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Genetics of the peroxidase isoenzymes in *Petunia*

8. Flower and root peroxidases

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Summary. In root and flower corolla tissue of *Petunia* several anodic moving peroxidase isoenzymes are present, which cannot be detected in other organs. Alleles of the gene *prxF* control the presence or absence of several peroxidases that are only present in flower corolla tissue. Alleles of the gene *prxG* code for two peroxidases that can only be detected in root tissue. In addition to mutations of *prxG* that cause a change in the electrophoretic mobility of the *PRXg* enzymes, a mutation was also found that causes the absence of expression in enzyme activity. Crossing experiments indicated that this mutation is located in the gene *prxG*. Peroxidases encoded by the gene *prxH* were only found in root tissue. Two alleles of *prxH* were identified by electrophoretic variation; one allele is responsible for a single band, whereas the other allele could be recognized by a double-banded phenotype. The double-banded *PRXh* phenotype is suggested to be caused by tandem duplication, followed by mutation in one of the genes. A third *prxH* allele could be identified by the absence of *PRXh* activity. The genes *prxF*, *prxG*, and *prxH* were shown to be located on chromosome VII, with the following gene order: *prxG-An4-lapB-gpiB-prxH-prxF*.

Key words: *Petunia* – Peroxidase isoenzymes – Flower corolla – Root – Gene localization

Introduction

In previous publications we have reported on the genetics of the major peroxidases that are present in leaves of *Petunia*. The three encoding genes, *prxA*, *prxB*, and *prxC* have been located on chromosomes III, I, and IV, respectively (van den Berg and Wijsman 1982a, b; van den Berg et al. 1982).

Here, we report on the genetics of peroxidase isoenzymes that can only be detected in flower corolla or root tissue. In flower corolla tissue one major and several minor peroxidases can be detected in addition to the enzymes encoded by the genes *prxA*, *prxB*, and *prxC*. The major flower corolla peroxidase, not detectable in other parts of *Petunia*, was present in only one of the *Petunia hybrida* cultivars investigated (van den Berg and Wijsman 1981).

In the present report we show that the presence of the flower corolla peroxidases is controlled by one gene, termed *prxF*. In root tissue the genes *prxB* and *prxC* are expressed, in contrast to *prxA*, whose expression can barely be detected. In addition, several other anodic moving peroxidases can be found in root tissue which are encoded by the genes *prxG* and *prxH*. The genetic experiments described here show that all three genes involved in the production of the flower and root specific peroxidase are located on chromosome VII.

Materials and methods

Plant material

The inbred lines used are presented in Table 1. Lines S12, S13, and S14 were derived from plants D660-1, D579-1, and D582-1, that were grown from seeds collected in South-America. The origin of these plants and of the other *P. axillaris* s.l. and *P. integrifolia* s.l. inbred lines has been given in previous papers (van den Berg and Wijsman 1982a, b; van den Berg et al. 1983).

Electrophoretic analysis

Sample preparation, electrophoretic separation of the peroxidase isoenzymes using gel system I, and staining for per-

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oxidase activity were carried out as described previously (van den Berg and Wijsman 1981, 1982b). Segregation for the genes *lapB* (leucyl-aminopeptidase) and *gpiB* (glucose phosphate isomerase) were determined as described by Wijsman and van den Berg (1982).

Nomenclature of the peroxidase genes and enzymes

The notation for the peroxidase structural genes and enzymes as given earlier is followed (van den Berg and Wijsman 1982a, b). This notation is extended to include genes the mutation of which results in the absence of peroxidase activity (e.g. *prxF*) unless it is shown that a regulatory gene is involved.

Results

The gene prxF

In flower corolla tissue several anodic moving peroxidase isoenzymes (one major and sometimes one or several minor bands) may be present that cannot be detected in other parts of *Petunia*. Among our collection of *Petunia hybrida* inbred lines, representing most classical cultivars, the major flower corolla peroxidase was only found in lines derived from the cultivar 'Pendula Cyanea' (van den Berg and Wijsman 1981; Table 1).

Crossing experiments indicated that presence versus absence of the flower corolla peroxidase shows monogenic segregation (Fig. 1). The allele *prxF1* that controls presence of the flower corolla peroxidases behaves dominant over the allele *prxF2* which is responsible for absence of the bands. The minor bands do not always accompany the major band, but absence of the major band goes hand in hand with absence of the minor ones (Fig. 1).

