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Quantitative and Qualitative Investigations on the Seed Proteins of Mutants and Recombinants of *Pisum sativum**

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Summary. The proteins of the seed flour of 26 X-ray induced mutants and 5 recombinants of the species *Pisum sativum* were analysed quantitatively and qualitatively. The values obtained were related to the seed yield of the genotypes In this way, the protein yield as well as the production of specific amino acids per genotype were determined. The following results were obtained.

1. The mean values for the character "total protein content of the seed flour" of the genotypes studied varied between 14 and 23%. The fasciated mutant 489C produced 18–23% more seed proteins than the initial line in three subsequent generations. The mean values of some other mutants and recombinants were 10–17% higher, the lowest value being 27% lower than that of the control values. In the material investigated there is no correlation between seed size and seed protein content.

2. The buffer-soluble seed proteins of 55 mutants were electrophoretically subdivided in different subfractions and the protein patterns were determined. They are extraordinarily variable. Distinct groups of mutants do not only differ from the initial line but also from one another with regard to the number, position and breadth of their bands. A correlation between the degree of morphological deviations of specific genotypes and the composition of their seed proteins was not observed. Moreover, differences in the concentration of specific protein fractions between the genotypes were densitometrically ascertained.

3. The globulins and albumins of some genotypes were quantitatively determined and electrophoretically subdivided into subfractions which differ between different mutants with regard to their number as well as their concentration. This is especially valid for the albumins which could be essentially stronger subdivided than the globulins.

4. The amino acid spectra of all the mutants investigated agree qualitatively with the spectrum of the initial line, however, clear quantitative differences in distinct amino acids were observed. The proportion of the essential amino acids is increased in two mutants by 5 and 20% in relation to the control line.

5. The protein production of the fasciated mutant 489C was 20–70% higher than the corresponding values of the initial line in three subsequent generations regarding its high seed production. The protein yield of the early flowering mutant 46C was about 20% lower and that of the bifurcated mutant 1201A 12–31% higher as related to the initial line. Mutant 1201A shows an equally favourable situation with regard to the total content of the essential amino acids. Its lysine production exceeded the control values by 20–40%.

6. The different components of the protein synthesis can negatively be influenced by the co-operation of the mutant genes. The protein production per plant, the total production of essential amino acids as well as the lysine production are lower in the recombinants 68C/176A and 68C/1201A as compared to the parental mutants. The combination of genes 68C and 46C does not show any negative interactions.

Introduction

In modern plant breeding, biochemical methods are increasingly being utilized for determining the breeding value of species, varieties and strains. Besides carbohydrates, fats, oils, pharmacological ingredients and minor components present in the seeds, proteins represent an important constituent as regards their use in animal and human nutrition (Altschul 1965, World Health Organisation 1965). Only relatively few details are known so far on the properties of "protein mutants" and their utilization in mutation breeding. The earliest investigations have been carried out in maize (Nelson *et al.* 1965, Nelson and Mertz 1973, Mertz *et al.* 1964, 1965). Further results in this field obtained in different crops are summarized in the proceedings of three symposia organized by the International Atomic Energy Agency (1969, 1970, 1973). Methodically it is not yet possible to select protein mutants directly. The only way to utilize genotypes of this kind consists in analysing the mutants already available biochemically in order to obtain data on the quantitative and qualitative situation of their seed proteins. This has been done in *Zea* (Mossé *et al.* 1966, a, b), *Oryza* (Tanaka 1969), *Hordeum* and *Triticum* (Favret *et al.* 1969, Gustafsson 1969) among others. But also mutants having an unfavourable protein make-up are of interest because they can be used for studying the genetic basis of protein production and protein composition (Boulter *et al.* 1967, Hynes 1968, Adriaanse

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et al. 1969, Gottschalk and Müller 1970, Müller 1973, Müller and Gottschalk 1973). Moreover, it is possible to study the co-operation of mutant genes in this context by comparing the biochemical situation of recombinants and thei parental mutants.

In the present paper an account of the relations between seed protein content and seed yield of a group of mutants and recombinants of *Pisum sativum* were analysed quantitatively as well as qualitatively. Also, an amino acid analysis of the proteins was made in order to study the action of the mutated genes on the composition of the seed proteins.

Materials and Methods

1. The Mutants

For the genetic and biochemical investigations 20 X-ray-induced mutants and 6 recombinants of the variety 'Dippes gelbe Victoria' of *Pisum sativum* were used. The following genotypes are of particular interest because of their useful breeding characters:

mutant 46C: early flowering and ripening;
mutant 68C: increased number of ovules per ovary;
mutant 176 A: small leaflets, stipules, flowers, pods and seeds;
mutant 251A: apical part of the stem relaxed fasciated, flowers and pods distributed over a relatively long part of the stem;
mutant 489C: apical part of the stem strongly fasciated, all the flowers and pods clustered at the top of the plant (the two genes for stem fasciation belong to a series of multiple alleles);
mutant 1000: large seeds;
mutant 1201A: dichotomous stem bifurcation, reduced penetrance;
recombinant R 176X: narrow leaflets, stem fasciation, late flowering (from a spontaneous hybridization of mutants 176A × × 489C);
recombinant R 177: stem bifurcation, stabilized penetrance (selected in an F_2-generation 489C × 1201A);
recombinant R 300: homozygous for genes 68C and 46C;
recombinant R 320: homozygous for genes 68C and 176A;
recombinant R 350: homozygous for genes 68C and 1201A;

The seed yields of these genotypes are graphically illustrated in fig. 1. The small-grained mutants 251A and 489C show an extraordinarily high seed production as a consequence of the stem fasciation. The seed production of the recombinants is in general less than that of the parental mutants. Moreover, the yielding capacity of recombinants R 176X and R 177 due to an extremely small size of their seeds is negatively influenced.

