin-free *Primula obconica* produced by Arends (Storck, 1944) and later by Mayer (1971) were either lost or were proved not to be free of primin (Fregert & Hjorth, 1977). According to Brachtendorf (1956) it should nevertheless be possible to obtain primin-free mutants, in analogy with mutants obtained in other genera for similar characters. It seems likely that the primin-free selections out of the reciprocal crosses 82261 and 82270 descended from a recessive mutation in an earlier generation.

Though the majority of the  $F_1$  crosses, the back crosses and the  $F_2$  populations suggest a monogenic inheritance with  $p^+$  being dominant over  $p^-$ , there are too many deviations to maintain this model:

- segregation of 5  $p^-$  plants out of 50 plants from reciprocal crosses of 2 plants of 'Donkerrose';
- segregation of 33  $p^-$  plants out of 108  $F_1$  plants in a cross between one of the 5 original  $p^-$  plants and a  $p^+$  plant of 'Stadt Zürich';
- segregation of too few  $p^-$  plants in 12 of the 55  $F_2$  populations and too many  $p^-$  plants in another two.

The available data do not allow a complete analysis, but the deviations observed indicate the presence of a second independently inheriting gen. The different possible genotypical combinations may represent different levels of primin content, but the method of analysing used did not allow a quantitative assessment.

Apparently, genotypes heterozygous for primin content occur in some of the varieties (e.g. 'Donkerrose' and 'Stadt Zürich').

The results Storck (1938) obtained, by crossing the primin-free plants he selected after crossing and backcrossing *P. obconica* with *P. sinolisteri*, can not elucidate the inheritance of primin production. He obtained  $p^+$  plants after crossing  $p^- \times p^+$  selections, but since the number of plants was relatively low and no information was given about the ploidy level, his suggestion of the presence of three independently inheriting factors, controlling the synthesis of primin production, is not convincing.

The results obtained by Horn & Eltorky (1989) with their *Primula obconica* genotypes are more or less comparable with the results obtained by Stock; these results can, however, not elucidate the inheritance of the primin production in *Primula obconica* and neither can they be explained by monogenic inheritance or by the segregation of two or three genes controlling the primin production.

The *Primula obconica* strains used in the present study were from a different origin than those used by Horn & Eltorky (1989). This may explain the differences with our present results.

## Conclusions

Plants heterozygous for primin content occur frequently in several commercial varieties.

Primin-free plants breed true for this character at least in the  $F_3$ ; this is in contrast with data of Horn & Eltorky (1989).

Two genes appear to be involved in primin production but, as one is often not

functioning (homozygous  $p^-$ ), many cases of apparent monogenic inheritance are found.

Absence of primin can be and has been combined with different flower colours.

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