Among plants of the putative progenitor species of *P. hybrida*, *P. axillaris* s.l. and *P. integrifolia* s.l., both alleles of the gene *prxF* were found (Table 1).

The gene *prxF* was shown to be located on chromosome VII (see below).

The gene prxG

The gene *prxG* codes for two anodic moving peroxidases that were only detected in root tissue. Among *P. hybrida* inbred lines and related species, four different alleles of the gene *prxG* were found (Fig. 2, Table 1). Three alleles could be recognized by variation in electrophoretic mobility of the *PRXg* enzymes, whereas a fourth allele could be identified by an absence of *PRX*g activity.

In the progeny of the cross $\{(\text{Vu6} \times \text{V23}) \times \text{Vu6}\} \times$ V35, linkage of *prxG* to the genes *An4* (involved in anthocyanin synthesis) and *lapB* (encoding a leucyl-aminopeptidase) was the case (Fig. 3, Table 2). The

Table 1. Distribution of alleles of the genes *prxF, prxG, prxH,* and *An4* among inbred lines of *Petunia hybrida,* derived from cultivars as well as from progeny of different cultivars (recombinant), and the related species *P. axillaris* (ssp. *axillaris* and *parodii*) and *P. integrifolia* (spp. *integrifolia* and *inflata*)

Line	Cultivar/species	Alleles			
R27	'Roter Vogel'	*prxF2*	*prxH1*	*prxG4*	*an4*
R51	'Royal Ruby'	*prxF2*	*prxH2*	*prxG3*	*an4*
M1	'Rose of Heaven'	*prxF2*	*prxH1*	*prxG4*	*An4*
M72	'Straub'	*prxF2*	*prxH2*	*prxG1*	*An4*
H11	'Silvery Blue'	*prxF1*	*prxH3*	*prxG4*	*An4*
V2	'Admiral'	*prxF2*	*prxH1*	*prxG4*	*An4*
V7	'Gottfried Michaelis'	*prxF2*	*prxH2*	*prxG4*	*An4*
V21	'Dark Purple'	*prxF2*	*prxH1*	*prxG4*	*an4*
V23	'Blauzwerg'	*prxF2*	*prxH2*	*prxG1*	*An4*
V28	'Purple Waters'	*prxF2*	*prxH2*	*prxG1*	*An4*
V30	'Dwarf Velvet Ball'	*prxF2*	*prxH2*	*prxG1*	*An4*
W4	'Pendula Cyanea'	*prxF1*	*prxH3*	*prxG1*	*an4*
W15	recombinant	*prxF2*	*prxH1*	*prxG4*	*an4*
W115	recombinant	*prxF1*	*prxH3*	*prxG4*	*an4*
Vu6	recombinant	*prxF2*	*prxH2*	*prxG2*	*an4*
A4	recombinant	*prxF2*	*prxH2*	*prxG4*	*An4*
V35	recombinant	*prxF2*	*prxH2*	*prxG4*	*an4*
S1	ssp. *axillaris*	*prxF2*	*prxH3*	*prxG4*	*an4*
S2	spp. *axillaris*	*prxF1*	*prxH1*	*prxG4*	*an4*
S6	ssp. *inflata*	*prxF2*	*prxH2*	*prxG1*	*An4*
S7	ssp. *parodii*	*prxF2*	*prxH2*	*prxG4*	*an4*
S8	ssp. *axillaris*	*prxF1*	*prxH3*	*prxG4*	*an4*
S9	ssp. *inflata*	*prxF1*	*prxH3*	*prxG4*	*An4*
S12	ssp. *integrifolia*	*prxF2*	*prxH2*	*prxG1*	*An4*

