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A New Colour Mutant in Salvia splendens KER-GAWL.

Nová barevná mutace u Salvia splendens KER-GAWL.

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Abstract — The present work explains the genetic determination of a new spontaneous mutation of the species *Salvia splendens* KER-GAWL., which phenotype differs from the known forms of the above mentioned species by the pigmentation of a part of its inflorescence. Further, the influence of environmental conditions on expressivity of the corresponding gene is discussed. Particularly the light effect on the synthesis of anthocyanins causing the colouring of the inflorescence is studied.

Salvia splendens KER-GAWL. is the ornamental plant, popular especially for the conspicuous pigmentation of its inflorescence. From the original form native in the tropical Brazilian forest with striking brilliant red inflorescence new forms have been developed by mutations. These are characteristic especially in different flower pigmentation.

Based on a detailed study and genetical analysis carried out at the Department of Genetics, Caroline University in Prague, four genes have been detected in the above species controlling the type of inflorescence pigmentation in differently coloured forms (HENDRYCHOVÁ-TOMKOVÁ 1964). It concerns the following genes: the gene for the formation of anthocyanin causing either a pigmented or unpigmented phenotype (*Pp*), the gene determining the chemical quality of the nonsugar component of the anthocyanin manifested either by a violet or red colour (*Vv*), the gene for the regular or limited distribution of pigmentation (*Ll*), and the gene for full or weak intensity of the pigmentation (*Int int*).

However, in one of the segregating offspring of the f. *violacea* × f. *rubra* hybrid a new mutation was found manifested by a phenotype with unpigmented calyces and bracts. Its occurrence proved a possibility of existence of other genes determining the presence or absence of synthesis of anthocyanins in individual organs.

The appearance of white calyces in the offspring of the above hybrid was not dependent on the monofactorial segregation of the violet and red pigmentation of the corollae. According to the pigmentation of the flower, there were distinguished plants of four types: with violet flowers, normally pigmented; red flowers, normally pigmented; plants with white calyces and violet corollae and plants with white calyces and red corollae. The white calyx persevered in the offspring, therefore, it was considered to be a segregated mutation. The recessive character of this spontaneous mutation was assumed because of the segregation ratio for the pigmented and unpigmented calyces already detected in the aberrantly bifactorial segregating F_2 progeny. This opinion was verified in the F_3 progenies of a number of F_2 plants selected as dominants from the aberrant F_2 progeny. The given analysis of the segregation ratios was carried out only for the offspring of the heterozygous plants in both studied characters i.e. in the pigmentation of the calyx (pigmented or unpigmented), and in the colour of flowers, resp. of the corolla (violet or red). See Table 1 (namely for 1179 plants of the total number of 2113).

For the pair of characters under investigation relations were found which are shown in Table 2. The results of analysis including the χ^2 and P-values are quoted there. In all cases, the values of probability (P) were considerably higher than the level of the significance $P = 0.05$, which is in agreement with theoretical assumption of recessive monofactorial nature of new mutation.

Table 1. F_3 progenies segregating in colour and pigmentation of calyx

The number of plant	Pigmentation				Total
	violet	KM violet	red	KM red	
3	46	21	25	7	99
4	72	22	27	13	134
5	77	24	27	7	135
6	61	29	29	6	125
7	69	19	17	9	114
8	47	19	15	8	89
11	63	22	26	8	119
12	83	17	26	9	135
15	71	26	17	3	117
17	59	22	22	9	112
Total	648	221	231	79	1179

Table 2. Segregation ratios in colour and pigmentation of calyx (deduction from the Table 1)

Investigated features	Segregation ratio	χ^2	N	P
colour of the flower and calyx pigmentation	dihybrid 9 : 3 : 3 : 1 ($v : KMv : ru : KMru$)	1.18	3	0.75
calyx pigmentation	monohybrid 3 : 1 ($(v + ru) : (KMv + KMru)$)	1.05	1	0.30
colour of the flower	monohybrid 3 : 1 ($(v + KMv) : (ru + KMru)$)	0.13	1	0.70
colour of the flower and calyx pigmentation	random assortment	0.001	1	0.95

For the explanation of the mutant phenotype of plants *Salvia splendens* with unpigmented calyces the existence of a gene controlling separately the pigmentation of the calyx and bracts was a priori presumed. In order to verify the presence of that gene (*Kk*) and to ascertain its genetic relation to other genes controlling the type of the flower pigmentation, the crossing of other colour mutants, i.e. alba (*A*), rosea (*RO*), carnea (*CA*), rubra (*RU*) and red-calyx (*KM* 3) forms with the violet calyx mutant (*KM* 7) was performed. During the flowering time of the F_2 generation segregation analysis of the pigmentation of the inflorescences was carried on (see Table 3).

