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LINKAGES IN *PISUM* L.
I. THE GENE *orp* (ORANGE POD)[1]

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Summary. A mutant characterized by orange colour of the pod schlerenchyma and the phloem—xylem tissue has been derived as a result of seed treatment of the pea variety Paloma (the catalogue number of this line at Wiatrowo Pea Genebank = Wt 3527) by a combined dose of fast neutrons and N-nitroso-N-aethylurea (200*r* + 0.014%). This mutant has been designated Wt 10263, "orange pod".

The origin test cross Wt 10263 × Wt 3527 (mutant × initial line) resulted in normal F_1 generation plants and monohybrid recessive segregation in the F_2 generation. The gene responsible for the orange pod was designated *orp*.

For analysis of linkage the linkage test between Wt 10263 (mutant) and Wl 1238 (a tester) line with gene-markers derived from the Weibullsholm Pea Collection of the Nordic Genebank) was carried out. The investigation of the F_2 generation showed clear linkages of the new gene *Orp* with the marker genes *K* and *Wb* in chromosome *2*. According to these investigations the sequence was *Wb* — *Orp* — *K* with the crossing over values: *K* — *Wb* = 18.7, *K* — *Orp* = 12.4, *Wb* — *Orp* = 10.0

A genetic analysis of any induced mutants usually involves three steps: origin test crosses, identity test crosses and linkage tests (Blixt, Gottschalk 1975, Święcicki 1980). A linkage analysis connected with elaboration of gene maps, are presently carried out in rather few institutions in the world. This work has been promoted by The Pisum Genetics Association which also proposes rules for and coordinates the work on gene nomenclature in *Pisum*. A Gene Symbol Coordinator was appointed to deal with the problems of priority, acceptance of symbols etc. (see: Preface in The Pisum Newsletter 1969 and Blixt 1977).

Gene markers with high penetrance, stable expression and easy to observe also in the seedling stage, are of particular interest in the genetic analysis of a plant. The character of orange pod was found to be an induced mutation after treating seeds of the pea variety Paloma with a combined dose of fast neutrons (200r Nf) + + N nitroso-N-eathylurea (0.014% NEU).

As a result of pea breeding and genetic investigations carried out at the Plant Breeding Station in Wiatrowo, many induced mutants have been produced (Święcicki 1983). Their utilization in breeding or genetic research or in both depends

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on the nature of individual mutants. This paper deals with a genetic analysis of a mutant. The main purpose was to present the results of crosses between a new mutant with orange pods and the initial line and the testerline leading to establishment of the new gene *orp* and its localization on chromosome *2*.

MATERIAL AND METHODS

The "orange pod" mutant (the catalogue number at Wiatrowo Pea Genebank = Wt 10263) has been found in an M_2 population as a result of treating seeds of the pea variety Paloma (Wt 3527) by a combined dose of 200 r Nf+0.014% NEU (Święcicki 1979). Both lines are available in the Pea Genebank at Wiatrowo (Święcicki et al. 1981). The main characteristic of the mutant is an orange colour of the pod wall schlerenchymatic tissues, which are normally colourless and phloem—xylem tissues. Thus, the colour becomes visible in the stem already before the appearance of flowers. When inflorescences develop, the orange colour goes through the peduncles to the pods. From the outside of the pod the colour of the schlerenchyma is visible as "dirty orange". On opening the pod the schlerenchyma is vividly orange and after removing the schlerenchymatic layer the parenchyma is normally green (see also: Nozzolillo et al. 1983, Święcicki 1982).

In this work the origin test cross was performed between the "orange pod" mutant (Wt 10263) and the initial line Wt 3527 (cv. Paloma).

No other similar mutants seem to be known from the world pea collections and, therefore, there was no possibility or need for identity test crosses.

The linkage test was carried out between the mutant Wt 10263 and the line Wl 1238, a testerline from the Weibullsholm Pea Collection of the Nordic Genebank (Blixt 1976). The genotype of the two lines involved were, with respect to the genes studied:

Wt 10263 — *a*, *B*, *bra*, *Cp*-1, *D*, *Dt*, *Gp*, *I*, *K*, *orp*, *Pr*, *R*, *S*, *T*, *Tl*, *u*, *Wb*;
Wl 1238 — *A*, *b*, *Bra*, *cp*-1, *d*, *dt*, *gp*, *i*, *k*, *Orp*, *pr*, *r*, *s*, *t*, *tl*, U^{st}, *wb*.