2. Biochemical Methods

The total nitrogen content of the seed meal (dry weight basis) was determined by the Kjeldahl method. The protein content was calculated by multiplying the nitrogen value by 6.25 (Keil and Sormova 1965). The soluble proteins were determined by the method described by Lowry et al. (1951).

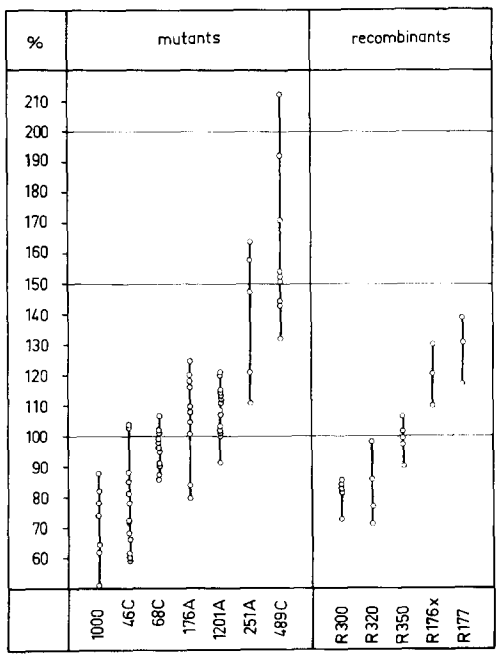

Fig. 1. The seed production of some pea mutants and recombinants in subsequent generations. Each point represents the mean for the character "number of seeds per plant" as percentage of the corresponding value of the initial line

The albumins and globulins were extracted from the seed meal according to Danielsson (1949, 1956). The protein content was determined by the Folin test (Lowry *et al.* 1951).

The analysis of the globulin fractions was carried out on gels containing 7.5% acrylamide according to Bloemendal (1967) and Maurer (1968). The albumins were analysed using the method of Loeschke and Stegemann (1966). The destained gels were compared, the R_f-values determined and the banding pattern schematically drawn. Furthermore, the gels were scanned densitometrically using the Chromoscan (Joyce-Loebl). The gel patterns of the different genotypes were compared with one another on the basis of optical density curves (Maurer 1968). For comparison, they were evaluated by measuring the area of the peaks (Müller 1970).

Samples of seed meal were hydrolysed according to Barton-Wright (1952), Keil and Sormova (1965) and Horn *et al.* (1947, 1955). The hydrolysates were analysed in an amino acid analyser (Aminolyzer, Optica) according to the procedures given by Mondino (1967, 1969a, b).

Results

The amount of the seed proteins as well as their qualitative composition is an important component for the yield of many cultivated plants. Their analysis can be carried out in two different ways. One way is to use equal amounts of seed flour of different mutants for determining the protein content and the proportion of specific amino acids. The advantage of this method lies in its possibility to compare a large number of different genotypes with one another, but the other components of yield cannot be considered in this way. The other possible approach is to combine this method with an estimation of factors such

