INHERITANCE AND LINKAGE RELATIONS OF A CRINKLED VARIANT IN PEAS

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(With Plates 6–8)

CONTENTS

Introduction

In the breeding programme being conducted at the Wisconsin Experiment Station, numerous rogue-type plants have been observed in seedling stands of field and canning peas. The most common of these has been a plant showing a crinkled mosaic-like condition from which the affected seedlings always seemed to recover. This paper is a report on the occurrence of this variant, its inheritance, and its linkage relations.

Methods and materials

The variant was observed in an autumn planting of genetic material consisting of 2330 F_3 lines and 578 F_2 plants grown for the study of the linkage relations of some foliage colour mutants referred to as **cl**$_1$, **cl**$_2$ and **cl**$_3$. These three mutants originated in the Alaska variety (Wells, 1949). The genetic constitutions of the linkage tester lines used in this study are shown in Table 1.

Table 1. *Genetic constitutions of the linkage tester lines*

Tester line	Genotypes with respect to marker genes
Grey Sugar	**vv, le le, AA,** $l_1 l_1$, $l_2 l_2$
Dwarf Telephone	**rr, le le, cp cp, bt bt**
White Canada	**II, fa fa**

The second and third generation material was planted from 21 to 25 September. The plants were pulled and examined in the first week in November. The product method (Immer & Henderson, 1943) was used in estimating linkage intensity.

The crinkled variant was first thought to be a type of low-temperature reaction because temperatures as low as $-0{\cdot}5°$ C. occurred during the 3 weeks following date of planting. To determine if temperature was the causal factor, test plantings were made. Five seeds were planted per pot of the **cl**$_2$ mutant line, hereafter known as Pale Green 2, involved in crosses segregating for the variant, and of White Canada, one of the linkage tester lines.

One pot was set in a constant temperature of 28° C. and allowed to remain there until the seedlings were large enough for examination. The pot was then moved to an air temperature of 16° C. to reduce the rate of growth in order that comparisons might be more easily made with slower growing seedlings from seed germinated at lower temperatures. A second pot was allowed to remain during the entire period at the 16° C. temperature. A third pot was at 16° C. except for a 9 hr. period in an outdoor air temperature of 3–5° C. during the first day. A fourth pot was held at 16° C. except for a 9 hr. period out of doors 48 hr. after planting when the air temperature was 5° C. A fifth pot was similarly held at 16° C. except for a 6 hr. period 72 hr. after planting when the outside temperature varied between 0 and 4° C. In addition to these plantings, five seeds of Pale Green 2 and White Canada were allowed to germinate over a 7-day period in the 4 and 12° C. incubators. These seedlings were then established in an air temperature of 20° C.

EXPERIMENTAL RESULTS

Nature of the variant

The variant occurred in three crosses having one parent in common, Pale Green 2, the foliage colour mutant carrying **cl**$_2$. The variant did not occur in progenies homozygous for **cl**$_1$ or **cl**$_3$, the other two foliage colour mutants, or in any of the F_2 or F_3 progenies from their crosses with Dwarf Telephone, White Canada and Grey Sugar.

Twelve seedlings of Pale Green 2 were available for examination. All of these were crinkled, though not quite to the same extent. Representative ones are shown in Pl. 6.

Some of the F_3 lines showed the same characteristics and some were even more severely affected, as shown in Pl. 7.

The leaves on individual plants at the stage of growth illustrated in Pl. 7 were uniformly affected and exhibited a mosaic-like pattern of irregularly intermingled light and dark green areas. The light green areas appeared to be responsible for some distortion causing the leaves to crinkle. The edges of occasional leaves were serrated as may be seen in Pls. 6 and 7. Leaves of the more severely affected plants as in Pl. 7 were reduced in size and were inwardly curved toward the lower surface. Plants were stunted, though not uniformly, and were also stiffened and darker green than normal, especially in Pale Green 2 which was normally a light green in colour. Leaves with dwarfed tendrils and leaves completely lacking tendrils may be seen in Pl. 7. The leaf symptoms of this variant were noticeable from the time the foliage emerged. As affected seedlings grew in height there was at first no change in the character of the unfolding leaves, for they were typically crinkled and mottled. The severity of the symptoms appearing on new foliage diminished with continued growth in height until normal leaves appeared. The net effect of this character was to retard growth, but ultimately normal plants developed. This type of variant has been observed both in the field and in the greenhouse. The occurrence of this variant appears to be of little commercial importance.

A variant occurring in the Alaska variety and described by Stubbs (1937) resembles the crinkled variant in some respects. The growing points of the seedlings he examined were either injured or completely destroyed, resulting in the development of axillary buds and the growth of normal stems. Of the 1586 crinkled seedlings examined, none exhibited a dead or noticeably injured growing point, and no axillary bud development was observed.

Lamm (1949) described a mutation in peas which he designated by the symbols **Cri cri** derived from the descriptive term 'Crispus'. Typical plants had crisp or folded leaves and

elongated obovate leaflets. The character was expressed in both seedlings and mature plants. The **cri** locus was found to be in the fifth linkage group in association with **Gp**. Unlike Crispus, the crinkled character is expressed only in the seedling stage and the leaves are not folded or elongated.