The symbols applied in Table 3 have been introduced above in part. An auxiliary designation of *KM-V* was introduced (for the violet calyx mutant, i.e. *bicolor violacea* — a name by Hendrychová-Tomková 1965), *KM-RU* (for the *f. bicolor rubra*), *CA-V* for the *f. carneoviolacea* (medium

Table 3. Segregation ratios in the F_2 generation of crossing of pigmented forms with *KM-V*

Crossing combination	Phenotype of F_1 generation	Segregation ratio found in F_2 generation	The corresponding theoretical segregation ratio	Genotype of F_1 generation
A × *KM* 7/1	violet (*v*)	47 : 26 : 16 : 12 : 3 *v : a : KMv : ru : KMru*	27 : 16 : 9 : 9 : 3	*Pp Vv* – – – – *Kk*
	red (*ru*)	41 : 14 : 21 *ru : KMru : a*	9 : 3 : 4	*Pp vv* – – – – *Kk*
RO × *KM* 7/1	violet (*v*)	91 : 36 : 24 : 21 : 9 : 8 : 10 : 2 *v : ru : ro-v : KMv : ro : KMru : KMro-v : KMro*	27:9:9:9:3:3:3:1	– *Vv Intint* – *Kk*
CA × *KM* 7/1	violet (*v*)	29 : 10 : 9 : 6 : 1 : 2 : 3 : 1 *v : ru : ca-v : KMv : ca : KMru : KMca-v : KMca*	27:9:9:9:3:3:3:1 –	– *Vv* – – *Ll Kk*
	red (*ru*)	53 : 19 : 15 : 6 *ru : ca : KMru : KMca*	9 : 3 : 3 : 1	– *vv* – – – *Ll Kk*
RU × *KM* 7/1	violet (*v*)	71 : 20 : 30 : 9 *v : ru : KMv : KMru*	9 : 3 : 3 : 1	– *Vv* – – – – *Kk*
KM 3/1 × *KM* 7/1	KM violet (*KMv*)	94 : 27 *KMv : KMru*	3 : 1	– *Vv* – – – – *kk*
	KM red (*KMru*)	–	–	– *vv* – – – – *kk*

violet, *PPVVIntIntll*), and *RO-V* for the *f. roseoviolacea* (light violet, *PPVVintintLL*, according to HENDRYCHOVÁ-TOMKOVÁ 1964). The corresponding phenotypes are introduced by means of corresponding small letters. Gene pairs the situation of which agrees with that of *f. violacea* (in all features homozygous dominant), are not quoted in the Table.

By the above genetic analysis assumed existence of the gene *Kk* was verified. Considering the gene *Kk*, the segregation ratios in F_2 may be fully explained. The recessive gene *k* in the presence of a normal genotype for the synthesis of anthocyanins (*PV*, *Pv*) causes an active inhibition of this synthesis in calyces and bracts. Thus was simultaneously excluded the alternative hypothesis for the explanation of the phenotype „unpigmented calyx" by the possibility of an incomplete mutation within the gene *P*, i.e. a partial fault in the function of the main gene for the synthesis of the anthocyanins. The inhibition of the function and not its deficiency is proved particularly by the phenotype of F_1 derived from the f. *alba* × f. *bicolor violacea* (*KM* violet) hybrid. The F_1 flowers were totally pigmented (corolla and calyx). Therefore, the gene *P* is present in the dominant state in the genotype of the violet calyx mutant. The *P* gene is introduced to the hybrid genotype by the calyx mutant only, not by the white form.

On the basis of the segregation ratio in the F_2 generation (crossing of differently pigmented forms with the violet calyx mutant) the random assortment of the gene pair *Kk* and *Vv*, and a recessive epistasis *kk* over *PV* and *Pv* was ascertained. By crossing f. *rosea* and f. *carnea* with the f. *bicolor violacea* the recessive epistasis *kk* over all other genes controlling the anthocyanin synthesis, that means the genes *Int int* and *Ll* was established, too.

