HortScience 24(3):501–502. 1989.

Inheritance of Red-spotted Petals and Golden Leaves in Zonal Pelargonium

A. Baset Almouslem and Richard A.E. Tilney-Bassett
School of Biological Sciences, University College of Swansea, Singleton Park, Swansea, SA2 8PP Wales, United Kingdom

Additional index words. *Pelargonium* ×*hortorum*, aurea leaves, petal trichomes

Abstract. **The pink flowers of the golden-leaved (aurea) zonal pelargonium (*Pelargonium* ×*hortorum* Bailey 'Verona') have small red spots on the upper epidermis. The spots are caused by an intensification of pigments at the base of trichomes and in adjacent, surrounding epidermal cells. Trichomes are found on petals of other colors, but without spots. Genetic analysis shows that 'Verona' is heterozygous for the red-spotted trichome trait, which is determined by a gene, *Rst/rst*, dominant over red spotless. The red-spotted gene segregates independently of the aurea gene, *Aur*$^+$/*Aur*, which conditions golden leaf color.**

In 1907, Baur (1) observed that the golden-leaf mutant of 'Verona' zonal pelargonium was inherited, similar to the aurea mutant of snapdragon (*Antirrhinum majus*), in which the golden-leaf trait was determined by a nuclear gene with incomplete dominance. The pelargonium golden leaf heterozygote, also known as aurea, *Aur*$^+$/*Aur* (3) likewise segregated, after selfing into a monohybrid ratio of 1 green : 2 aurea : 1 white progeny, except that there was a deficit of inviable white seedlings. This was confirmed by several authors using other golden-leaf cultivars (7–9, 12, 13). Compared with the green-leaf homozygote, the golden-leaf heterozygote has a reduced chlorophyll content and, due to a deficiency of chlorophyll *b*, a raised chlorophyll *a*/*b* ratio (6, 14). Much of the published work on genetics of zonal pelargoniums was reviewed by Craig (2) and Harney (5).

We have recently discovered a new trait in 'Verona' (VER), in which the pink petals have small red spots just visible on the upper epidermis. Our objective was to determine the inheritance of red-spotted pink petals, and to test the independence between this character and that of the green- vs. golden-leaf traits.

We grew our plants in John Innes No. 1 or 2 potting compost in 13-cm (1 liter) plastic pots in a greenhouse under natural daylight and temperature conditions. The behavior of 'Verona' was analyzed by comparing it with other cultivars. 'Foster's Seedling' (FOS), 'Hills of Snow' (HS), and a male-sterile hybrid seedling (MS1H) had pink flowers without red spots. 'J.C. Mapping' (JCM) had white flowers with a pink blush and no spots, and 'Dolly Varden' (DV) and 'Flower of Spring' (FS) had red flowers without spots. Except for 'Verona' and the male-sterile hybrid, these cultivars exist as both green and variegated forms. We checked by chromosome counts of root tip squashes that all are diploid, 2n = 2x = 18.

The red spots are more frequent in the lower three petals than the upper two of the slightly zygomorphic flowers, and they tend to be concentrated in the lower half of the petal. Microscopic examination reveals that the spots occur with the position of a trichome arising from the upper epidermis. Each trichome consists of a bright red, dense central cell, which is elongated into a glandular hair with a unicellular tip. The central cell is surrounded by five to 10 reddish epidermal cells, more strongly pigmented than other epidermal cells. Presumably, the pigments are anthocyanins similar to the petal pigments. We have looked for trichomes on red, salmon, pink, and white petals of other cultivars. They vary from absent to quite frequent. Sometimes they occur on the lower epidermis as well as the upper. When present, except on pink petals, they are not associated with red spots; there is no intensification of the pigment in and around the trichome, so they are difficult to see with the naked eye. Hence, it is the existence of the associated red spot, rather than the mere existence of a trichome, that is unique to 'Verona'. Hall et al. (4), using an improved scanning electron microscopy technique, failed to detect trichomes on petals of *P. zonale* (unnamed), but they did observe trichomes on adaxial surfaces of leaves, which appeared similar to the petal trichomes that we have observed. Trichomes were also observed by scanning electron microscopy on leaf surfaces of diploid and autotriploid clones of *P. odoratissimum* (10). Similarly, Peterson and Vermeer (11) have observed the leaf trichomes of *P. graveolens* by scanning electron microscopy and have investigated their histochemistry.