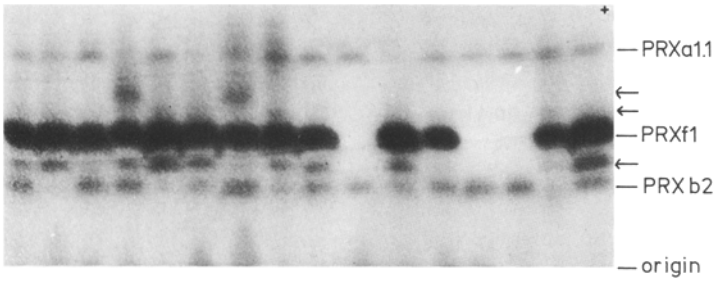

Fig. 1. Starch gel showing segregation of the gene *prxF* among the progeny of the backcross (W4 × W28) × W28. Flower corolla tubes of mature plants were analysed. Because of the variation in number and mobility of the minor bands, only the major PRXf enzyme was numbered. The minor bands are indicated with *arrows*. Segregation found: *prxF1/F2 : prxF2/F2* = 68 : 82 ($\chi^2_{1:1} = 1.31$; $P = 0.26$)

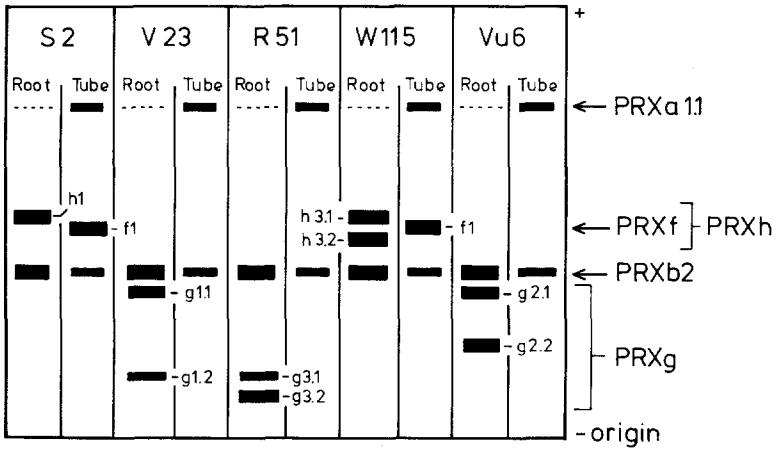

Fig. 2. Composite drawing showing the anodic peroxidases of root and flower corolla tube tissue. Genotypes: S2: *prxA1/A2, prxB2/B2, prxF1/F1, prxG4/G4, prxH1/H1.*
V23: *prxA2/A2, prxB2/B2, prxF2/F2, prxG1/G1, prxH2/H2*
R51: *prxA1/A1, prxB2/B2, prxF2/F2, prxG3/G3, prxH2/H2*
W115: *prxA1/A1, prxB2/B2, prxF1/F1, prxG4/G4, prxH3/H3*
Vu6: *prxA1/A1, prxB2/B2, prxF2/F2, prxG2/G2, prxH2/H2*

genes *An4* and *lapB* have been located on chromosome VII by Maizonnier and Moessner (1979) and Wijsman and van den Berg (1982), respectively. From these results we conclude that *prxG* is located on chromosome VII, and that the order of the genes is *prxG-An4-lapB*.

In several lines of *P. hybrida* and its putative progenitor species, a mutation was found that causes absence of expression in *PRXg* activity (Table 1). The results of the cross $\{(Vu6 \times V23) \times Vu6\} \times V35$ indicate that mutation of the gene *prxG* is the case, because only the *PRXg* phenotypes of Vu6 and V23 were observed among the progeny (see discussion).

The gene prxH

The gene *prxH* is involved in anodic moving peroxidases that were only detected in root tissue. In some of our inbred lines one *PRXh* band is present (responsible allele *prxH1*), whereas other lines showed a double-banded phenotype (responsible allele *prxH3*), or virtual absence of *PRXh* activity (allele *prxH2*) (Fig. 2, Table 1).

The assumption that only one gene is involved in these three different phenotypes stems from the results of the crossing experiments presented in Tables 3–5. The following observations were considered.

1. The double-banded *PRXh* phenotype inherits as an unit (only double-banded phenotypes were found among the progeny of the cross with genotype *prxH3/H2* × *prxH2/H2* (Fig. 4, Table 3).

2. Presence versus absence of the single-banded as well as the double-banded phenotype was monofactorial (Tables 3 and 4).

3. The allele *prxH3* shows a similar linkage to *prxF1* as the allele *prxH1* (Tables 3–5).

4. Only double-banded phenotypes were observed among the progeny of (W115 × R4) × R4, whereas single-banded phenotypes were observed among the progeny of (W115 × R4) × W15.