A short description of the effects of these genes (after Blixt 1972, 1977) is given below (number in paranthesis refers to chromosome):

A — *a*	(1)	Basic gene for development of anthocyanin, *a*-plants develop no anthocyanin.
B — *b*	(3)	With dominance in the gene *A* and other flower colour genes, flower colour dull purple — deep rose pink.
Bra — *bra*	(3)	Inflorescence with bracts — without bracts.
Cp-1 — *cp*-1	(5)	Straight pods — concavely curved pod.
D — *d*	(1)	Recessive *d* extinguishes the anthocyanin-coloured ring at the basis of the stipules.
Dt — *dt*	(0)	Inflorescence with long peduncles — short peduncles.
Gp — *gp*	(5)	Pod colour green — yellow.
I — *i*	(1)	Cotyledons orange yellow — green.
K — *k*	(2)	Wings normal — reducet.

Orp — orp		Pod schlerenchyma and phloem—xylem tissue uncoloured green — orange.
Pr — pr	(0)	Inflorescence long — short.
R — r	(7)	Seeds smooth — wrinkled.
S — s	(2)	Normal — with excretion of tragacanth on the outside of the seed-coat.
T — t	(4)	Recessive thin stem.
Tl — tl^w	(7)	Leaves with 2 - 3 pairs of tendrils — leaflets instead of tendrils.
U^{st} — u	(5)	With *A*, *Z* seed-coat with blackish to brownish violet stripes — — without such colour.
Wb — wb	(2)	With *Wa*, *Was*, *Wlo*, *Wsp* normal — with very little wax, pods waxless.

The analysis of linkage was done in the F_2 generation which is more favourable for peas than a back-cross model *Drosophila*. Calculations of Chi^2, linkage and crossing-over (Cr-0) values were performed on the Wang-computer at Weibullsholm, Landskrona, Sweden, according to the product-ratio method (Stern 1933).

RESULTS AND DISCUSSION

Plants of the F_1 generation of the origin test cross Wt 10263 (orange pod) × Wt 3527 (initial line) were all normal. The F_2 generation segregated into quite undisturbed 3 normal plants: 1 orange pod (140 : 48 — found; 141 : 47 — expected; Chi^2=0.028). This indicated a monogenic recessive inheritance of the mutant. So, the suggested gene symbol *orp* for the orange pod character was approved by the Pisum Genetics Association Gene Symbol coordinator (Święcicki 1982).

It is of interest that the origin variety Paloma is recessive for the gene *a* (produces no anthocyanin). The gene *orp* may also be the first gene found to produce colour in the schlerenchyma; other known colour genes, e.g., *Pu*, *Pur*, *gp*, *dp* affect parenchyma. *Pu* and *Pur* are furthermore hypostatic to *a*. As expected *orp* and *gp* (yellow pod) did not seem to interact. In *p*, *v* genotypes (pods without schlerenchyma) orange colour of the gene *orp* appears only in phloem—xylem tissue.

The F_2 generation of the linkage test (Wt 10263 × Wl 1238) was grown in a greenhouse which (as is normally the case) affected the expression of several of the genes studied. The gene *s* causing the so-called "caterpillar" character, i.e., the seeds glue together, showed a very large deficit of recessives. This is a well-known effect of the small seed size sometimes caused by green-house conditions preventing contact between seeds necessary for glueing process. It is in accordance with the experience of other investigators (Blixt 1966). Also for several other genes studied the monohybrid segregations were not satisfactory, and therefore calculations for linkage were restricted to genes with a clear expression and well-studied by other researchers. The monohybrid segregations of those are presented in Table 1.