Temperature relations

All Pale Green 2 plants in the several temperature treatments developed the crinkled character. However, at the lower temperatures, the variant was more strongly expressed at time of emergence, and the degree of expression persisted for a longer time. All seedlings of the White Canada variety were normal in appearance. The Pale Green 2 seedlings in the lower temperature treatments showed essentially the same intense expression of the crinkled character. The lower leaves of Pale Green 2 seedlings in the 28° C. temperature were crinkled. Newer growth at the top of the plant was normal in appearance. Pale Green 2 plants from seed germinated at 4 and 12° C. and planted in a house where the air temperature was 20° C. were similar in appearance to those grown at 16° C.

Table 1. *Summary of ratios for the factor pair,* **Cr cr**, *in all cultures studied and P values from* χ^2 *for goodness-of-fit to* 3:1 *ratios*

Normal parent	Generation	Culture no.	No. plants or lines in crosses with Pale Green 2 given as F_2 phenotypes **Cr**	**cr**	Total	Range of *P*
Blackeye	F_2	10	20	4	24	0·50–0·30
Dwarf Telephone	F_2	5	24	7	31	0·95–0·50
Dwarf Telephone	F_2	6	28	7	35	0·50–0·30
Dwarf Telephone	F_2	7	26	3	29	0·10–0·05
Total			98	21	119	
White Canada	F_3	106	31	8	39	0·95–0·50
White Canada	F_3	107	80	18	98	0·20–0·10
White Canada	F_3	108	46	7	53	0·05–0·01
White Canada	F_3	109	106	17	123	<0·01
Grey Sugar	F_3	112	58	13	71	0·20–0·10
Dwarf Telephone	F_3	115	167	22	189	<0·01
Dwarf Telephone	F_3	117	162	15	177	<0·01
Total			650	100	750	<0·01

Inheritance and linkage relations

The crinkled variant has been assigned the genetic symbols **Cr cr** derived from the word 'crinkled'. The total observed ratio for **Cr cr** in four small F_2 families planted 23 October and examined on 7 December was 98 **Cr**:21 **cr**, a fairly satisfactory fit to a 3:1 ratio ($P = 0{\cdot}10$–$0{\cdot}05$). Each of the component ratios compared favourably with a 3:1 ratio as shown in Table 1.

These F_2 populations and the strains Pale Green 2, Dwarf Telephone, White Canada, and Grey Sugar were planted at this late date, 23 October, to obtain a check on the reaction of the parents not included in the earlier plantings and to see if segregation for **Cr cr** could be observed in F_2. Grey Sugar seedlings failed to emerge, apparently because the seed was not viable. Seedlings of Dwarf Telephone and White Canada were normal, and seedlings of Pale Green 2 showed typical mottling and crinkling.

F_3 data for **Cr cr**, given in Table 1 for all the families studied, are listed as F_2 phenotypes rather than F_2 genotypes. The reason for this was that there was such a bunching of lines in the **Cr Cr** class that only very poor comparisons with 1:2:1 ratios were obtained. Three of the smaller families gave ratios that were in accord with single factor expectation.

The remaining five families and the total for F_3 data exhibited a marked deficiency of **cr cr** lines. It may be that modifying factors were responsible for these results. Some of the F_3 lines classified as crinkled were homozygous for only a slight manifestation of the character, others were homozygous for a severe manifestation, and others were segregating for normal *v.* slight, normal *v.* severe, and normal *v.* both slight and severe.

Plants representative of the crinkling character are shown in Pl. 8 from one of the latter type of F_3 line. The intensity of expression of the character increases from left to right. The plant on the left is normal except for a few light coloured areas on the leaves and a general surface irregularity. The plant on the far right is severely affected, showing stunting, intense mottling and crinkling, and misshapen leaves. An analysis of the factors conditioning this behaviour was not attempted.

Data are summarized in Table 2 for associations of **Cr cr** with marker genes in four of the seven linkage groups proposed by Lamprecht (1948).

Table 2. *Associations of* **Cr cr** *with factors in four chromosomes of peas, P values for the single-factor ratios involved, and recombination percentages*

