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NEUTRONIC MUTATIONS IN PEAS

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ABSTRACT

Pea seeds were irradiated with neutrons. At least 9 mutations have been found. Probably all are recessive. Some are undesirable, some have practical value. At least one mutant represents a completely new characteristic. One mutation occurred more than once. Three times two mutations occurred in one seed.

Irradiation with neutrons is considered to present a valuable means in plant breeding.

1. INTRODUCTION

Since in 1928 MULLER with *Drosophila* and STADLER with maize demonstrated that mutations can be artificially induced by X-rays, the number of mutagenic factors has increased considerably. Of course this is of great importance for plant breeding, because new mutants may be the starting-point for a breeding-project. Relatively very little work has been done in the Netherlands with neutrons as a factor for inducing mutations. This is the reason why in 1957 the writer started an experiment with neutrons in peas, his collaborator K. VERKERK in tomatoes. The object was a first orientation on the value of neutronic mutations for plant breeding. The results with tomatoes will be described in a separate publication (5).

2. MATERIAL AND METHODS

Originally 2 pure lines were used as starting-material: 'Dominant' and 'Elfjespeul'. Both are my own selections. The former has all genetical characters in dominant position, as far as known. The latter is a sugar pea of very good quality, but with strongly developed strings. It has wrinkled cotyledons.

Dry seeds of these lines were irradiated in the Kjeller reactor of the former "Joint Establishment for Nuclear Energy Research" (JENER) at Lillestrøm, Norway. The intensity was $5.1 \times 10^8 n/cm^2/sec$ with a pile level of 0.5 kW. In a first series samples of 100 seeds were irradiated during 0, 4 or 8 hours, yielding 99, 70 or 63 germ plants of 'Dominant', 97, 47 or 20 germ plants of 'Elfjespeul', respectively. Evidently a considerable difference in resistance to radiation occurs. This is in harmony with LAMPRECHT (2), who established that wrinkled seeded cultivars are much more sensitive to X-rays than non wrinkled ones. GELIN *et al.* (1) ascribe this difference to the lower water content of wrinkled seeds.

The growth of the germ plants was very poor and especially their roots were severely damaged. The plants did not react to gibberellic acid and 5 weeks from sowing all had died.

Although the season was rather late for peas, a second series was irradiated, this time samples of 200 seeds of 'Dominant' during 0, $\frac{1}{2}$, 1 or 2 hours respectively. These seeds were sown on 18th May 1957 and 4 weeks later 192, 164, 2 or 0 X_1-plants could be transplanted. The growth of the plants from irradiated seeds was poor. All were quite abnormal in habit, chlorotic and resembled plants with virus diseases. This made it impossible to make reliable observations. The seed set was poor, doubtlessly also on account of the late season. Fourty two plants yielded enough seeds to be grown as individual X_2-lines. These were numbered N_1, N_2,......N_{42}. The 877 seeds of the remaining plants were put together as mass and indicated as N_{43}. These N-numbers are used for reference only. For comparison 5 lines of non-radiated 'Dominant' were grown. All X_2-plants, besides some semi-lethal mutants, grew quite well, so that the direct damage in X_1 has been only somatical.

3. Results

The X_1-motherplants of N_1 and N_2 had a fasciated stem. Among their 32 and 28 X_2-plants respectively this characteristic did not occur and hence does not represent a mutation.

The average germination of the X_2-seeds was 90%. Seven lines germinated considerably worse, averaging 74% only. This might suggest the segregation of a lethal gene. In one case 21 X_3-lines with 4,101 seeds were studied and they germinated for 99%. This clearly opposes segregation of lethality.

The above two negative cases have been mentioned for completeness' sake. We shall now proceed to the positive cases.

3.1. *Mutations observed in X_2*

1. "Bushy".– In the X_2-mass N_{43} one plant was found with a habit of growth which can best be indicated as "bushy". Several branches arise from the bottom of the plant and these branches remain short and bear small leaves. The type resembles somewhat LAMPRECHT's fruticosa (4). The X_2-plant yielded 3 seeds only, from which 2 X_3-plants were obtained, both typically bushy, but unfortunately not bearing seeds. It is the more unfortunate that this type was lost, since breeders are interested in heavily branching types with regard to mechanical harvesting for processing purposes.