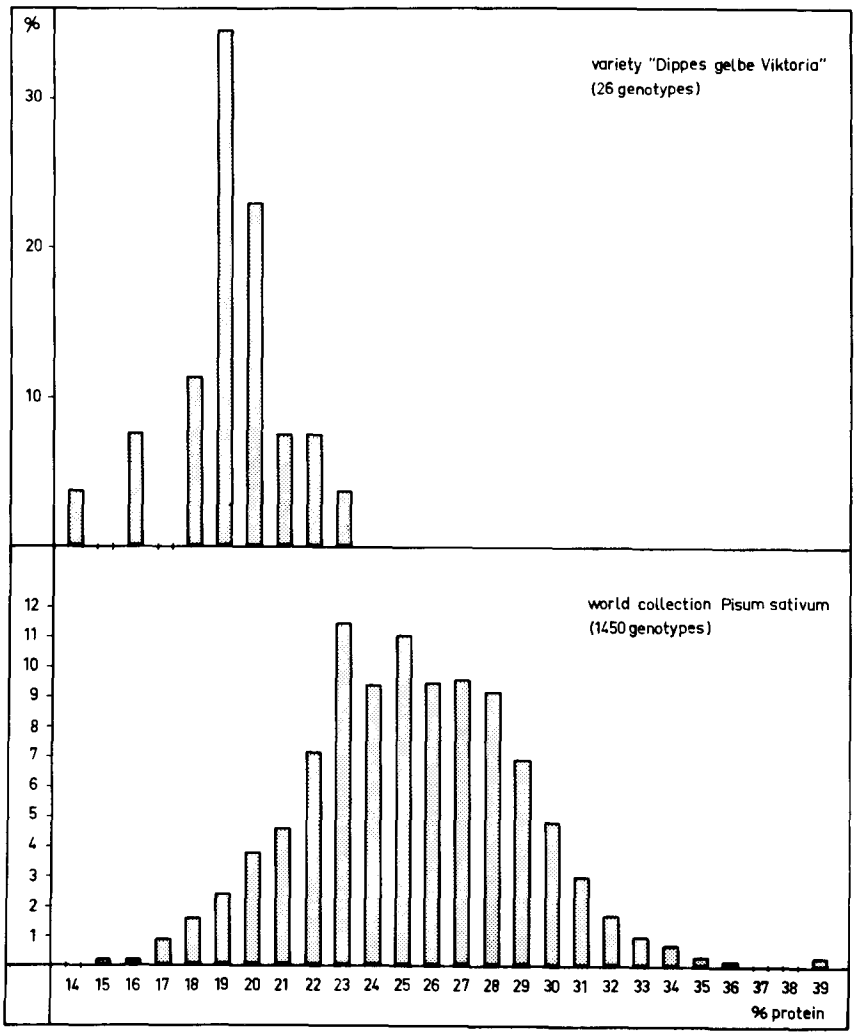

Fig. 2. The frequency distribution of the protein content of 1450 genotypes of the world collection of *Pisum sativum* (below) in comparison to 26 mutants and recombinants of our assortment (above).
Horizontal axis: Total protein content in percent of the dry weight of the seeds.
Vertical axis: Proportion of the genotypes in the different protein classes

as the number of seeds per plant and the seed size which take part in seed production of the plant in order to calculate seed weight per plant. This value can be related to the protein and amino acid values. In this way, mean values for the protein production per plant as well as for the total production of distinct amino acids can be obtained. The yielding components mentioned above vary considerably in different mutants in subsequent generations and thus protein production is deviated positively as well as negatively. The present work gives an account of the results got after utilizing both the above given methods for a group of mutants and recombinants of *Pisum sativum*.

1. *The Quantitative and Qualitative Analysis of the Proteins of the Seed Flour*

a) The Total Seed Protein Content. The legumes because of their high protein content are of great economical importance especially in countries with widespread vegetarian diet. The total protein content of the seeds of most cereals varies between 9 and 13 precent of the dry weight. In contrast to this, in legumes like *Glycine max* and *Arachis hypogaea* the values reach up to 45 percent and are comparable to some protein-rich oil crops such as *Sesamum, Linum, Gossypium, Helianthus* (see Altman and Dittmer 1968). The garden pea occupies an intermediate position in this respect by showing a variation from 14—39 percent. Majority of the cultivars and strains of the world collection tested so far have a total protein content of 22—29 percent. The distribution of the character "total seed protein content" is graphically presented in the lower part of figure 2. The diagram is based upon the mean values of 1450 different genotypes of the world collection of *Pisum sativum* without consideration of mutants (Slinkard 1972).

The initial line 'Dippes gelbe Viktoria' of *Pisum sativum* used for our radiation genetic experiments has a total protein content varying between 19 and 22% and occupies the lower protein range in the world collection. Precise comparison of our mutants and recombinants with the existing cultivars and strains of the world collection is difficult because only

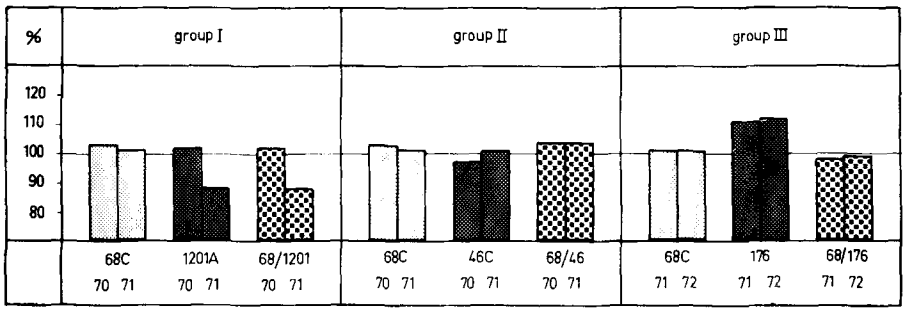

Fig. 3. The total protein content in the seed flour of *Pisum mutants* and recombinants of three different hybrid combinations as related to the values of the initial line = 100% in two subsequent generations. In each combination the recombinant is compared with its two parental mutants

26 genotypes of our assortment have been analysed so far. If we relate this relatively small number of findings to the corresponding data of world collection (upper part of fig. 2), the balance appears to be negative. However, considering the relatively bad biochemical situation of the initial line a considerable variation of the mean values of the different genotypes is evident. This shows that the amount of seed proteins can considerably be altered in positive or negative direction under the influence of a single gene or a small number of genes.

The most protein-rich genotype of our assortment is the fasciated mutant 489C that produces 18—23% more total protein as compared with the initial line related to equal amounts of seed flour. In this value, the high seed production of the mutant is not yet considered (see section 2 of the present paper). Some other mutants and recombinants show likewise an increase of the protein content by 10—17%. The lowest value of 73.4% of the control value was found in the recombinant R177 having a dichotomous stem bifurcation in combination with a high penetrance of one of the two genes involved.

A comparison of some recombinants with the respective parental mutants is of particular interest with regard to the co-operation of mutated genes as far as the protein synthesis is concerned. The results of three different hybrid combinations are represented in fig. 3. Mutant 68C was one of the parents in all the three crossings; mutants 1201A (stem bifurcation), 46C (earliness) and 176A (small grains) were used as the second partner.

The seed protein content of mutant 68C is similar to that of the initial line while mutant 1201A showed varying values in 1970 and 1971 (fig. 3, left-hand part). The recombinant R 350 homozygous for genes 68C and 1201A shows in both these growing seasons the protein values equal to that of mutant 1201A. Thus, in 1971 the full protein content of mutant 68C was not reached in the recombinant. From these findings certain negative interactions between the two mutated genes become discernible. As this situation was only realised in one of the two years of study it can be assumed, that the gene interaction is vulnerable to some environmental factors. Further work in this direction is in progress. The same situation is valid for hybrid group III (fig. 3, righthand part). Also in this case the high protein content of the small-grained mutant 176A is not reached by the recombinant R 320 (= 68C/176A). The protein level of this recombinant is likewise similar to that of the parental mutant which has a low protein content. Group II contains two mutants of equal protein content. The values of the recombinants lie in the same range; thus, the genes involved do not show any positive or negative interaction with regard to the character "total content of the seed proteins".