It should be particularly noted that though the monohybrid segregations of the genes *B*, *K*, *S*, and *I* are disturbed, the use of the corrected expected Chi^2 and the

Table 1. Monohybrid segregation for genes observed in F_2 population of the linkage test — Wt 10263 (*orp*) × Wl 1238 (testerline)

Gene	Allele		Total	*Chi*² (3:1)
	dominant	recessive		
B (3)	228.75	76.25	305 Expected	9.05
	206.00	99.00	305 Found	
K (2)	332.25	110.75	443 Expected	12.91
	365.00	78.00	443 Found	
Wb (2)	356.25	118.75	475 Expected	0.03
	358.00	117.00	475 Found	
Tl (7)	356.25	118.75	475 Expected	1.55
	368.00	107.00	475 Found	
Gp (5)	348.00	116.00	464 Expected	0.74
	340.00	124.00	464 Found	
S (2)	171.00	57.00	228 Expected	22.48
	202.00	26.00	228 Found	
I (1)	260.25	86.75	347 Expected	6.30
	240.00	107.00	347 Found	
Orp	342.75	114.25	457 Expected	1.00
	352.00	105.00	457 Found	

Table 2. Distribution of phenotypes in F_2 population of the linkage test — Wt 10263 (*orp* × Wl 1238) (testerline). Joint segregation of gene markers in chromosome *2*

Pair of gene markers	Phase[1]	Phenotypes				Total	Joint *Chi*²	Cr-O-value (per cent)
		D D	*D r*	*r D*	*r r*			
K — Wb	C	315	49	23	55	442 Found		
		278	86	60	18	442 Corrected expected segregation		
*Chi*²		4.92	15.92	22.82	75.06		118.72	18.7±2.1
K — S	C	179	11	23	15	228 Found		
		168	22	34	4	228 Corrected expected segregation		
*Chi*²		0.72	5.50	3.56	30.25		40.03	21.8±3.2
K — Orp	R	259	205	77	0	441 Found		
		277	87	59	18	441 Corrected expected segregation		
*Chi*²		1.17	3.72	5.49	13.00		23.38	12.4±4.7
Wb — S	C	182	4	20	22	228 Found		
		165	21	37	5	228 Corrected expected segregation		
*Chi*²		1.75	13.76	7.81	57.80		81.12	11.1±2.2
Wb — Orp	R	239	105	112	0	456 Found		
		265	79	86	26	456 Corrected expected segregation		
*Chi*²		2.55	8.56	7.86	26.00		44.97	10.0±4.6
S — Orp	R	150	52	23	3	228 Found		
		153	49	20	6	228 Corrected expected segregation		
*Chi*²		0.06	0.18	0.45	1.50		2.19	36.4±5.7

[1] C = coupling phase
R = repulsion phase

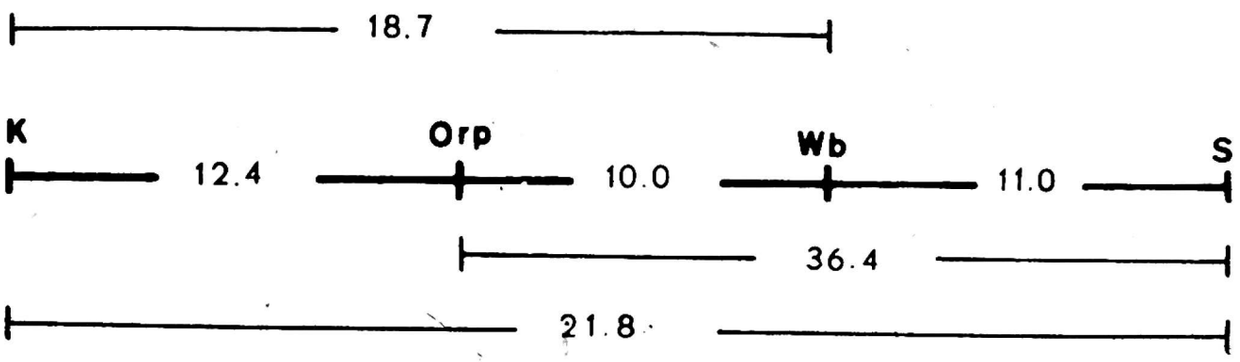

Fig. 1. The map distances in *Orp* segment of chromosome *2*

product-ratio method (Stern 1933) still makes it possible to use these data to establish linkage, though the Cr-O-value, of course, is less reliable. It should be noted that the segregation for the gene *orp*, though not so good as that under field conditions (see: results of the origin test cross), is still satisfactory, confirming the monohybrid recessive nature of the mutation.