2. Emerald.– Also in the X_2-mass N_{43} one emerald plant was found, lacking the waxy coating on stems and leaves. This plant yielded 2 seeds only, from which 2 X_3-plants were obtained, both emerald. Again, these plants did not produce any seeds.

3. Chlorina.– In N_{35} 41 X_2-plants out of 44 seeds were grown and 7 of these were yellow and died in a very early stage of growth. The ratio of 34 green : 7 yellow corresponds with the 3 : 1-expectation of (31) : (10).

All but one of the green X_2-plants yielded many seeds and could be grown as X_3

which was done in the fall of 1958 immediately upon harvest. The seeds were sown in flats in a greenhouse. Results:

13 lines (1,952 seeds, 1,922 plants) did not segregate;
20 lines (3,580 seeds, 3,509 plants) segregated into:
2,756 green : 753 yellow
exp. (2,632) : (877)

The ratio of 13 constant : 20 segregating lines corresponds well with the expected (11) : (22), but the ratio green : yellow in the segregating lines shows a considerable deficiency of recessives. Most probably this points to lethality in an early embryonic stage. Anyhow, the conclusion is justified that the recessive "chlorina" has mutated.

4. Dwarf.– The X_2 N_{12} consisted of 28 plants and 3 of these were so underdeveloped that the indication "dwarf" is still much too strong. The original 'Dominant' may reach a stem length of more than 2 meter, but the mutants became 4 or 5 cm long and succumbed without having flowered. A fall-sown X_3 of 21 normal (long) X_2-plants yielded the following results:

13 lines (2,558 seeds, 2,532 plants) did not segregate;
8 lines (1,553 seeds, 1,529 plants) segregated into:
1,159 long : 370 dwarf
exp. (1,147) :(382)

The ratio 13 constant : 8 segregating does not correspond with the expected (7) : (14), actual deviation divided by standard deviation being 4.0. However, in the segregating lines the 3 : 1-expectation is closely approached.

An attempt was made to grow the dwarfs into seed by nursing them carefully. Flower buds appeared, but no fruits were formed. Fortunately, the dwarfs reacted well to a treatment with gibberellic acid and reached stem-lengths of about 30 cm. These plants produced fruits and seeds so that the mutant has been fixed.

5. Spoon-leaf.– The original 'Dominant' cultivar has broad and sessile stipules like most cultivars. The mutant which has been called "spoon-leaf" has narrow and elliptical stipules with a petiole which may reach the same length as the lamina. See figures 1 and 2.

Closer observation of germ plants has shown that the lowest five nodes have no stipules at all, while the sixth (or sometimes seventh) and following nodes bear the spoon-leaves. Quite remarkably the spoon-leaf characteristic is always accompanied by flowers in which pistil and stamens are not covered by the keel so that the flowers are open.

The mutation has occurred at least twice and probably more often. It was found in the X_2-line N_3 and also in no less than 13 plants of the mass N_{43}. Since this mass is a mixture of lines with 16 or fewer seeds, the 13 mutants could belong to one line and represent one mutation, but this does not sound very probable.

In N_3 with 19 seeds and 18 plants there were 5 normal : 13 spoon-leaf. This would suggest a dominant mutation which has been overlooked in X_1. Of course this is possibly due to the abnormal growth of the plants in X_1; however, it is improbable, because the mutants in question are very conspicuous. Extensive studies in X_3 and X_4 did not confirm the dominant character of spoon-leaf, but nevertheless gave partly quite puzzling results. For the moment the most plausible explanation is that spoon-leaf is a monogenic recessive, but that the open flowers give rise to frequent spontaneous crossing which disturbs the results. Some years must elapse before this explanation will be tested in carefully isolated material. During the very abnormally hot and dry summer of 1959 all isolated flowers failed to set seed. We shall summarize the experimental data on the assumption that spontaneous crossing occurred among the open flowers of the spoon-leaf mutants.

Out of the 13 mutants from the mass, 12 yielded seed which was fall-sown as an X_3. Two X_3-lines with 3 plants each bred true, while the remaining 10 lines gave 100 normal : 203 spoon-leaf. These normals are supposed to be the result of spontaneous crossing.

The X_3 of N_3 yielded the following results:

5 normal X_2's segregated into 245 normal: 95 spoon-leaf, expected as (255) : (85) according to 3 : 1.

3 spoon-leaf X_2's bred true with 8, 6 and 30 plants respectively.