The genotype with *kk* is manifested with variable expressivity in relation to external conditions. The relations between *kk* and *PV* (*Pv*) may, therefore, be designated as an incomplete, eventually, variable recessive epistasis. The mutated calyx is, namely, pure white only under certain conditions, i.e. under light of a weak intensity, during winter. With the increasing light intensity and with the prolonged illumination time the calyces take on various degrees of colour of the same hue as the corolla, however, always much weaker. Therefore, studied was the question of the effect of light and of the corresponding gene (*Kk*) on the synthesis of the flower pigments — the anthocyanins — in the calyces of the mutated plants. The effect of light on the synthesis of the anthocyanins by means of the production of assimilates (sugars, as the building material for the formation of anthocyanins) was studied. The question of this non-specific or specific (i.e. direct support of the formation of anthocyanins) effect was investigated.

The orientation test was carried out in four variants (with five plants in each variant):

1. with shading the total plant
2. with shading the inflorescence only
3. with shading the leaf area
4. without shading (control).

From the test is derived that the pigmentation of the calyx is dependent on the direct illumination of the inflorescence itself, not on the illumination of the assimilation area.

In order to clear the investigated problem the amount of anthocyanins was colorimetrically determined in the calyces of the involved inflorescences as well as the content of free sugars (by the Lisicyn method of determination of reducing sugars — LISICYN 1950). The separation of glucose and fructose by the Kolthoff method was done (KOLTHOFF 1932). The material for both tests were the calyces of inflorescences of plants shaded by the same method as described above in the orientation test.

Table 4. Mean values of the sugar content in calyces of the inflorescences of the tested plants

Sample	Method of shading	Percentage content of sugars			
		Glucose	Fructose	Saccharose	Total
Calyces KM violet	control	0,007	0,056	0,107	0,170
	whole plants	0,025	0,018	0,022	0,065
	leaves	0,050	0,089	0,183	0,322
	inflorescence	0,053	0,107	0,035	0,195
Leaves KM violet	control	0,036	0,200	0,118	0,354
	whole plants	0,006	0,078	0,066	0,150
	leaves	0,006	0,034	0,002	0,042
Calyces KM red	control	0,038	0,038	0,014	0,091
	whole plants	0,027	0,029	0,019	0,075
	leaves	0,146	0,069	0,093	0,309
	inflorescence	0,052	0,091	0,089	0,233
Leaves KM red	control	0,032	0,324	0,108	0,464
	whole plants	0,010	0,090	0,024	0,124
	leaves	0,003	0,078	0,006	0,087

Table 4a. Differences between sugar content values in calyces of the inflorescences and in leaves of tested plants. Derived from the Table 4. (t-test values)

Sample	Method of shading	control		whole plants		leaves		inflores-cence	
		t	P	t	P	t	P	t	P
Calyces KM violet	control			3,23	0,01	4,01	0,01	0,98	—
	whole plants	3,23	0,01			5,61	0,01	3,28	0,01
	leaves	4,01	0,01	5,61	0,01			3,50	0,01
	inflorescence	0,98	—	3,28	0,01	3,50	0,01		
Leaves KM violet	control			3,40	0,01	4,87	0,01		
	whole plants	3,40	0,01			3,91	0,01		
	leaves	4,87	0,01	3,91	0,01				
Calyces KM red	control			1,89	—	4,25	0,01	3,31	0,01
	whole plants	1,89	—			5,01	0,01	4,40	0,01
	leaves	4,25	0,01	5,01	0,01			2,72	0,05
	inflorescence	3,31	0,01	4,40	0,01	2,72	0,05		
Leaves KM red	control			3,95	0,01	6,11	0,01		
	whole plants	3,95	0,01			2,13	—		
	leaves	6,11	0,01	2,13	—				

Table 5. The comparison of the contents of sugars and anthocyanins in the calyces of tested plants

Material	Degree of shading	Percentage content of sugars				Content of anthocyanins (absorbance)
		Glucose	Fructose	Saccharose	Total	
KM violet (calyces)	Control (without shading)	0,007	0,056	0,107	0,170	32,8
	leaf area	0,050	0,089	0,183	0,322	34,0
	inflorescence	0,053	0,107	0,035	0,195	61,0
KM red (calyces)	control (without shading)	0,038	0,038	0,014	0,091	2,4
	leaf area	0,146	0,069	0,093	0,309	3,1
	inflorescence	0,052	0,091	0,089	0,233	66,0

Table 5a. Differences between anthocyanins content values in calyces of the inflorescences of tested plants. Derived from the Table 5. (t-test values)