In cereals a negative correlation between seed size and protein content is known since long; the smaller the seeds, the higher their total protein content. This correlation could not be confirmed in our material. The distribution of the mean values of 23 mutants and recombinants seems to suggest a certain trend in this direction. However, it was not possible to support this assumption statistically by means of the regression analysis separately for mutants, recombinants and the total material. Thus, it can be concluded, that the selection of small-grained pea mutants does not result in a selection of protein-rich genotypes. Also, there are no statistically significant correlations between seed size and protein content. A particularly clear example in this respect is the recombinant R 177 mentioned above. It is one of the most small-grained genotypes of our assortment

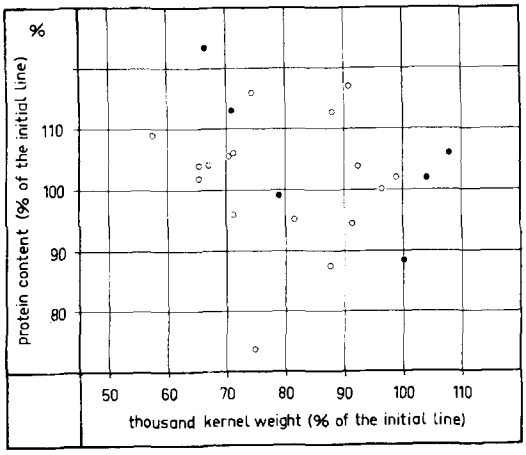

Fig. 4. The relations between thousand grain weight and total protein content of the seeds of 23 mutants and recombinants of *Pisum sativum*

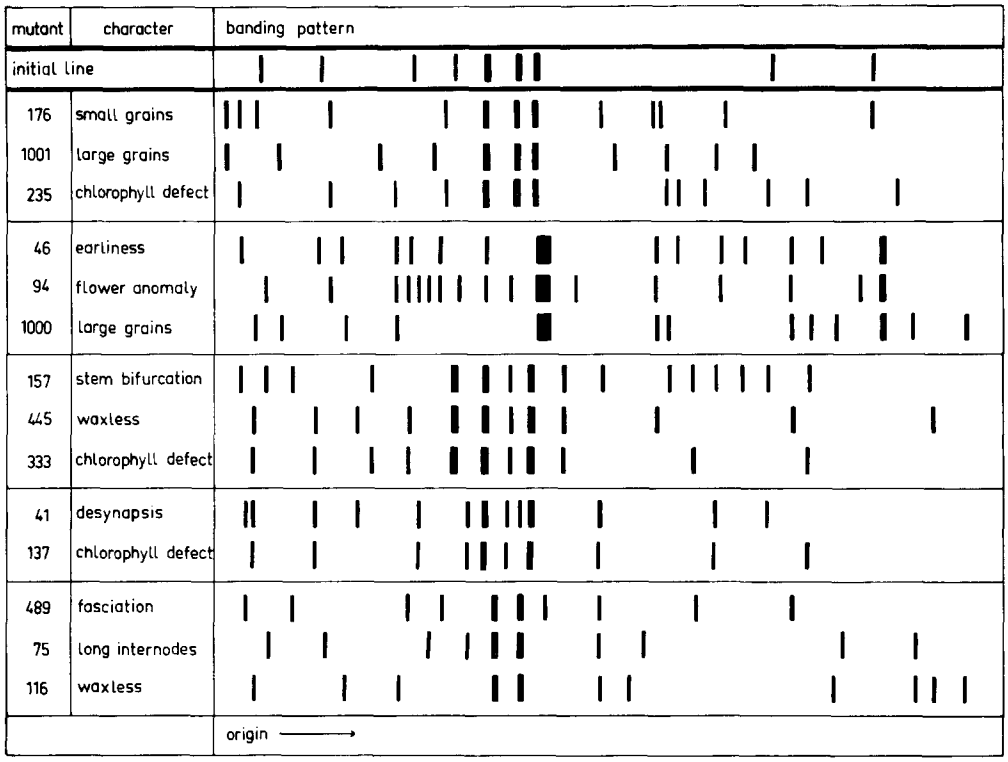

Fig. 5. The banding patterns of the soluble seed proteins of some pea mutants in comparison to the initial line

(thousand kernel weight = 75.2% of the initial line), but it shows the lowest protein content of all genotypes studied so far (73.4% of the initial line). On the other hand, the large-grained mutant No. 1000 exceeds the control values only by 6%.

b) The Protein Patterns. Using the acrylamide-gel-electrophoresis it is possible to separate the soluble seed proteins in a series of distinct fractions. After treatment with specific dyes they become discernible in the gel in a specific distance from the starting point. In this way, a protein pattern occurs which is specific for each genotype with a high degree of reproducibility.

So far, 55 mutants of our assortment have been analysed in this respect. As shown in fig. 5, an extraordinarily broad diversity of the protein patterns was obtained. Using this method, the total number of bands varied between 12 and 18 in the mutants studied. In some strikingly broad bands further separation using a more sophisticated method seems to be possible. Using standardized conditions, 55 different protein patterns were obtained for the 55 different mutants. 14 of them are schematically illustrated in fig. 5.