9 spoon-leaf X_2's "segregated" into 38 normal : 160 spoon-leaf, which resembles 1:3 according to (49) : (149). The 38 normals could also have arisen from spontaneous crossing.

Two X_3-lines were studied in X_4 as completely as possible. Both came from normal X_2's which had segregated in X_3 in normal : spoon-leaf as 58:30 and 56:27 respectively. The X_4-results of the former are summarized as follows:

12 normal X_3's bred true (1,084 plants);

44 normal X_3's segregated into:

	2,983 normal	: 1,003 spoon-leaf
exp.	(2,990)	:(996)

The ratio of 12 constant : 44 segregating is theoretically (19) : (37). The deviation is rather large, but tolerable, actual deviation divided by standard deviation being 2.0. Therefore, these X_4-data suggest monohybrid recessive inheritance of spoon-leaf. However, continuing the X_4-data, we have:

4 spoon-leaf X_3's bred true (55 plants);

25 spoon-leaf X_3's gave 245 normal : 1,100 spoon-leaf, the percentage of normal varying from 7%–35% in different lines.

Again, the most simple explanation is that the 245 normals are the result of spontaneous crossing.

The second X_4 was grown primarily because in its X_3 an acacia mutant appeared for the first time. This point will be discussed in 3.2. For the moment the data with regard to the spoon-leaf characteristic will be summarized:

18 normal X_3's bred true (1,050 plants);

32 normal X_3's segregated into:

	1,597 normal	: 543 spoon-leaf
exp.	(1,605)	:(535)

The ratio 18 constant : 32 segregating closely corresponds to the expected (17):(33) and the data as a whole point to monogenic recessive inheritance of spoon-leaf. Out of 23 spoon-leaf X_3's, 4 bred true with 134 plants and 19 gave 86 normal : 725 spoon-leaf. Again, the normals are supposed to be the result of spontaneous crossing.

Since in the X_4 just discussed acacia also segregated, some data could be collected to study the interrelation between spoon-leaf and acacia. Twenty normal-tendril X_3's segregated in X_4 for both characters as follows:

	normal tendril		normal acacia		spoon-leaf tendril		spoon-leaf acacia
	674	:	307	:	273	:	56
exp.	(709)	:	(272)	:	(238)	:	(91)

These data hint to linkage, of which the percentage of crossing-over would approach 38%. However, the material at hand is not suited to give definite proof, so that further confirmation is necessary.

The material as a whole certainly suggests monogenic recessive inheritance of spoon-leaf, with spontaneous crossing of the open flowers of spoon-leaf plants frequently disturbing the breeding true of spoon-leaf. However, further proof is needed and therefore no genetical symbol for spoon-leaf is proposed for the moment.

6. Uncoloured leaf-axil.– The original 'Dominant' has coloured leaf-axils. In N_{21} with 19 seeds and 18 plants, 3 plants with a colourless leaf-axil occurred. Since the flowers of these plants were purple like in 'Dominant', the mutation refers to the well known gene *d*. The X_2-ratio of 15 coloured : 3 uncoloured fits a 3:1-expectation of (14):(4).

In X_3 2 uncoloured X_2's bred true (200 plants). Out of 11 coloured X_2's:

6 bred true (499 plants);

5 segregated into:

237 coloured : 75 uncoloured
exp. (234) : (78)

The ratio 6 constant : 5 segregating is expected to be (4):(7). The X_3-data are in harmony with monogenic segregation.

7. Early flowering.– 'Dominant' is a very late flowering cultivar, forming its first flowers from the 16th node. In N_{12} – where according to point 4 dwarfs mutated – 2 plants were found which flowered considerably earlier than 'Dominant', the difference being estimated to be at least one week. Both plants yielded a sufficient number of seeds and could be grown into X_3. None of these X_3-lines segregated dwarfs. Observations of the flowering time in comparison with 'Dominant' yielded the following data:

'Dominant' flowered 16/6/59 – 22/6/59 = 7 days,
out of 16th node.

One X_3-line flowered 4/6/59 – 10/6/59 = 7 days,
out of 10th node (53 plants).

One X_3-line flowered 1/6/59 – 22/6/59 = 22 days,
out of several nodes averaging the 14th (81 plants).

Evidently the first X_3-line is constant for early flowering. The difference with 'Dominant' is 12 days and this is certainly considerable. It is also important from a practical point of view, since 'Dominant' is frequently used in crosses and the late flowering is a drawback. Probably 'Dominant' can be replaced by the new line.

