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THE CONCENTRATION OF S-PROTEIN IN STIGMAS OF *BRASSICA OLERACEA* PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR A GIVEN S-ALLELE

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Summary

From the measurement of end-point dilution of stigma extracts of *B. oleracea* tested against S-protein antisera it was found that stigmas homozygous for a particular S-allele contained more specific S-protein than those heterozygous for the same S-allele. There was generally twice as much S-protein in the homozygote as in the heterozygote.

1. Introduction

NASRALLAH AND WALLACE (1967*a*, *b*) described a method whereby antisera were produced in rabbits to stigma extracts of *B. oleracea* genotypes of known S-allele constitution. Some sera contained an antibody thought to be specific for a particular S-allele and the antigen was called the S-protein. To test the association between S-allele and S-protein, plants that had been used to induce serum production were crossed to other S-genotypes and the F_1 and F_2 progenies raised and tested. It was found that the capability of any offspring to stimulate production of the specific S-protein was correlated absolutely with presence of the specific S-allele (Nasrallah, Barber and Wallace, 1969; Nasrallah, Wallace and Savo, 1972). This paper reports the measurement by serological techniques of the relative concentration of S-protein in plants homozygous and heterozygous for S-alleles.

2. Materials and methods

S-allele antisera were produced in rabbits, as described by Sedgley (1974, in press), to stigma extracts of two kale plants, one homozygous for the

TABLE 1

End-point dilutions of the S_{16}-protein in Stigma extracts of $S_{16}S_{16}$, $S_{16}S_{23}$ and $S_{16}S_2$

Titre of S_{16}-antibody (ab.)	$S_{16}S_{16}$			$S_{16}S_{23}$			$S_{16}S_2$		
	End-point of S_{16}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$	End-point of S_{16}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$	End-point of S_{16}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$
$\frac{1}{16}$	$\frac{1}{64}$	4·94	4	$\frac{1}{32}$	4·56	2	$\frac{1}{32}$	4·56	2
$\frac{1}{16}$	$\frac{1}{48}$	5·44	3	$\frac{1}{24}$	5·37	1·5	$\frac{1}{32}$	4·57	2
$\frac{1}{16}$	$\frac{1}{32}$	5·04	2	$\frac{1}{16}$	4·70	1	$\frac{1}{16}$	4·69	1
$\frac{1}{16}$	$\frac{1}{64}$	4·89	4	$\frac{1}{24}$	4·49	1·5	$\frac{1}{32}$	4·50	2
$\frac{1}{16}$	$\frac{1}{32}$	4·47	2	$\frac{1}{24}$	4·67	1·5	$\frac{1}{16}$	4·83	1

TABLE 2

End-point dilutions of the S_{23}-protein in stigma extracts of $S_{23}S_{23}$, $S_{16}S_{23}$ and $S_{23}S_{45}$

Titre of S_{23}-antibody (ab.)	$S_{23}S_{23}$			$S_{16}S_{23}$			$S_{23}S_{45}$		
	End-point of S_{23}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$	End-point of S_{23}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$	End-point of S_{23}-protein (ag.)	Total protein content (mg/ml)	Ratio $\frac{\text{ab.}}{\text{ag.}}$
$\frac{1}{8}$	$\frac{1}{32}$	5·54	4	$\frac{1}{16}$	5·06	2	$\frac{1}{16}$	4·87	2
$\frac{1}{8}$	$\frac{1}{64}$	5·55	8	$\frac{1}{32}$	5·48	4	$\frac{1}{32}$	5·07	4
$\frac{1}{8}$	$\frac{1}{32}$	4·90	4	$\frac{1}{16}$	5·11	2	$\frac{1}{16}$	5·36	2
$\frac{1}{8}$	$\frac{1}{32}$	5·28	4	$\frac{1}{24}$	4·44	3	$\frac{1}{16}$	4·60	2

S_{23}-allele and the other for the S_{16}-allele. These two kale plants were selfed, hybridised with each other and with plants of other S-genotypes, to give the two homozygotes ($S_{23}S_{23}$ and $S_{16}S_{16}$), the hybrid ($S_{16}S_{23}$) and the kale-brussels sprout hybrids ($S_{23}S_{45}$ and $S_{16}S_2$). The S_{23} and S_{16} alleles are of high dominance whereas S_2 and S_{45} are intermediate (Thompson and Taylor, 1966). The genotypes of all plants were checked by controlled pollinations to plants of known S-allele status followed by an examination of pollen behaviour with the aid of ultraviolet microscopy (van Hal and Verhoeven. 1968).