Let us compare mutant 94 and the initial line as an example for the diversity of two genotypes. The mutant has eight bands more; furthermore, it is characterized by a particularly broad band that in the control material exists in the form of a narrow streak (fig. 5, group II). Moreover, rapidly moving fractions occurring in the mutant are absent in the initial line (righthand part of the figure). Thus, it is evident that the seed proteins of mutant 94 are partly composed of certain different subfractions than those of the initial line. A single gene pair is responsible for these differences. A similar situation was observed for many other mutants analysed earlier (Gottschalk and Müller 1970). Interestingly, this is also valid for typical "micromutations" that can be identified in segregating families only with great difficulties. Also with regard to their seed production they are in many cases comparable with the initial line. Thus, there are no correlations between the degree of the morphological deviations and the composition of the seed proteins. For further details concerning the differences of the protein patterns see fig. 5.

The findings just mentioned refer to the qualitative situation of the seed proteins. Furthermore, a quantitative evaluation was carried out by a direct densitometric scanning of the gels. From the intensity of the staining of the various protein fractions certain conclusions concerning the respective quantities can be drawn. Thus, the densitometrically scanned absorption curves can be used for calculating the quantitative situation. In fig. 6 the absorption curves of two mutants are compared with that of the initial line. Strong differences between the three genotypes are particularly discernible in the lefthand part of the

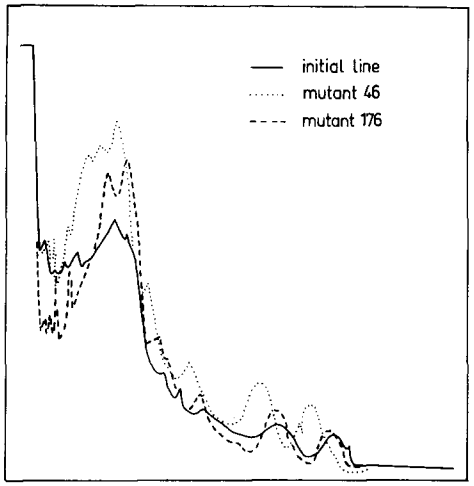

Fig. 6. The direct densitometric evaluation of the protein bands of the initial line and of mutants 46C and 176A

curves, i.e. in the pattern of the slowly moving proteins. The curves show, that proteins with similar moving speed can be present in differing amounts depending on the presence of mutant genes. Analogous findings have already been obtained with regard to other mutants of our collection earlier (see Gottschalk and Müller 1970).

c) The Protein Fractions. The seed proteins of the *Leguminosae* consist mainly of the salt-soluble globulins and the water-soluble albumins as the main fractions. 75% of the total protein of our initial line are globulins. They represent the most essential reserve proteins of the plant which are mobilized during seed germination and are responsible for the maintainance of the earliest stages of the ontogenetic development. The albumins are the enzymatically active seed proteins mobilizing the globulins. Both the fractions can be electrophoretically subdivided into their subfractions.

An evaluation of the globulins of some mutants and recombinants has shown that the genotypes differ from one another and from the initial line with regard to the number of subfractions as well as their concentration. Considering the genotypes analysed eight different subfractions could be found according to their position at the gels. Their number and concentration vary in different genotypes. Some examples are given in fig. 7. The initial line shows 4 bands of distinguishing breadth and migration velocity at the gel. They are given in figure 7 according to their distance from the starting point. The height of the columns is a measure for the concentration of the respective fractions and has been obtained by scanning the gels. The area of the various peaks was planimetrically determined and was related to the total area of all the peaks present (= 100%).

If we compare the fasciated mutant 251A with the initial line there is a concordance of the two genotypes with regard to the number of the globulin subfractions, but fraction no. 4 is present in mutant 251A in an essentially lower concentration. The opposite relation is valid for fraction no. 1 while fractions no. 6 and 7 differ only slightly in the two genotypes. The recombinant R 177 belongs to the same group. The second group of genotypes (fig. 7, middle part) shows only three