Material	Method of shading	control		leaves		inflorescence	
		t	P	t	P	t	P
KM violet (calyces)	control			0,45	—	2,92	0,05
	leaves	0,45	—			2,79	0,05
	inflorescence	2,92	0,05	2,79	0,05		
KM red (calyces)	control			0,23	—	6,21	0,01
	leaves	0,23	—			5,97	0,01
	inflorescence	6,21	0,01	5,97	0,01		

As follows from Table 4 the sugar content is the lowest in the calyces of plants totally shaded and the highest (even higher than in the control, unshaded plants), in the calyces of plants shaded except for the inflorescences. The calyces of the latter variant became light green some time after the shading of the assimilation area, which proves that they partly overtake the assimilating function. Thus also, the increased sugar content in calyces shaded except for the inflorescences may be explained.

When determining the sugars in the leaves of differently shaded plants, the highest content was found in unshaded control plants, while the lowest content was ascertained in leaves of plants totally shaded, or those with shaded assimilation area except for the inflorescence. In the latest variant, the sugar content was even lower than in the plants totally shaded (see Table 4a).

By means of colorimeter, it was found that the anthocyanin content is lower in the calyces of plants with the inflorescences shaded than in the calyces of plants with inflorescences exposed, or in control plants (Table 5 and 5a).

It follows from comparing the sugar and anthocyanin contents in the parallel test variants that the synthesis of anthocyanins does not depend quantitatively on the sugar synthesis directly. The light affects specifically the anthocyanin formation also in *KM*, namely by the contrary effect to the inhibiting gene *kk*. According to this observation it may be concluded that the investigated gene, inhibiting, in its recessive state the biosynthesis of anthocyanins, interfers specifically in the stage of the actual formation of the pigment molecule.

In spite of the present results it cannot be more accurately determined at which phase, and in which way does this gene act. Unsolved remains also the question of the relation of light to the action of the gene-inhibitor, namely, whether the light affects directly the formation of anthocyanins and blocks thus the function of that gene (whose counter-action is not sufficient to prevent the formation of anthocyanins, strongly supported by light activity), or whether it affects directly the function of that particular inhibiting gene (*kk*).

Summary

It has been ascertained that the mutant "white calyx" of the species *Salvia splendens* Ker-Gawl., is caused by a recessive mutation of a special gene (*K*) controlling separately the pigmentation of the calyx and bracts. In the relation to genes *Pp* and *Vv* (determining the synthesis of anthocyanins and their quality) the gene *kk* is freely recombinating. The gene *kk* is in recessive epistasis with *PV* and *Pv* and all other genes involved in synthesis of anthocyanins.

As far as the phenotype effect is concerned, the recessive gene *kk* possesses a variable expressivity related to external conditions, foremost to light.

The physiological function of the recessive alleles *kk* is the inhibition of the biosynthesis of anthocyanins. However, the inhibiting effect is easily disturbed by the action of light of higher intensities, so that the remaining genetic apparatus for the synthesis of anthocyanins can, to a considerable extent, become functionally valid, and to form phenotypes of different shades of calyx pigmentation.

Souhrn

Práce vysvětluje genetickou determinaci nové spontánní mutace druhu *Salvia splendens* Ker-Gawl., fenotypově odlišné zbarvením částí květenství od známých forem uvedeného druhu. Dále se zabývá studiem podmíněnosti fenotypové expresivity příslušných mutovaných vloh vnějšími podmínkami, zejména působením světla na syntézu antokyanů, podmiňujících zbarvení květenství.

Bylo zjištěno, že mutace „bílý kalich" je recesivní mutací samostatného genu, ovládajícího separátně zbarvení kalichů a listenů. Ve vztahu ke genům určujícím samotnou syntézu antokyanů (*Pp*) a jejich kvalitu (*Vv*) jeví genový pár *kk* volnou kombinovatelnost a recesivní epistasi nad *PV* a *Pv* a nad všemi ostatními geny, uplatňujícími se při syntéze antokyanů (tj. *Ll* a *Int int*).

Co do fenotypového účinku jeví recesivní vloha *kk* variabilní expresivitu v závislosti na existenčních podmínkách, a to především na světle.

Fyziologickou funkcí recesivních alel *kk* je inhibice biosyntézy antokyanů. Inhibiční účinek je však působením světla vyšších intenzit snadno rušen, takže ostatní genový aparát pro syntézu antokyanů se může do značné míry funkčně uplatnit a vytvářet fenotypově různé odstíny ve zbarvení kalichu.

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