Stigma extracts were clarified by freezing and the total protein content measured by the method of Lowry *et al.* (1951). Quantitative serological tests were carried out only on stigma extracts of comparable protein content. The S_{16}-serum, after absorption with S_8S_8, $S_{17}S_{17}$ and $S_{25}S_{25}$ kale stigma extracts, had a titre of $\frac{1}{16}$ as measured by Sedgley (1974, in press). However the S_{23}-serum absorbed similarly contained too low a concentration of S_{23}-antibody for adequate resolution of precipitin bands and was concentrated by removing 75 per cent of the water and low molecular weight substances using " Lyphogel ", a polyacrylamide gel. The serum then had a titre of $\frac{1}{8}$ and could be used in gel tests. Clarified extracts from all the plants were tested against the sera to check that the S-proteins were present in the S_1 and F_1 progeny. Dilutions of $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{1}{6}$, $\frac{1}{8}$, $\frac{1}{12}$, $\frac{1}{16}$, $\frac{1}{24}$, $\frac{1}{32}$, $\frac{1}{48}$ and $\frac{1}{64}$ were prepared of each extract and these were loaded in order of dilution into the 4 mm diameter outer wells of the 0·5 per cent agarose gels. Absorbed serum was placed in the 8 mm diameter centre well which was at a distance of 4 mm from the outer wells. The gels were kept for 48 hours at 24° C. and then observed by reflected light on a black background. In the S-protein dilution plates, the greatest dilution at which the S-protein-antibody precipitation band was visible was taken as the end-point of the S-protein in the stigma extract.

3. Results

A reaction of identity developed between the $S_{23}S_{23}$, $S_{23}S_{45}$ and $S_{23}S_{16}$ stigma extracts when reacted against the absorbed S_{23}-serum and between the $S_{16}S_{16}$, $S_{23}S_{16}$ and $S_{16}S_2$ stigma extracts when reacted against the absorbed S_{16}-serum.

Total protein contents varied between 4·4 and 5·6 mg/ml (tables 1 and 2). In each experiment the dilution end-point for plants homozygous for an S-allele was higher for plants heterozygous for the same S-allele, and in

Table 3

Average S-protein-antibody titre/S-protein end-points and total protein contents

Extract	S-allele to which antibody was produced	Average ratio $\frac{\text{ab.}}{\text{ag.}}$	Average total protein content (mg/ml)
$S_{16}S_{16}$	S_{16}	3	4·96
$S_{16}S_{23}$	S_{16}	1·5	4·76
$S_{16}S_2$	S_{16}	1·6	4·63
$S_{23}S_{23}$	S_{23}	5	5·32
$S_{16}S_{23}$	S_{23}	2·75	5·02
$S_{23}S_{45}$	S_{23}	2·5	4·98

most experiments the end-point of the homozygote was two end-points higher, indicating twice as much S-protein as in the heterozygote. The ratio of the S-protein-antibody titre to the S-protein end-point was calculated (table 3); this gave an estimate of the concentration of the two S-proteins taking into account the different titres of the S-protein-antibodies.

Application of the *t*-test to the results showed a significant difference between the end-points of the homozygotes and heterozygotes; a two-fold difference was highly probable.

4. Discussion

Twice as much of the respective S-protein was regularly found in the stigmas of plants homozygous for either the S_{23} or S_{16} allele as compared with the heterozygote containing only one of these alleles. The ratio of S-protein-antibody titre to S-protein end-point was higher for the S_{23}-protein than for the S_{16}-protein (table 3). This difference may be due to differences in the absorption and concentration processes rather than genotypic differences and may explain the variability between the results from different experiments, since results within an experiment were fairly constant.

The alleles S_{23} and S_{16} and also S_{16} and S_2 show equal action in the stigma (Thompson and Taylor, 1966) and the S_{23}-allele is dominant to the S_{45}-allele (Hodgkin, personal communication) but in spite of this no more S_{23}-protein was detected in the $S_{23}S_{45}$ than in the $S_{23}S_{16}$ stigmas. Thus, one S_{23}-allele appears to code for a certain amount of S_{23}-protein, twice as much being produced by the two S_{23}-alleles of the homozygote; the two different alleles of the heterozygote do not interact to affect the amount.

It is possible that in *B. oleracea* the dominance of the allele may have some effect on S-antibody stimulation in the rabbit. Nasrallah and Wallace (1967*a*, *b*) produced three S-antibodies, one of high titre to an allele of high dominance, one of low titre to an allele of intermediate dominance and a third also of low titre to an allele whose dominance relationships are unknown. This may indicate that the alleles of higher dominance produce S-proteins with a larger or more stable structure, which as well as being more antigenic in the rabbit may also have a competitive advantage for substrates in the stigma.

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