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ABERRANT LEAF MORPHOLOGY IN MUTANTS OF SILENE PRATENSIS (CARYOPHYLLACEAE)

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SUMMARY

Mutants of *Silene pratensis* which are unable to O-glycosylate isovitexin in the petals often possess leaves with a typically curled shape. These leaves are mainly found on the elongated flower stem and not in the rosette. Increased light levels stimulate the occurrence of the aberrant leaf shape. In older plants a rosette-tied O-glycosylation may get lost which results in curled rosette leaves.

1. INTRODUCTION

Silene pratensis (Rafn) Godron et Gren.*), the white campion or white cockle, is a common weed widely distributed over Europe. Within this species chemical races occur which differ in flavone glycosylation in the petals (MASTENBROEK et al. 1982). The main flavonoid "aglycones" found in this species are the flavone C-glycosides isovitexin and its hydroxylated and methylated products iso-orientin and isoscoparin (VAN BREDERODE et al. 1980, VAN BREDERODE & KAMPS-HEINSBROEK 1981). In general, these compounds occur as their 7- and/or 6-(2″)-O-glycosides. One genotype, gg glgl fgfg, however, is unable to glycosylate isovitexin in the petals. At the same time plants of this genotype are distinguished from other genotypes by the occurrence of flowers with much smaller petals, which also curl in a typical way (VAN BREDERODE & VAN NIGTEVECHT 1972). Genetical evidence points to a direct relation between accumulation of the non O-glycosylated flavone and the morphogenetic effect. The flavone is accumulated in the upper epidermis (NIEMANN et al. 1983 and unpublished results) and, contrary to its O-glycosides, isovitexin interferes with the cell metabolism. This leads to the appearance of aberrant inclusions (VAN GENDEREN et al. 1983) and finally to cell death (VAN BREDERODE et al. 1982). The inhomogenous distribution of isovitexin over the epidermis thus causes local disruption followed by curling of the petals.

Sometimes a comparable morphogenetic aberrancy was also observed in the leaves but here it is much less common than in the petals. On further investigation different causes appeared to be responsible for the comparatively infrequent occurrence of leaf curling. The present paper deals with the appearance of this typical effect and its background.

* formerly: Silene alba (Mill) E. H. L. Krause, nom illeg. (McNeill & Prentice 1981).

2. MATERIAL AND METHODS

2.1. Plant material

Genotypes of *Silene pratensis* were obtained by the research group "Oecological and biochemical genetics of plants" of the University of Utrecht by inbreeding and selection. They are homozygous for certain flavone glycosylation genes. Seedlings of line no 88D, genotype gg glgl fgfg, were grown in an experimental garden, in a greenhouse or in a climate chamber. In the climate chamber the temperature varied from 24°C at daytime to 15°C at night, with a light-dark regime of 16–8 hours at 8000, 17000 and 30000 lux.

2.2. Flavone analysis

Fresh leaves were extracted three times with 80% methanol (10 ml/g fresh weight). For a rough estimate of total flavone the amount was measured by UV spectrophotometry using the absorption at 335 nm with a test curve of vitexin (log ε 4.33) as reference. An identical molecular extinction was assumed for vitexin and isovitexin and derivatives. The solutions were evaporated to dryness and the residue was taken up in methanol (½ ml/g fresh). This solution was used for thin layer chromatography (TLC) and/or high-performance liquid chromatography (HPLC). For TLC 4 µl samples were spotted on cellulose plates (Merck Fertigplatten, 0.1 mm) and developed with 15% acetic acid in water. The spots were located by their absorption under UV light. Afterwards the plates were sprayed with a 1% ethanolic solution of flavone reagent (Fluka) and again viewed under UV. With this reagent the compounds become fluorescent, greenish for isovitexin and its O-glycosides, yellow for iso-orientin and orange for iso-orientin 7-O-glycosides.

HPLC was performed with a Dupont 830 chromatograph, using a 4.2 × 240 mm Zorbax ODS column eluted with a gradient (convex 2, from 5–55% at 3% per min.) of methanol – 0.1% phosphoric acid in water at room temperature and at a flow of 0.5 ml/min. (pressure around 1100 psi or 7500 kPa). Naringenin was used as internal standard. Peak areas were measured with an Infotronics 304 computing integrator. For calculation of absolute amounts a known solution of vitexin was used as a reference.

3. RESULTS AND DISCUSSION

Seedlings of *Silene pratensis* start with the formation of a rosette having leaves with a clear petiole. Later a flower stem arises with sessile leaves. Auxilliary buds at the stem base give, after or during flowering of the first stem, rise to new rosettes and in due time to flowering stems.