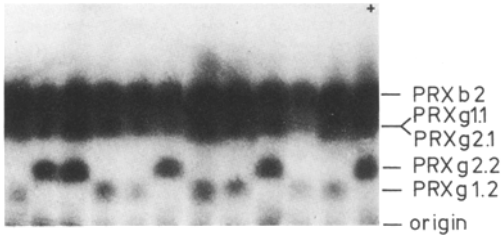

Fig. 3. System I starch gel showing segregation of *prxG* among the progeny of the cross $\{(Vu6 \times V23) \times Vu6\} \times V35$ (genotype *prxG1/G2* × *prxG4/G4*). All plants have genotype *prxH2/H2*. Root tissue of mature flowering plants was analysed

Table 2. Segregation for the genes *prxG, An4* and *lapB* showing linkage of the three genes

Cross:	{(Vu6 × V23) × Vu6} × V35			
Genotype:	*prxG1, An4, lapB1* / *prxG2, an4, lapB2* × *prxG4, an4, lapB1* / *prxG4, an4, lapB1*			
Genotypes and nos. found				
	An4an4 lapB1/B1	*An4an4 lapB1/B2*	*an4an4 lapB1/B2*	*an4an4 lapB1/B2*
prxG1/G4	37	0	0	2
prxG2/G4	0	0	2	24
Segregation chi square tests				
prxG	39 : 26	$\chi^2_{1:1}=2.60$	$P=0.11$	
An4	37 : 28	$\chi^2_{1:1}=1.25$	$P=0.26$	
lapB	39 : 26	$\chi^2_{1:1}=2.60$	$P=0.11$	
Linkage chi square tests				
prxG – An4	$\chi^2_{1:1:1:1}=41.7$	$P<10^{-3}$		
prxG – lapB	$\chi^2_{1:1:1:1}=36.3$	$P<10^{-3}$		
An4 – lapB	$\chi^2_{1:1:1:1}=41.7$	$P<10^{-3}$		
Genetic distances				
prxG – An4	3.1 ± 2.2 cM			
prxG – lapB	6.2 ± 3.0 cM			
An4 – lapB	3.1 ± 2.2 cM			

The latter point favours the possibility of gene duplication that resulted in the double-banded *PRXh* phenotype instead of modification of one of the *PRXh* enzymes.

Crossing experiments show that *prxH* is located on chromosome VII, linked to the genes *gpiB* and *prxF* in the order *gpiB-prxH-prxF* (Tables 3–5).

The order of the genes on chromosome VII

The order of the genes located on chromosome VII as indicated by the crosses presently described is shown in Fig. 5. The order of the genes shown is based on three-point tests involving *prxG-An4-lapB* (Table 2), *An4-lapB-gpiB* (Wijsman and van den Berg 1982), and *gpiB-prxF-prxH* (Table 4). Maizonnier (1976) has located *An4* in the proximity of a chromosomal terminus. Therefore, it could be assumed that *prxF* and *prxH* would be distal to *gpiB* with respect to *An4*. In fact, in a F2 progeny that showed segregation for *prxF* and *An4*, no evidence for linkage was found (unpublished results).

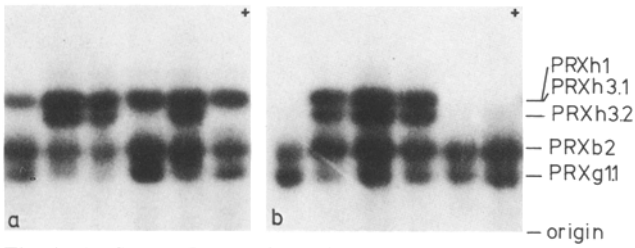

Fig. 4a, b. System I starch gels showing segregation of *prxH* among the progeny of the cross (W115 × R4) × R4 **(a)** (genotype *prxH3/H3 × prxH2/H2*) and of the cross (W115 × R4) × W15 **(b)** (genotype *prxH3/H2 × prxH1/H1*). Root tissue of mature flowering plants was analysed

Table 3. Inheritance in a monogenic fashion of presence versus absence of the double-banded PRXh phenotype and linkage of *prxF* to *prxH*