The second X_3-line was not at all constant and this opposes the recessive nature of early flowering. Of course the data at hand are far too meagre to be of value for obtaining genetical insight in such a complicated character as flowering-time. However, the mutation in itself is clear enough and it is noteworthy that the two mutations, dwarf and early flowering, have occurred in one seed. Since in the X_3 the two mutants did not segregate simultaneously, their mutual relationship could not be studied.

3.2. *Mutations observed in X_3*

Two mutations have been observed *for the first time* in X_3. This has led to the concept that the radiation has directly induced a mutation of mutagenic genes, with as a consequence the indirect induction of mutation of other genes. Full details have been discussed in another publication (6), so that for the moment a summary will do.

1. Chlorina.– In the X_3 of N_{21}, according to point 6 in 3.1 studied for colourless leaf-axil, chlorina germ plants were observed which in X_4 behaved as recessives, however with a strong deficiency of chlorina, probably due to lethality of chlorina in an early embryonic stage. This is similar to the chlorina mutant as described in point 3 of 3.1, which mutant has arisen in another X_1, however. The mutations of the mutagenic gene for chlorina and of colourless leaf-axil have occurred in one and the same X_1.

2. Acacia.– The well known recessive characteristic of absence of leaf-tendrils, indicated as "acacia", was observed in the X_3 of N_3, studied for spoon-leaf (see point 5 in 3.1). In X_4 it behaved as a simple recessive characteristic. In this case the mutations of the mutagenic gene for acacia and of spoon-leaf have occurred in one and the same X_1.

4. Discussion

The mutants which were described in the foregoing, are: bushy, emerald, chlorina, dwarf, spoon-leaf, uncoloured leaf-axil, early flowering, mutagenic gene for chlorina, mutagenic gene for acacia. Most of these characteristics are already known in peas. This is doubtful for bushy and for dwarf, although they resemble characteristics described by LAMPRECHT (3). Our chlorina differs from LAMPRECHT's in being lethal. Spoon-leaf is a completely new characteristic, hitherto unknown in peas. The same holds true for the possible mutagenic genes.

From the breeder's standpoint some of the mutants are decidedly undesirable. Bushy might have been valuable, if it had been maintained. Early flowering is certainly very valuable. Considering that the primary object of the investigations was a first orientation, the results are promising. Anyhow it is quite easy to obtain a number of mutants and of course the chance to find valuable mutants increases when the work is done on a larger scale.

It is impossible to indicate the percentage of mutations accurately, but considering that among 42 X_2-lines 7 mutants were found, a minimum percentage of about 17 can be accepted.

Some observations of general interest are that spoon-leaf has occurred more than

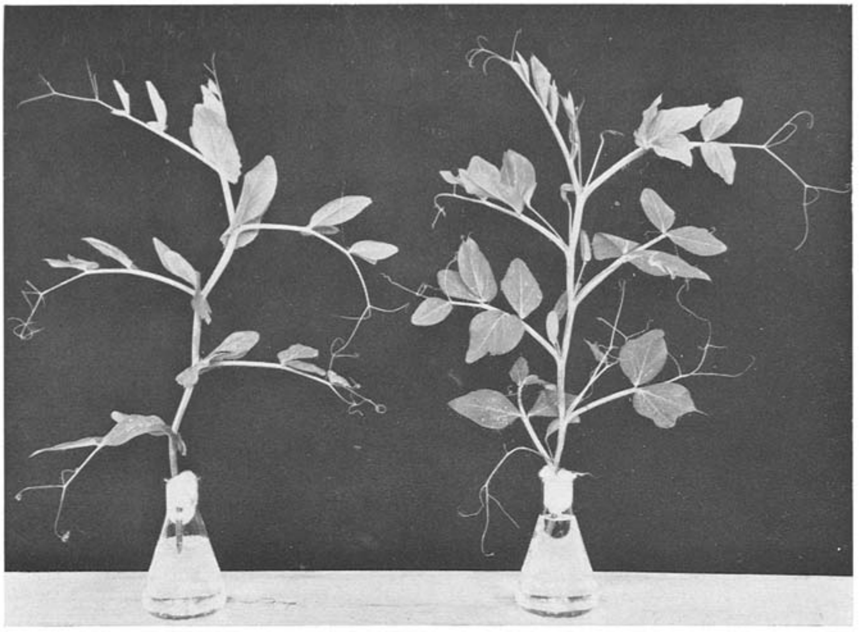

FIG. 1. THE LEFT PLANT REPRESENTS THE ORIGINAL CULTIVAR 'DOMINANT' WITH BROAD AND SESSII STIPULES. THE RIGHT PLANT IS A "SPOON-LEAF" MUTANT WITH ELLIPTICAL PETIOLATE STIPULES