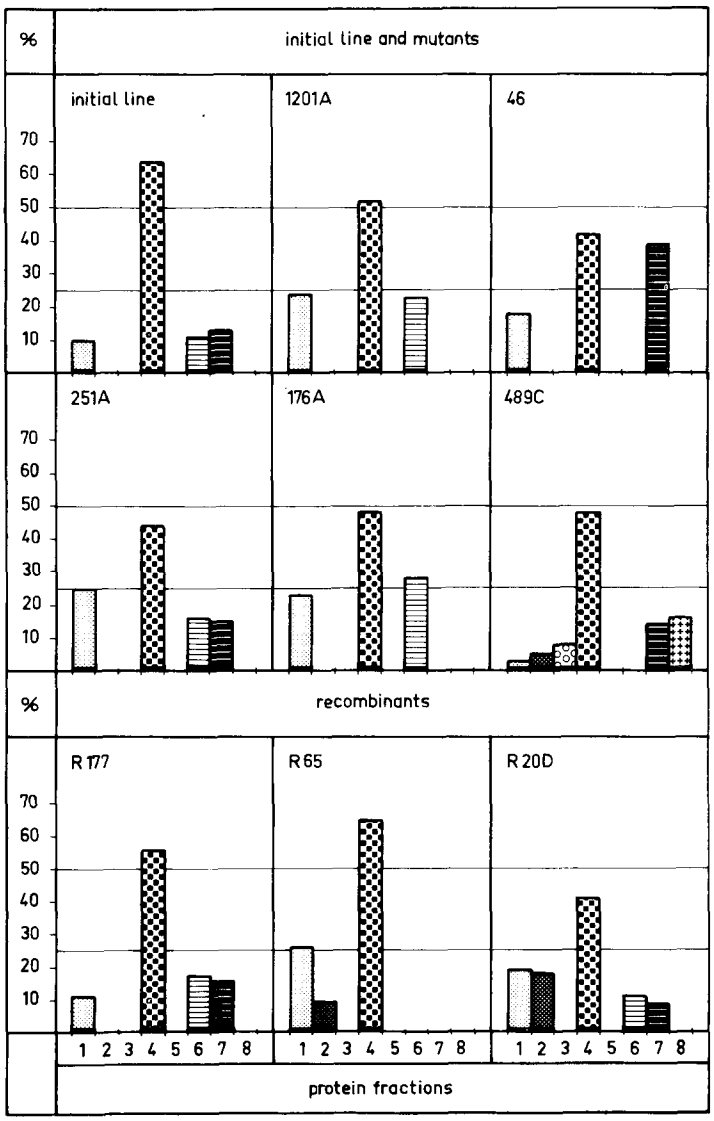

Fig. 7. The electrophoretically obtained globulin subfractions of five mutants and three recombinants of *Pisum sativum* in comparison to the initial line. The height of the columns were obtained by a densitometric evaluation of the bands. The sequence of the columns corresponds to the position of the bands in the gels

subfractions which nearly agree in mutants no. 1201A and 176A (subfractions no. 1, 4, 6). In the recombinant R 65, however, the subfraction no. 6 is absent, but the subfraction no. 2 is present which could not be found in the other genotypes analysed so far. The early flowering mutant 46C with its subfractions 1, 4, 7 belongs likewise to this group. The fasciated mutant 489C having 6 globulin subfractions with a characteristic distribution is of particular interest. Subfraction no. 8 of this mutant could not be found in the other genotypes studied. The comparison of the 5 mutants and 3 recombinants of figure 7 shows clearly that the globulins in legumes do not consist of only two subfractions as described in the literature (Danielsson 1949, 1956). Furthermore, the figure demonstrates strong genetically conditioned variations of the globulin patterns.

The subfractions of the albumins can be stronger separated from one another using the disc gel electrophoresis. 35 bands were found by means of staining with Coomassie Blue. Also in this respect, clear quantitative differences of specific subfractions were stated between different mutants of our assortment. Detailed investigations are in progress; the results will be published elsewhere.

The co-operation of some genes has been tested with regard to the quantitative distribution of the globulins and albumins by comparing the recombinants with their parental mutants. The data of two hybrid groups available so far show a diverging behaviour (fig. 8). Let us at first consider group II (crossing 68C × 46C). The globulin content of mutant 68C lies only slightly, the albumin content considerably below the values of the initial line. Mutant 46C possesses higher amounts of both these fractions. The recombinant R 300 (= 68C/46C) corresponds to mutant 46C with regard to the globulin as well as the albumin production. Thus, gene 46C exerts its full action on the globulin and albumin production even when gene 68C is present in the genome. Hence, the negative influence of gene 68C on the two protein fractions is ineffective in the recombinant 68C/46C.

The situation is more complicated in the hybrid group I (68C × 1201A; fig. 8). The globulin production is clearly reduced under the combined action of both the genes; the value for recombinant R 350 (= 68C/1201A) is only 82% of the corresponding mean of the initial line while the values of the parental mutants are 93 or 102%, respectively. From these findings an interaction between the two mutant genes influencing the globulin production negatively becomes obvious. The albumins, however, are in a different way influenced by the combined action of the two genes. The albumin content of the recombinant R 350 (= 68C/1201A) lies considerably below of that of mutant 1201A, but it is higher than that of 68C. Thus, the negative effect of gene 68C gets reduced by the presence of gene 1201A.

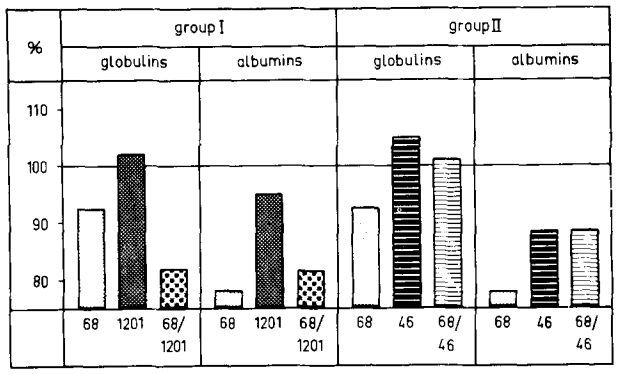

Fig. 8. The quantities of globulins and albumins of two hybrid combinations as related to the control values of the initial line = 100%. The values of the recombinants are in both the groups compared with those of the parental mutants

d) The Amino Acid Composition of the Proteins. The proteins of the legumes consist of 20—25 amino acids, 16 of them can be quantitatively determined by means of an amino acid analyser using acid hydrolysates. As expected, no qualitative differences between different genotypes could be found considering the analytical data of the initial line, four mutants and some recombinants. All the amino acids are present in their seed proteins, but there are clear genetically conditioned quantitative differences with regard to specific amino acids.