Cross:	(W115 × R4) × R4			
Genotype:	*prxF1, prxH3* / *prxF2, prxH2* × *prxF2, prxH2* / *prxF2, prxH2*			
Genotypes and nos. found				
	prxF1/F2	*prxF2/F2*		
prxH3/H2	51	2		
prxH2/H2	3	36		
Segregation chi square tests				
prxF	54 : 38	$\chi^2_{1:1}=2{,}78$	$P=0.10$	
PrxH	53 : 39	$\chi^2_{1:1}=2{,}13$	$P=0.14$	
Linkage chi square test			Genetic distance	
$\chi^2_{1:1:1:1}=80.0$	$P<10^{-3}$		5.5 ± 2.4 cM	

Table 4. Segregation for the genes *prxF, prxH,* and *gpiB* showing linkage

Cross	(D3001 B-4 × S2) × A4		
Genotype:	*gpiB1, prxF1, prxH1* / *gpiB2, prxF2, prxH2* × *gpiB2, prxF2, prxH2* / *gpiB2, prxF2, prxH2*		
Genotypes and nos. found			
		gpiB1/B2	*gpiB2/B2*
prxF1/F2, prxH1/H2		24	6
prxF1/F2, prxH2/H2		1	2
prxF2/F2, prxH1/H2		2	0
prxF2/F2, prxH2/H2		5	34
Segregation chi square tests			
gpiB	32 : 42	$\chi^2_{1:1}=1.35$	$P=0.25$
prxF	33 : 41	$\chi^2_{1:1}=0.86$	$P=0.35$
prxH	32 : 42	$\chi^2_{1:1}=1.35$	$P=0.25$
Linkage chi square tests			
gpiB – prxF:	25 : 7 : 8 : 34	$\chi^2_{1:1:1:1}=28.4$	$P<10^{-3}$
gpiB – prxH:	21 : 6 : 6 : 36	$\chi^2_{1:1:1:1}=36.5$	$P<10^{-3}$
prxF – prxH:	30 : 3 : 2 : 39	$\chi^2_{1:1:1:1}=57.6$	$P<10^{-3}$
Genetic distances			
gpiB – prxF	20.3 ± 4.7 cM		
gpiB – prxH	16.2 ± 4.3 cM		
prxF – prxH	6.8 ± 2.9 cM		

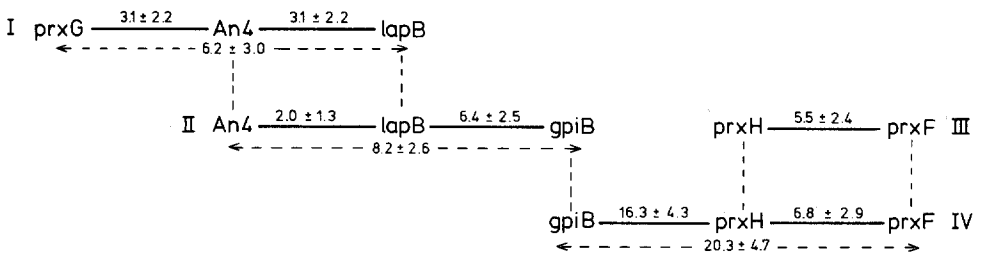

Fig. 5. Genetic map of the genes on chromosome VII. The data are derived from Tables 2 (I), 3 (III), 4 (IV), and from Wijsman and van den Berg (1982) (II)

Table 5. Segregation for the genes *prxF* and *prxH* showing linkage

Cross:	(W115 × R4) × W15		
Genotype	$\frac{prxF1, prxH3}{prxF2, prxH2} \times \frac{prxF2, prxH1}{prxF2, prxH1}$		
Genotypes and nos. found			
	prxF1/F2	*prxF2/F2*	
prxH3/H1	53	11	
prxH2/H1	10	112	
Segregation chi square tests			
prxH	64 : 122	$\chi^2_{1:1} = 18.1$	$P < 10^{-3}$
prxF	63 : 123	$\chi^2_{1:1} = 19.4$	$P < 10^{-3}$
Linkage chi square test			
prxH – prxF		$\chi^2_{2\times2} = 104.4$	$P < 10^{-3}$
Genetic distance			
prxH – prxF:	11.3 ± 2.3 cM		