From observations in the experimental garden it appeared that the typical curling only occurred in the sessile leaves and not in rosette leaves (*fig. 1*). These stem leaves first start to fold in the form of a boat, whereas in a later stage brown, dead areas may appear at the leaf top and the leaf edges may wrinkle (*fig. 2A*). Often plants grown in the greenhouse did not show any leaf aberrancy

Fig. 1. *Silene pratensis*, genotype gg glgl fgfg, grown in the experimental garden, with typically curled stem leaves.

at all. If, however, a number of these plants was transferred to the open they formed curled leaves within three weeks. This effect is clearly illustrated in *fig. 2A* and *B*. It is known that in *Silene* a higher light level results in an increased flavone concentration in the upper epidermis (NIEMANN et al. 1983). Accumulation of flavone was supposed and could be confirmed by analysis. A typical analysis of the isovitexin content of comparable leaves of a plant of each series (greenhouse *vs.* experimental garden) is given in *table 1*. Practically all stem leaves of the plant transferred to the open have a much higher isovitexin level than the greenhouse control.

That indeed a change in light level and no other changes were responsible was confirmed in another experiment. Plants grown in the climate chamber under three different light levels clearly showed the aberrant leaf form after 6 weeks under the highest level (30000 lux) only, at 17000 lux the effect tended to develop whereas it was totally absent at 8000 lux. Total flavone per g fresh weight in comparable leaves was found to decrease from about 3.3 mg at 30000 lux, via 2.2 at 17000 to 0.7 mg at 8000 lux. Here again leaf curling seems directly related to flavone content.

Care has to be taken with the interpretation of total flavone since both the composition and the localization in the leaf play an important part. It may be assumed that the greater part of the flavone is found in the upper epidermis (NIEMANN et al. 1983) and thus could be directly responsible for curling in a

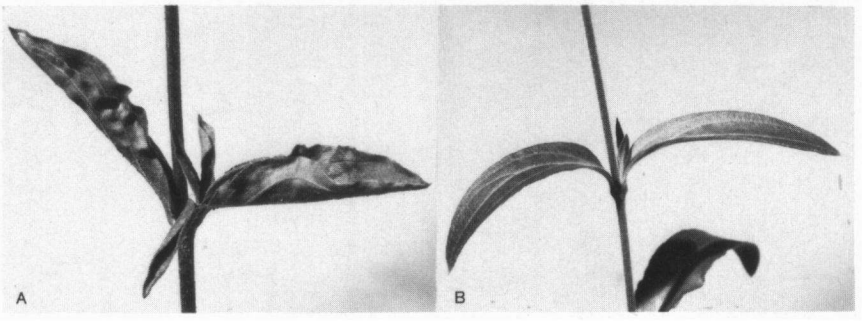

Fig. 2A. Curled leaves of *Silene pratensis*, genotype gg glgl fgfg, formed in three weeks time after transference from the greenhouse to the experimental garden.
Fig. 2B. Comparable leaves of a typical plant of the same genotype as shown in *2A* but kept in the greenhouse.

way similar to that found in the petals (VAN BREDERODE et al. 1982). The flavone composition in the leaves, however, may differ from that in the petals. Although the mutant investigated is unable to glycosylate isovitexin in the petals it has recently been shown (STEIJNS et al. 1983) that some special O-glycosylation occurs in the first leaves, apparently restricted to the rosette type (cf. NIEMANN, in preparation). This also provides an explanation for the fact that the morphological phenomenon is hardly observed in rosette leaves. The total flavone concentration is not essentially lower in rosette leaves than in stem leaves, but the composition differs. Accordingly, in rosettes of young plants curling was never observed.

In older plants, with secundary rosettes, curled rosette leaves could sometimes be found. When such leaves were analysed for their flavone composition it appeared that the plant had lost part of its potency to O-glycosylate isovitexin in the rosette leaves. This is illustrated in *fig. 3* which shows a diagram of the TLC analysis of both stem and rosette leaves of two 4½ months old plants, C and D, of which one (D) had both curled rosette and stem leaves, whereas the

Table 1. Amounts of flavone in corresponding stem-leaves of *Silene pratensis* plants, genotype gg glgl fgfg, respectively transferred to the open (A) and greenhouse control (B). The leaves of plant A show leaf curling.

	iv		iv/io		iv + io[1]	
	A	B	A	B	A	B
Old leaf at the stem base	1.8	0.8	78/22	79/21	2.3	1.0
Leaf at the top of the main flowering stem	4.3	0.7	89/11	64/36	4.9	1.3
Young leaf under apex of a side stem	3.6	1.0	85/15	59/41	4.2	1.7
Ibid. at the apex of the same side stem	3.2	1.5	92/8	52/48	3.5	2.9
Ibid. at another side stem	2.7	0.9	92/8	53/47	2.9	1.7

[1]iv = isovitexin, io = iso-orientin, iv/io = the ratio isovitexin/iso-orientin, all expressed in mg/g fresh leaf weight; iv + io may also include some isoscoparin.