The amino acid spectrum of the initial line and of mutants 1201A, 68C, 46C and 176A is graphically illustrated in figures 9 and 10 separately for non-essential and essential amino acids. The amino acids are arranged in the same order in which they were registered by the amino acid analyser. The figure

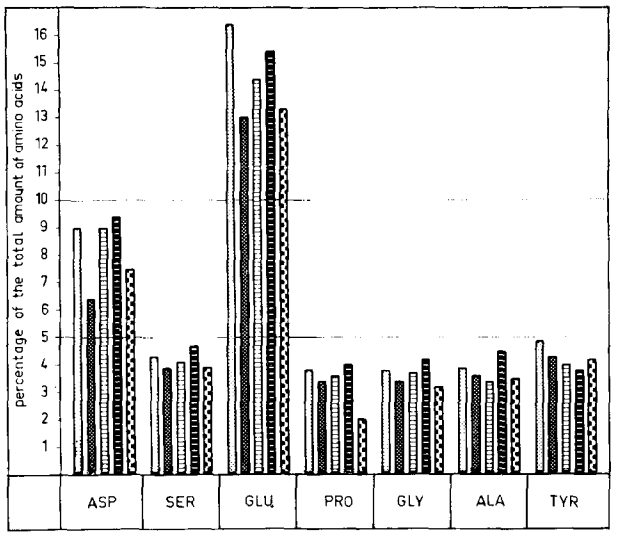

Fig. 9. The percentage of the non-essential amino acids as related to the total amount of the amino acids in the initial line and in four *Pisum* mutants.
Column 1: initial line; Column 2: mutant 1201A; Column 3: mutant 68C; Column 4: mutant 46C; Column 5: mutant 176A

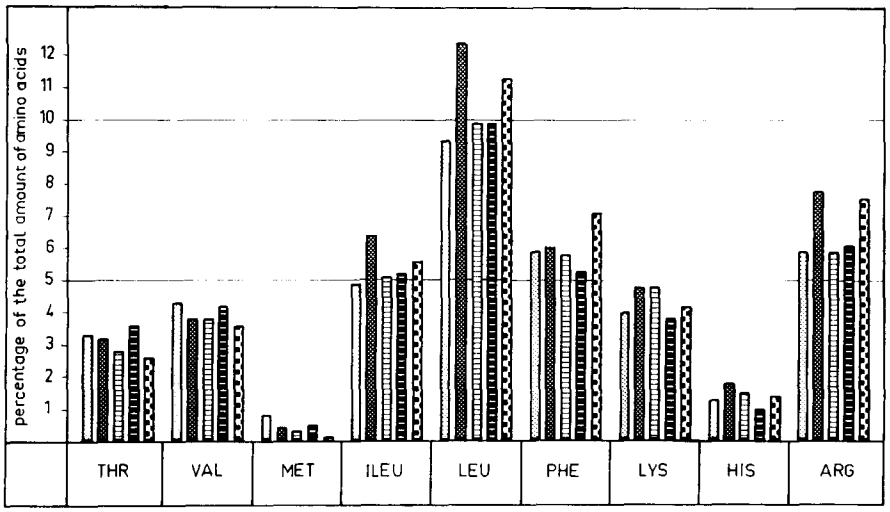

Fig. 10. The percentage of the essential amino acids as related to the total amount of the amino acids in the initial line and in four *Pisum* mutants (arrangement as in figure 9)

shows the quantitative situation of the various components of the total hydrolysate which is more or less similar in all the genotypes studied. Glutamic acid occupies the highest proportion of the total amount of the amino acids with 13—16 percent while the proportion of methionine is very low reaching a mean of nearly one percent only. With regard to the problem discussed in the present paper the genetically conditioned differences between the various mutants are of particular interest. They are low for most of the amino acids; however, clear deviations could be found in single cases. Mutants 1201A and 176A, for instance have higher leucine and arginine than that in the other three genotypes but the amounts of glutamic and aspartic acid are reduced in both of these mutants. The small-grained mutant 176A shows considerable deviations with regard to specific amino acids as compared with the other genotypes studied; phenylalanine is present in an increased and proline in a reduced amount. A corresponding quantitative variability was likewise observed for the other mutants analysed presently (fig. 9 and 10).

The protein quality depends on the proportion of the essential amino acids constituting the seed proteins. Therefore, the influence of some mutant genes on the production of this group of substances should be specifically discussed considering again the co-operation of the genes involved. In the upper part of fig. 11 the total content of all the essential amino acids of the three hybrid combinations mentioned in the preceding sections is illustrated. Mutant 68C does not differ in this respect from the initial line. Mutant 46C produced four percent less, mutants 1201A and 176A five and twenty percent more essential amino acids, respectively. All these values refer for equal amounts of seed meal while the seed yield of the respective genotypes is not yet considered.

The co-operation of gene 68C with genes 1201A, 46C and 176A leads to different results. The recombinant R 350 (= 68C/1201A) shows a similar production of essential amino acids as compared to mutant 1201A. Thus, there is no negative interaction between genes 68C and 1201A in this recombinant. The combination of genes 68C and 46C results in an even more favourable situation because the recombinant R 300 (= 68C/46C) exceeds both the parents by 25