Linkage phase	Linkage group	**Y y** Generation	**Y y** P*	**Cr cr** Generation	**Cr cr** P*	Factor pair **Y**	Factor pair **y**	No. of plants **Cr** **Y**	**Cr** **y**	**cr** **Y**	**cr** **y**	Total	P from χ^2 for independence	% recombination
C	I	F_3	<0·01	F_3	<0·01	**I**	**i**	210	100	49	21	380	0·95–0·50	
C	I	F_2	0·20–0·10	F_3	0·20–0·10	**A**	**a**	52	6	6	7	71	<0·01	22·2±5·7
C	I	F_3	0·95–0·50	F_3	0·20–0·10	$\mathbf{L_1}$ or $\mathbf{L_2}$	$\mathbf{l_1}$ or $\mathbf{l_2}$	55	3	12	1	71	0·95–0·50	42·5±8·5
R	IV	F_2	0·50–0·30	F_3	0·20–0·10	**V**	**v**	49	9	7	6	71	0·05–0·01	
R	IV	F_3	0·05–0·01	F_3	0·20–0·10	**Le**†	**le**	50	8	11	2	71	0·95–0·50	
R	IV	F_3	<0·01	F_3	<0·01	**Le**‡	**le**	268	46	45	7	366	0·95–0·50	
R	IV	F_2	<0·01	F_3	<0·01	**Fa**	**fa**	223	39	43	5	310	0·50–0·30	
C	IV	F_3	0·20–0·10	F_3	<0·01	$\mathbf{Cl_2}$	$\mathbf{cl_2}$	473	177	72	28	750	0·95–0·50	
R	V	F_2	<0·01	F_3	<0·01	**Cp**	**cp**	273	43	29	6	351	0·95–0·50	
R	VII	F_2	<0·01	F_3	<0·01	**Bt**	**bt**	205	107	21	12	345	0·95–0·50	
R	VII	F_3	0·05–0·01	F_3	<0·01	**R**	**r**	238	100	26	10	374	0·95–0·50	

* From χ^2 for independence.
† Internode length factor carried by Grey Sugar.
‡ Internode length factor carried by Dwarf Telephone.

Most of the single-factor ratios involved in these comparisons do not satisfactorily compare with a 3:1 ratio. This is not unexpected, for it is difficult to take readings on **Fa fa**, **Cp cp**, **Bt bt** and $\mathbf{L_1}$ $\mathbf{l_1}$ $\mathbf{L_2}$ $\mathbf{l_2}$. These factors denote fasciated stem, pod curvature, pod-tip shape, and dimpling of the mature seed, respectively. The ratio for seed colour was 259 **I**:121 **i**. There was no apparent explanation for this marked excess of green-seeded types. The ratio for internode length in the Dwarf Telephone cross is 313 **Le**:53 **le**, a considerable deficiency of **le** types. This character was difficult to classify, presumably because of the action of genes which modified the primary effect of **Le le**. F_3 lines shorter than Dwarf Telephone were easily included in the **le le** class, but the contrast was so great between those very short lines and **le le** lines lacking such modifiers for shorter stature that many of the latter type were classed as segregating or tall. Failure of **Cr cr** ratios in the larger populations to fit the 3:1 ratio has been discussed.

The data indicate association to exist between **Cr cr** and **A a** in the first linkage group with 22·2±5·7 % of recombination and between **Cr cr** and one of the two factors for dimpling *v.* smooth seed surface, $\mathbf{L_1}$ $\mathbf{l_1}$ and $\mathbf{L_2}$ $\mathbf{l_2}$, with 42·5±8·5 % of recombination. The P value from χ^2 for independence for the association of **Cr cr** with one of the dimpling factors was not significant, but since one of the dimpling factors has been shown to be

associated with **A** in a larger population (Wells, 1949) with $7{\cdot}1 \pm 1{\cdot}2$ % of recombination, this latter linkage value was calculated.

Discussion and summary

A rogue-type plant that is very common in both field and canning peas was described. This variant was so abundant in the field in seedling stands as to be considered as a low-temperature effect, partly because the plants exhibited the crinkling and mosaic-like appearance only in the seedling stage when low spring temperatures often resulted in frost injury. Freezing injury leads to the formation of necrotic lesions on stems and leaves and often death of the growing points (Walker, 1939). The results of the present investigation suggest that the crinkled variant is the result of a simple mutation of high frequency and that its inheritance differs from normal by a single factor.

Apparently peas have some very unstable loci. In addition to the crinkled factor, there are known two other common mutants affecting vegetative form of the plant, the rabbit-ear rogue character (Brotherton, 1923) known to occur only in some varieties of wrinkled seeded canning peas and the late-rogue character only occurring to the writer's knowledge in Alaska type varieties.

It is apparent from the temperature studies reported here that the crinkled character is more strongly developed at low than at high temperatures. This explains in part why this characteristic of the foliage colour mutant, Pale Green 2, was not observed until the autumn of 1948 when generally low temperatures led to the prominent expression of crinkled in Pale Green 2 and in some F_3 lines.

The genetic symbols **Cr cr** have been assigned to the factor pair responsible for the crinkled character. The **Cr cr** factor pair appears to be located in the first linkage group in association with **A a** with $22{\cdot}2 \pm 5{\cdot}7$ % of recombination.

Even though the growth of affected seedlings is retarded, occurrence of this variant is of little commercial importance because plants ultimately assume normal growth.

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EXPLANATION OF PLATES

Plate 6

Representative plants of Pale Green 2, the crinkled parent line.

Plate 7

Representative seedlings from an F_3 line of the cross White Canada × Pale Green 2 showing severe expression of the crinkling character.

Plate 8

Seedlings representative of an F_3 line homozygous for **cr cr** but showing various expressions varying from slight on the left to severe on the right.