ORANGE COTYLEDONS (Orc) AND ORANGE LEAVES (orl): NEW GENES ON CHROMOSOME 1

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Routine observations of seed samples in the Pea Genebank at Wiatrowo revealed large differences in hue among accessions with yellow cotyledons, from white to light-orange. Moreover, in the accession obtained from China ('Ren-shou-da-bai Wan-dou'= WT 11145), after removing of the seed coat, the cotyledons have a clear orange color. This character was first described at the workshop of pea geneticists and collection curators at Alnarp, Sweden (4). Orange cotyledons are of particular interest because they apparently have not been noticed although they've existed for a long time. For the most part, varieties have been classified as having either yellow (I) or green (i) cotyledons.

Preliminary results of crosses involving WT 11145 were reported earlier (1,5). It was stated that the character orange cotyledons is controlled by a dominant gene Orc (independent from I). It is quite possible to suppose that this was a primary feature in Pisum evolution. It was also stated that recessive allele i is epistatic to both Orc and orc; genotypes Orc i as well as orc i have green seeds.

Moreover, plants with orange-yellow Leaf color were observed in segregating populations, but especially in the pure line Wt 11145. Therefore the possibility of a modifying effect of another color gene was considered. But in the locus/allelism tests Wt 11145 (Orc) x Wt 10006 (o) and Wt 12134 (py) the F1 plants had normal leaf color and orange cotyledons.

Simultaneously the carotenoid content of seeds was investigated (3). Seeds of line Wt 11145 contained an additional carotenoid fraction not found in the standard yellow seeds of Wt 3527.

Wt 11145, the type Line for Orc, was crossed with two tester lines, WL 1267 and WL 1393 from the Weibullsholm Pea Collection, to test for linkage. The F1 plants were normal and fully fertile (normal karyotype). F2 generations of both crosses were grown in the field in 1984.

Most markers showed an undisturbed monohybrid segregation, including the gene Orc. Unexpectedly, the yellow-orange leaf color segregated independently as a monogenic recessive (Table 1A). For this character the name orange leaves (orl) was proposed. From the F2 population Wt 11145 (Orc orl) x WL 1393 (orc Orl) recombinants Orc Orl and orc orl were selected. Therefore two genes were found in accession Wt 11145.

An analysis of the dihybrid segregat ion produced another surprise. In the population Wt 11145 x WL 1393 no evidence of linkage was detected between Orc or Orl and the following markers: A (chr. 1); K,Mifo,0h,S,Wb (chr. 2); B,St (chr. 3); N (chr. 4); and Te,U (chr. 5). However, both Orc and Orl showed linkage with D on chromosome 1 (Table IB, Fig. 1). In the F2 population of Wt 11145 x WL 1267, linkage between Orc and Orl was also detected (Cr0=23.3±4.2). It has not been possible to determine the CrO value with D because both parent lines have the same allele at D. A second gene-marker is necessary to determine on which side of the D locus the new genes are localized. In the D-segment Rup,

Table 1. Phenotypic distribution in F2 population segregating for Orange cotyledons and orange leaves from WT 11145 x WL 1393

A. Monohybrid F_2 segregation

D	d	Total	Chi-square (3:1)
382	102	484	3.977
Orc	orc		
478	160	638	0.002
Orl	orl		
490	124	614	7.559

B. Joint segregation of Orc and Orl with D

Orc D	Orc d	orc D	orc d	Total	Joint chi-square	Recomb. fract.	S.E.
321	40	61	62	484	84.81	24.21	2.29
Orc Orl	Orc orl	orc Orl	orc orl				
337	122	153	2	614	45.25	13.14	3.95
D Orl	D orl	d Orl	d orl				
274	90	92	10	466	10.70	34.73	4.01

Table 2. Phenotypic distribution in F2 population segregating for Orange cotyledons from Wt 11145 x Wt 11073.

A. Monohybrid F_2 - segregation

D	d	Total	Chi-square (3:1)
187	46	233	3.43
Orc	orc		
159	43	202	1.48
Orl	orl		
236	62	298	2.79
Idh*	idh		
225.	75	300	0.00
I	i		
202	52	254	2.78

B. Joint segregation of Orc with D and Idh

D Orc	D orc	d Orc	d orc	Total	Joint chi-square	Recomb. fract.	S.E.
106	15	17	16	154	18.9	26.2	4.2
Orc Idh	Orc idh	orc Idh	orc idh				
111	47	40	3	201	10.0	27.1	6.4
D Idh	D idh	d Idh	d idh				
131	56	46	0	233	18.0	15.5	6.4

* - Because of co-dominant inheritance for 3:1 ratio in calculations the fast-variant has been added to heterozygotes.

Sru, and Pur are mapped but the most useful variant may be the isozymic locus Idh. Weeden and Marx (6) showed close linkage of Idh with D and Pur with the suggestion that Idh is located between these two. So a cross between Wt 11145 (Orc I orl D Idh-slow) x Wt 11073 (orc i Orl d Idh-fast) was made at Wiatrowo. In F2 (field 1986) an undisturbed monohybrid segregation was found for all genes (see Table 2A and the footnote). The electrophoretic separation for Idh was done in the Biochemical Laboratory at Wiatrowo.

The linkage of Orc with markers of chromosome 1 has been confirmed, both with D and Idh (Table 2B). The CrO value for the gene-pair Orc-D is almost identical with that from the previous cross combination (Table IB). But despite undisturbed monohybrid segregation there was no evidence of linkages for Orl in this cross.

If Idh is located between D and Pur (6), then the data from Tables IB and 2B suggest the following gene order: Pur - Idh - D - Orc - Orl?

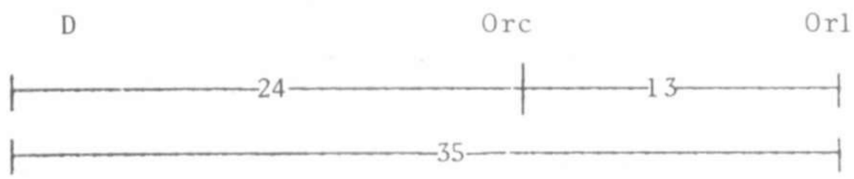

Fig. 1. The map distances in D segment on chromosome 1

Acknowledgement:
I wish to express my sincere gratitude to Mr. B. Wolko, M. Sc. from Wiatrowo's Biochemical Laboratory, for electrophoretic separation of Idh.

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x Editor's Note: x
x Recently, while inspecting seed samples of about a dozen x
x USDA Plant Introduction (P.I.) accessions, I noticed that x
x P.I. 143484 (from Iran) has orange cotyledons. x
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