Discussion

The present paper describes the tissue-specific expression and chromosomal localization of the genes *prxF, prxG,* and *prxH*. The experiments described indicate that the genes *prxG* and *prxH* are structural genes, whereas in the case of *prxF* we do not know whether presence versus absence of the flower peroxidases is caused by mutation of the structural gene or by mutation of a regulatory gene that affects expression of the structural gene. It is remarkable that all three genes, responsible for tissue-specific peroxidases and characterized by mutations causing absence of peroxidase activity, are located on the same chromosome. Structural genes for the other peroxidases are located on other chromosomes (van den Berg and Wijsman 1982 a, b; van den Berg et al. 1982).

The presence of null alleles seems to be a characteristic of the peroxidase system. Garcia et al. (1982) argued that the high number of null alleles for peroxidase, as observed for barley and wheat, could be related to the broad substrate specificity of peroxidases: the absence of specific peroxidases would not be lethal because its function can be taken over by another peroxidase. However, differences in cellular location of the peroxidases (Hendriks et al., in preparation; van den Berg and van Huystee 1984), and the fact that each peroxidase may have a specific function (van den Berg et al. 1984), indicates that this is not likely. With this in mind the role of peroxidase in the degradation of flavonoids (e.g. in the case of *PRXf;* Patzlaff and Barz 1978) and action in defense against micro-organisms and toxic substances (Seevers et al. 1971) (particularly in the case of seed and root peroxidases) can be mentioned.

Preliminary experiments involving a variant of *PRXh* that has a mobility similar to *PRXh3.2,* together with the results presented in Tables 3 and 4 – which exclude modification causing the double-banded *PRXh* phenotype since single band phenotypes were observed among the progeny of (W115 × R4) × W15 – provide the evidence that *prxH* is a structural gene.

In the case of *PRXf* we do not know whether a regulatory or structural gene is involved. In Wijsman (1983), the genes *prxF* and *prxH* were synonymized because: (a) at that time, based on a small number of progeny, no crossover between the two genes had been observed and (b) because the mobility difference between *PRXf* and *PRXh* is very slight, though consistent. The present evidence forces us to withdraw the assumption.

The mutation found in several inbred lines which is responsible for absence of *PRXg* activity may be located outside the gene *prxG,* and then would be trans-acting. On the other hand, the mutation might be located in the gene *prxG.* The results of the analysis of the progeny of {(Vu6 × V23) × Vu6} × V35 favour the latter possibility. If the mutation is located outside gene *prxG* then it is reasonable to assume independent inheritance, or, in the case of linkage, that crossing over could be expected between the assumed trans-acting gene and the gene *prxG.* In that case, the unknown genotype of line V35 (*prxG4/G4*) would be observed among plants of the progeny. Since only the *PRXg* phenotypes of Vu6 (*prxG2/G2*) have been observed, we assume that the mutation that caused absence of *PRXg* activity is located in the structural gene *prxG.*

In previous papers we reported on the modification of peroxidases encoded by the genes *prxA* (van den Berg and Wijsman 1981, 1982 b) and *prxC* (van den

Berg et al. 1982). The possibility of tightly linked duplicated genes was excluded since mutation of the encoding gene resulted in changes in mobility of the multibanded phenotype as a unit. In the case of the genes *prxF* and *prxG*, gene duplication cannot be ruled out, since for *prxF* no mobility variant has been found, whereas for *prxG* the phenotypes cannot be regarded as units shifted by mutation of the gene.

Wijsman and van den Berg (1982) have shown conservation of linkage of the genes *gpiB* and *An4* on chromosome VII of the *P. hybrida* genome. Regarding the distribution of alleles of the genes *prxF*, *prxG*, *prxH*, and *An4* among *P. hybrida* cultivars (Table 1), conservation of linkage is the case only for *prxF* and *prxH*.

In previous papers as well as in the present one we have dealt with the peroxidase genes *prxA*, *prxB*, *prxC*, *prxF*, *prxG*, and *prxH*, of which five are structural genes. In addition to these five structural genes, we have located the gene *prxD* (unpublished results). We suppose that apart from the genes involved in the production of the *PRXe* and *PRXf* enzymes, no other structural peroxidase genes are involved.

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