The analysis of the dihybrid segregations showed no linkage with the genes *B*, *Tl*, *Gp* or *I*. A clear linkage was found with genes in chromosome *2* and the relevant segregations are presented in Table 2. The corrected expected segregations are extremely useful when analysing F_2 data with disturbed monohybrid segregations. When calculating Chi^2 based on the theoretical expected segregations a disturbed monohybrid segregation may easily lead to high Chi^2 and perhaps to wrong conclusions. This can be avoided using the following corrected expected formula of Stern (1933):

$$AB=\frac{(AB+Ab)\times(AB+aB)}{N}$$

$$Ab=\frac{(Ab+AB)\times(Ab+ab)}{N}$$

$$aB=\frac{(aB+ab)(aB+AB)}{N}$$

$$ab=\frac{(aB+ab)\times(Ab+ab)}{N}$$

The Cr-O-values are given $\pm$ standard errors. The Cr-O-values found relating to *K*, *Wb* and *S* are in agreement with earlier findings (Blixt 1977). It is, therefore possible to localize the gene *orp* in chromosome *2* without any doubt. Bearing in mind the uncertainty of the values for *S* due to the deficit the detailed analysis of the Cr-O-values of Table 2 can be summarized as in Fig. 1.

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SPRZĘŻENIA GENÓW U GROCHU (*PISUM* L.)
I. GEN *ORP* (ORANGE POD)

Streszczenie

W wyniku traktowania nasion grochu odmiany Paloma (numer katalogowy linii w banku genów grochu w Wiatrowie=Wt 3527) kombinowaną dawką szybkich neutronów i N-nitroso-N-etylomocznika (200r+0,014%) otrzymano mutanta, którego charakteryzowało pomarańczowe zabarwienie sklerenchymy strąków oraz tkanki sitowo-naczyniowej łodygi. Mutacji nadano nazwę ,,orange pod", a dla linii w banku genów numer katalogowy Wt 10263.

W wyniku skrzyżowania mutanta Wt 10263 z linią wyjściową Wt 3527 (tzw. the origin test cross) uzyskano normalne, niezmienione rośliny F_1. W pokoleniu F_2 stwierdzono recesywne dziedziczenie mutacji. Dla genu warunkującego cechę ,,orange pod" przyjęto symbol *orp*.

W celu zanalizowania sprzężeń nowego genu (tzw. the linkage test) skrzyżowano mutanta Wt 10263 z linią Wl 1238 (linia testowa z genami-markerami pochodząca z kolekcji grochu Nordyckiego Banku Genów w Weibullsholm). Badania pokolenia F_2 wykazały sprzężenia nowego genu *Orp* z genami-markerami *K* i *Wb* w chromosomie *2*. Uzyskano kolejność genów *Wb-Orp-K* z następującymi wartościami crossing-over: $K - Wb = 18{,}7$, $K - Orp = 12{,}4$, $Wb - Orp = 10{,}0$.

СЦЕПЛЕНИЯ ГЕНОВ У ГОРОХА
I. ГЕН *ORP* (ORANGE POD)

Резюме

В результате комбинированного действия дозы быстрых нейтронов и N-нитрозо-N-этиломочевины (200 *г*+0,014%) на семена гороха сорта Paloma (номер каталога линии в банке генов гороха в Вятрове — Wt 3527) был получен мутант, который характеризовался оранжевой окраской скле

ренхимы и сетчато-сосудистой ткани стручков. Этот мутант назвали orange pod (оранжевый стручек), а для линии в банке генов был дан номер каталога Wt 10263.

В результате скрещивания мутанта Wt 10263 с первоначальной линией Wt 3527 (так-называемый the origin test cross) были получены нормальные, неизмененные растения F_1. В поколении F_2 была обнаружена рецессивная наследственность мутации. Ген, обуславливающий признак оранжевого стручка (orange pod) был обозначен символом *orp*.

Чтобы проанализировать сцепления нового гена (так называемый the linkage test cross), мутант *Wt* 10263 был скрещен с линией *Wl* 1238 (тестовая линия с генами-маркерами из коллекции гороха Северного Банка Генов в Weibullsholm). Исследования поколения F_2 обнаружили сцепление нового гена *Orp* с генами-маркерами *K* и *Wb* в хромосоме *2*. Получена очередность генов *Wb-Orp-K* со следующими значениями кроссинг-овуэра: $K-Wb=18.7$, $K\text{-}Orp=12.4$, $Wb\text{-}Orp=10.0$.