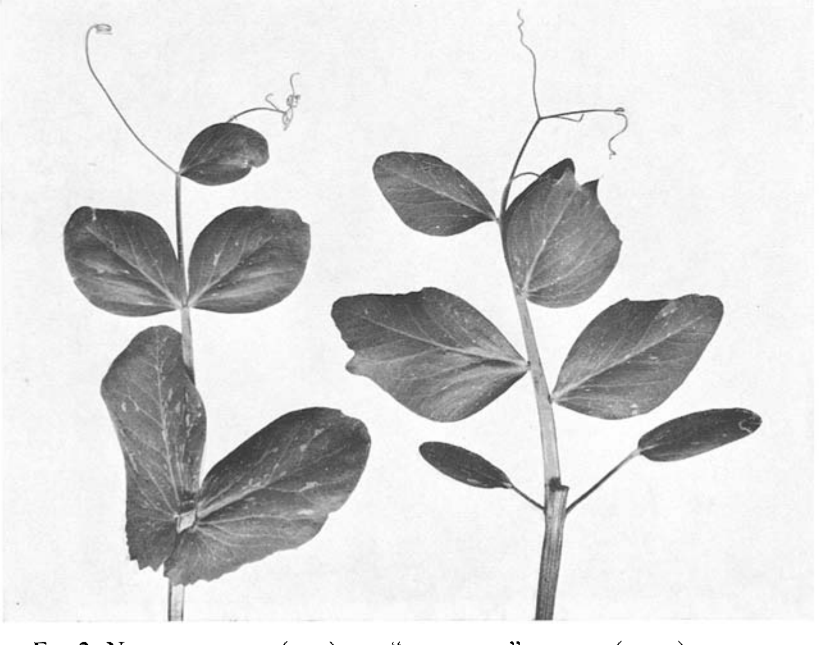

FIG. 2. NORMAL STIPULES (LEFT) AND "SPOON-LEAF" STIPULES (RIGHT) IN DETAIL

once, while in no less than 3 cases 2 mutations occurred in one seed, namely dwarf and early flowering, spoon-leaf and mutagenic gene for acacia, uncoloured leaf-axil and mutagenic gene for chlorina.

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SAMENVATTING

Neutronenmutaties bij erwten

Teneinde ervaring op te doen over de waarde van neutronen voor het verkrijgen van mutanten, werden droge zaden van erwten bestraald met een intensiteit van 5.1 × × 10^8 n/cm^2/sec; een bestralingsduur van slechts $\frac{1}{2}$ uur bleek toelaatbaar. In X_2 werden de navolgende mutanten gevonden, waarschijnlijk alle recessief:

1. "Bossig", zeer sterk vertakkende planten.
2. Wasloos, zonder een wasovertrek op stengel en blad.
3. Chlorina, gele kiemplanten, die spoedig afsterven.
4. Dwergen, uiterst kleine planten, die zonder speciale behandeling geen zaad zetten; na toediening van gibberellazuur strekte de stengel en had zaadzetting plaats.
5. "Lepelblad", steunbladen die smal elliptisch en gesteeld zijn, gelijkend op een lepel; steeds gepaard met "open bloemen", waarin stamper en meeldraden vrij liggen van de kiel.
6. Ongekleurde bladoksel.
7. Vroege bloei, 12 dagen eerder dan het uitgangsras, uit de 10e stengelknoop in plaats van uit de 16e.

Twee mutanten, chlorina (in ander materiaal dan sub 3) en acaciablad, werden pas in de X_3 voor het eerst gevonden. Verondersteld is, dat zij ontstaan zijn na mutatie van "mutagene genen".

Sommige der mutanten zijn zeker waardevol vanuit een veredelingsstandpunt en als eerste oriëntatie zijn de resultaten hoopvol. Eén mutatie is vaker dan eens opgetreden. Driemaal hebben 2 mutaties in 1 zaad plaats gehad.

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