When 'Verona' was self-pollinated, its progeny segregated into plants having pink flowers with red spots and pink flowers without red spots; the segregation gave an acceptable fit to a 3:1 ratio (Table 1). Self-pollinating a hybrid between 'Verona' and 'Dolly Varden', which had red spots, and crossing this spotted selection to 'Verona', gave similar results (Table 1). The acceptable fit to a 3:1 ratio indicated that the presence of red spots was dominant over their absence. Dominance was confirmed by the 1:1 ratio that was observed following backcrosses between 'Verona' and several spotless cultivars, including a spotless selection from the previously mentioned cross VER x DV. We attach no significance to the rather poor fit of one cross, VER x MS1H, which we attribute to chance (Table 2). We have therefore symbolized the gene as *Rst*/*rst* to represent red-spotted vs. spotless trichomes on the upper epidermis of pink petals. Although we have experience with many cultivars that are true-breeding for spotless, we have not yet attempted to isolate a line true-breeding for spotted.

For the backcrosses between golden 'Verona' with red spots on petals and green-leaf cultivars without red spots, we scored progeny initially for green vs. golden-colored leaves and later, when flowering, for the presence or absence of red-spotted trichomes. Hence, we were able to test the joint segregation for goodness-of-fit with an expected ratio of 1 green spotted : 1 green spotless : 1 golden spotted : 1 golden spotless; this ratio is expected from crosses between a double heterozygote and a double homozygote, assuming independent assortment of two genes. As the data indicate, the fit was acceptable (Table 2). The crosses VER x FS, VER x JCM, and VER x (VER x DV) had about one-half red- and one-half pink-flowered progeny, but only the data for the pink-flowered progeny are shown in the table, as the red-spotted phenotype is not expressed on red petals. Our conclusion was further confirmed by the analysis of progeny that resulted from self-pollinating 'Verona' (Table 3). The expected ratio of 3:1:6:2 is rather unusual due to the peculiar properties of aurea, in which the heterozygote is dis-

Table 1. Segregation of red-spotted and spotless progeny from selfs within 'Verona' (VER), and from a red-spotted hybrid between 'Verona' (VER) and 'Dolly Varden' (DV).

Combination	Progeny			χ^2		
	Spotted	Spotless	Total	3:1[z]	df	*P*
VER self	41	13	54	0.0000	1	1.0
(VER x DV)[y] self	6	3	9	0.0374	1	0.9–0.5
(VER x DV)[y] x VER	10	4	14	0.0000	1	1.0
Total				0.0374	3	
Pooled	57	20	77	0.0390	1	0.9–0.5
Heterogeneity				---	2	1.0

[z]Data tested for goodness-of-fit with the 3:1 monohybrid ratio after applying Yate's correction.
[y]Red-spotted selection.

Received for publication 24 Nov. 1987. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Table 2. Independence of green : golden leaf color from red-spotted : spotless petals in crosses between 'Verona' and several green leaved, spotless cultivars, or hybrids.

Combination	Progeny: Green spotted	Green spotless	Golden spotted	Golden spotless	Total	χ^2 1:1:1:1	df	P
VER x FS[z]	10	9	13	12	44	0.9091	3	0.9–0.5
VER x JCM	20	12	14	16	62	2.2581	3	0.9–0.5
VER x FOS	16	22	17	12	67	3.0299	3	0.5–0.1
VER x HS	5	4	3	5	17	0.6471	3	0.9–0.5
VER x MS1H	6	17	16	7	46	8.7826	3	0.05–0.01
VER x (VER x MS1H)[y]	10	10	10	12	42	0.2857	3	0.99–0.9
VER x (VER x DV)[y]	5	3	5	5	18	0.6667	3	0.9–0.5
Total						16.5792	21	
Pooled	72	77	78	69	296	0.7279	3	0.9–0.5
Heterogeneity						15.8495	18	0.9–0.5
Partitioned								
1 Green : 1 golden						0.0135	1	0.95–0.9
1 Spotted : 1 spotless						0.0541	1	0.95–0.9
1 Parental : 1 recombinant						0.6621	1	0.5–0.1

[z]DV = 'Dolly Varden', FOS = 'Foster's Seedling', FS = 'Flower of Spring', HS = 'Hills of Snow', JCM = 'J.C. Mapping', MS1H = A male-sterile plant, VER = 'Verona'.
[y]Spotless selection.