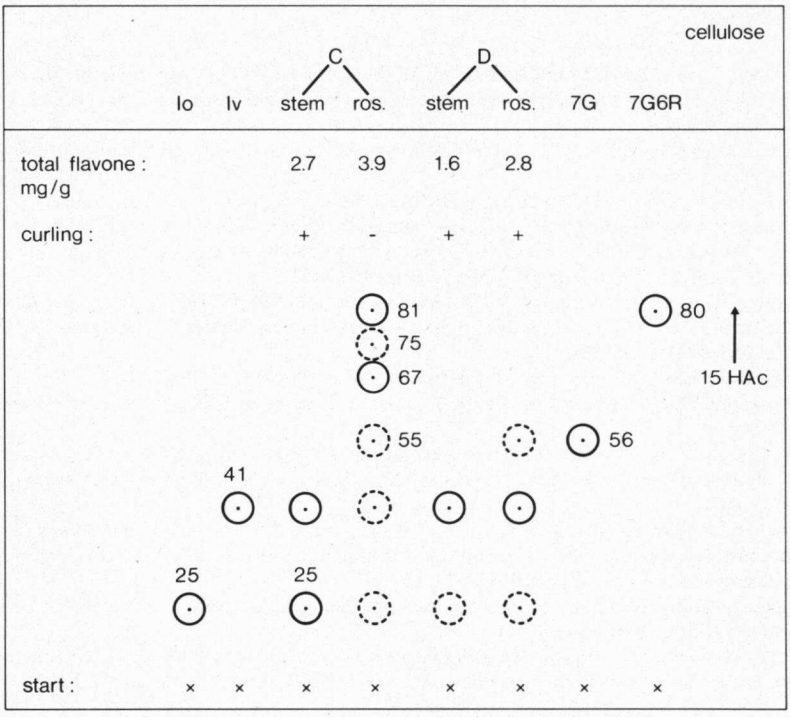

Fig. 3. Diagram of a thin layer sheet with extracts of stem and rosette leaves of older *Silene* plants with respectively normal (C) and curled (D) rosettes. Referents: iso-orientin (Io), isovitexin (Iv), isovitexin 7-O-glucoside (7G), and isovitexin 7-O-glucoside 6-O″-rhamnoside (7G6R), dotted spots indicate trace amounts and the figures give the $R_f \times 100$.

other had normal rosette leaves. In spite of the high flavone concentration in C rosette leaves of 3.9 mg/g curling does not occur, but the concentration of 2.8, mainly consisting of isovitexin in D rosette leaves cause the effect.

In general it appears that factors which influence the concentration of isovitexin in the leaf, such as enhanced light level or may be decreased O-glycosylating ability with increasing plant age, have a similar effect on leaf aberrancy. This seems quite well to explain the variability in its occurrence.

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REFERENCES

BREDERODE, J. VAN, H. H. VAN GENDEREN & W. BERENDSEN (1982): Morphological effect of the flavone isovitexin in a non-glycosylating genotype of Silene pratensis (Caryophyllaceae). *Experientia* **38**: 929–931.

— & R. KAMPS-HEINSBROEK (1981): Methylation of 3′, 4′di-OH C-glycosylflavones in Silene. *Z. Naturforsch.* **36c**: 486–487.

—, G. J. NIEMANN & G. VAN NIGTEVECHT (1980): Genetic, ontogenetic and geographical variation in the C-glycosylflavone patterns of Silene alba and S. dioica. *Planta Med.* **39**: 221.

— & VAN NIGTEVECHT (1972): Flavone-glycosides in the petals of Melandrium album: structure, genetic control and biosynthesis. *Genen Phaenen* **15**: 3–8.

GENDEREN, H. H. VAN, G. J. NIEMANN & J. VAN BREDERODE (1983): Globular, crystal- and clew-like structures in the epidermis cells of mutants of Silene pratensis unable to glycosylate isovitexin. *Protoplasma* **118**: 135–139.

MASTENBROEK, O., J. W. MAAS, J. VAN BREDERODE, G. J. NIEMANN & G. VAN NIGTEVECHT (1982): The geographic distribution of flavon-glycosylating genes in Silene pratensis. *Genetica* **59**: 139–144.

MCNEILL, J. & H. C. PRENTICE (1981): Silene pratensis (Rafn) Godron & Gren, the correct name for White Campion or White Cockle (Silene alba (Miller) E. H. L. Krause, nom. illeg.). *Taxon* **30**: 27–32.

NIEMANN, G. J., J. W. KOERSELMAN-KOOY, J. M. J. M. STEIJNS & J. VAN BREDERODE (1983): Flavone distribution in leaves of different genotypes of Silene pratensis (Rafn) Godron and Gren. (Caryophyllaceae). *Z. Pflanzenphysiol.* **109**: 105–112.

— (in preparation): The influence of light on leaf morphology and flavonoid glycosylation in Silene pratensis (Caryophyllaceae).

STEIJNS, J. M., G. VAN NIGTEVECHT, G. J. NIEMANN & J. VAN BREDERODE (1983): Differential regulation of flavone glycosylation during ontogeny of Silene pratensis. *Z. Naturforsch.* **38c**: 544–548.