

POLLEN PIGMENTATION AND THE ACTION OF MUTANT yp

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Murfet (1) identified a recessive gene, yp, determining yellow pollen which had arisen as a spontaneous mutant in the cultivar 'Vinco' (Hobart line 51). Chemical tests have now shown that the change in color from orange in the wild type pollen (Yp) to yellow in the mutant is the result of a quantitative rather than a qualitative effect on pigment (carotenoid) production in the pollen. The yp pollen contains approximately 5% of the carotenoid level occurring in Yp pollen.

For the interest of workers with Pisum we report here the results of chemical analyses of the carotenoid pigments, made on our behalf by D. G. Britton, Biochemistry Department, The University of Liverpool, U.K. (Table 1)

Table 1. TLC and spectral data of pollen carotenoids.

Compound	Rf	TLC system	Spectral peaks nm		
			Observed	Literature	
phytofluene	0.2	a.i	(green fluorescence - U.V.)	329,347,365	331,348,367
B-cryptoxanthin	0.4	b.i	(orange-yellow)	427,452,483	425,451,483
	0.6	a.ii			
B-carotene	0.2	b.ii			
	0.25	a.i			
dihydroxy-xanthophyll	0.3	a.iii			

Isolation and analysis of pigments

Pollen was treated with 15% KOH in ethyl alcohol : water, 1:1, overnight, at room temperature, in the dark under nitrogen. Diethyl ether and water were added dropwise, alternately, to give two phases. The water layer was re-extracted with ether, and the combined ether layers washed with water, then evaporated to dryness under nitrogen. The residues so obtained were examined by TLC on

- a) Silica Gel G (incorporating 0.1% KOH) using the solvent systems
 - (i) petroleum ether (40°-60°)
 - (ii) 30% diethyl ether/petroleum ether
 - (iii) diethyl ether
- h) MgO/Keiselguhr, 1/1 (incorporating 0.1% KOH) using solvent systems
 - (i) 5% benzene/petroleum ether
 - (ii) 20% acetone/petroleum ether

These treatments resolved the presence, in both Yp and yp pollen, of two major carotenoids, phytofluene and B-cryptoxanthin. Two minor pigments, B-carotene and an unidentified dihydroxyxanthophyll, were also present. Confirmatory evidence of pigment identity was obtained by eluting spots from TLC plates with acetone and determining absorption spectra.

The co-occurrence of phytofluene and B-cryptoxanthin as major carotenoid constituents in a plant tissue is somewhat unusual. By comparison examination of vegetative tissue (leaves) of Vinco showed a "normal" mixture of carotenoid with 3-carotene; lutein, violaxanthin and neoxanthin occurring as major pigments, together with a trace amount of a-carotene.

(1) Murfet, I. C. 1967. *Heredity* 22:602-607.