Table 3. Independence of green : golden leaf color from red-spotted : spotless petals after selfing 'Verona' (VER).

Combination	Progeny: Green spotted	Green spotless	Golden spotted	Golden spotless	Total	χ^2 3:1:6:2	df	P
VER self	12	4	29	9	54	0.3704	3	0.95–0.9
Partitioned								
1 Green : 2 golden						0.3333	1	0.9–0.5
3 Spotted : 1 spotless						0.0247	1	0.9–0.5
1 Parental : 1 recombinant						0.0123	1	0.95–0.9

tinguishable from the green homozygote, while the white homozygote dies and so cannot be scored for the spotted petal character. Hence, the expected 3:1 spotted : spotless segregation is superimposed on the 1 : 2 green : golden segregation. The acceptable fit confirms the agreement between the observed and expected segregation. Together, the two analyses show that the genes *Aur*$^+$/*Aur* and *Rst*/*rst* are segregating independently of each other.

Literature Cited

1. Baur, E. 1907. Untersuchungen über die Erblichkeitsverhältnisse einer nur in Bastardform lebensfähige Sippe von *Antirrhinum majus*. Ber. Deutsch. Bot. Ges. 25:442–454.
2. Craig, R. 1971. Cytology, genetics and breeding, p. 315–346. In: J.W. Mastalerz (ed.). Geraniums. A manual on the culture, diseases, insects, economics, taxonomy, and breeding of geraniums. Pennsylvania Flower Growers, Pennsylvania State Univ., University Park.
3. Hagemann, R. 1964. Genetike Grundlagen, Ergebnisse und Probleme in Einzeldarstellungen. 4. Plasmatische Vererbung. Gustav Fischer Verlag, Jena.
4. Hall, S.J., E.J. Skerrett, and W.D.E. Thomas. 1978. Critical point drying scanning electron microscopy: A semi-automatic method of preparing biological specimens. J. Microsc. 113:277–290.
5. Harney, P.M. 1976. The origin, cytogenetics and reproductive morphology of the zonal geranium: A review. HortScience. 11:189–194.
6. Herrmann, F. and R. Hagemann. 1971. Struktur und Funktion der genetischen Information in den Plastiden: III. Genetik, Chlorophylle und Photosyntheseverhalten der Plastommutante 'Mrs Pollock' und der Genmutant 'Cloth of Gold' von *Pelargonium zonale*. Biochem. Physiol. Pflanz. 162:390–409.
7. Imai, Y. 1936. Geno- and plasmotypes of variegated Pelargoniums. J. Genet. 33:169–195.
8. Noack, K.L. 1924. Vererbungsversuche mit buntblättrigen Pelargonien. Verh. Phys.-Med. Ges. Würzb. 49:45–93.
9. Noack, K.L. 1925. Weitere Untersuchungen über das Wesen der Buntblättrigkeit bei *Pelargonium*. Verh. Phys.-Med. Ges. Würzb. 50:47–97.
10. Payet, J. and Y. Cauderon. 1982. Etude d'un clone autotriploide de *Pelargonium odoratissimum* (L.) L'Héritier ex Aiton. Bul. Soc. Bot. Fr. Lettres Bot. 129:207–216.
11. Peterson, R.L. and J. Vermeer. 1984. Histochemistry of trichomes, p. 71–94. In: E. Rodriguez, P.L. Healey, and I. Mehta (eds.). Biology and chemistry of plant trichomes. Plenum, New York.
12. Roth, L. 1927. Untersuchungen über die periklinal bunten Rassen von *Pelargonium zonale*. Z. Vererbungsl. 45:125–159.
13. Tilney-Bassett, R.A.E. 1962. Cytoplasmic inheritance and virus diseases. D. Phil. Diss., University of Oxford, Oxford, U.K.
14. Tilney-Bassett, R.A.E. 1984. The genetic evidence for nuclear control of chloroplast biogenesis in higher plants, p. 13–50. In: R.J. Ellis (ed.). Chloroplast biogenesis. (Soc. Expt. Biol. Sem. Ser. 21). Cambridge Univ. Press, Cambridge, U.K.