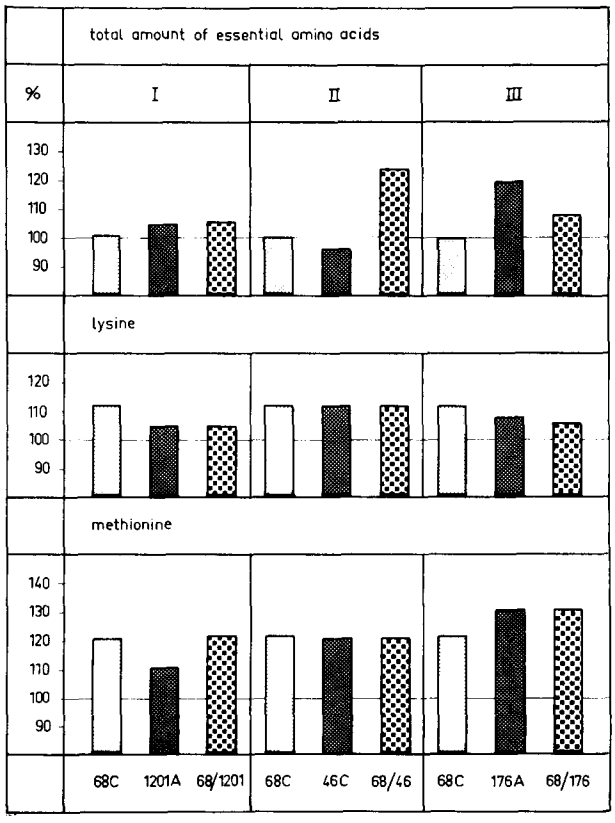

Fig. 11. Comparison of the total amount of the essential amino acids and the lysine and methionine content in three different hybrid combinations. All the values are related to the initial line; the recombinants are again compared with the respective parental mutants

to 30%. Therefore, its seeds should be valuable especially as far as its nutritional status is concerned. In the third hybrid combination (68C × 176A) a compromise is reached between the two genes involved for the total content of essential amino acids lies between corresponding values of both the parental mutants.

Finally, the co-operation of the genes just mentioned with regard to two specific amino acids will be described. We have chosen methionine as the limiting essential amino acid of the proteins of the legumes and lysine because of its high nutritive importance. The quantitative situation of both these amino acids in the mutants and recombinants studied is graphically illustrated in the middle and lower part of fig. 11. The simplest situation is realised in the hybrid combination II (68C × 46C). Both the mutants are producing both these amino acids in essentially higher quantities as compared with the initial line. Thus, they can be considered positive as far as their nutritional value is concerned. The combination of both the genes in the same plant organism has neither positive nor negative consequences. In hybrid combination I (68C × 1201A), however, the lysine production of the recombinant is similar to that of the low parental mutant (1201A). Thus, the favourable conditions of mutant 68C do not become effective in this combination. The same situation is realised in hybrid combination III (68C × 176A). In contrast to the lysine behaviour the methionine production shows a more uniform behaviour. In the recombinants R 350 (68C0 1201A) and R320 (68C/176A) that gene dominates which is responsible for the formation of the higher methionine amount.

2. *The Relations between Seed Yield and Protein Production*

All the data presented in the preceding sections on proteins, protein fractions and amino acids are related to equal amounts of seed flour irrespective of the seed production of the genotypes studied. From a breeder's standpoint, however, the seed protein content should be related to the seed yield of the genotypes. Using the criteria "number of seeds per plant" and "seed size" (thousand grain weight) the mean for the character "seed weight per plant" can be calculated. If we relate this value to the protein content of the seeds the protein production per plant can be estimated.

Let us demonstrate the relations between the criteria just mentioned using a clear example. The highest yielding genotype of our assortment is the fasciated mutant 489C. Considering 9 generations the mean values for the character "number of seeds per plant" varied between 132 and 212 percent of the corresponding means of the initial line. Fig. 12 gives the mean values for the years 1969—1971, these vary between 152 and 212% of the control values. The seeds are very small, but the seed yield of the mutant is higher than that of the initial line in majority of the years. This is because of the high seed number per plant of the mutant. The seed protein content is 20 to 25% higher resulting in 20—70% of increased protein production per plant over that of the initial line. A second fasciated mutant of our collection shows a similarly favourable situation (Müller and Gottschalk 1973). The nutritive value of these seeds tested by Eggum in Denmark through rats feeding technique indicated no differences between mutant and initial line. Thus, the increased protein production is in this case really equivalent with an increase of biologically utilizable protein.

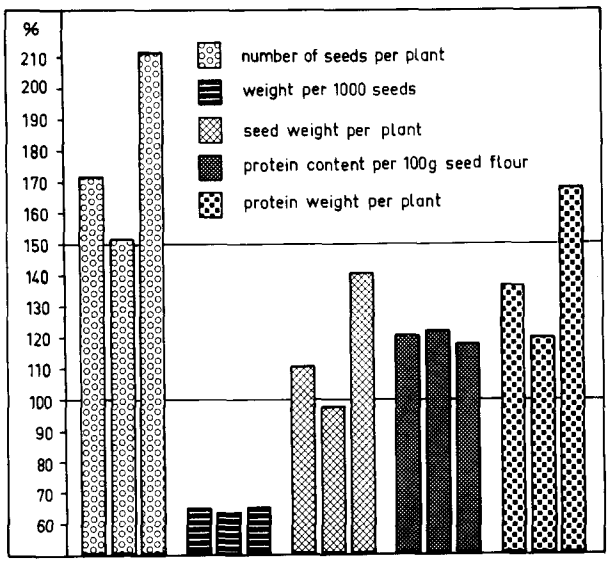

Fig. 12. Comparison of different criteria of the protein yield of mutant 489C in three subsequent generations

Let us now discuss the correlations between seed production and different criteria of the protein production in those mutants and recombinants already described in the preceeding sections. From the protein content of the seed flour and the seed production of the plant the total amount of the seed proteins per plant was calculated. Moreover, the total amount of seven essential amino acids was commonly considered (valine, leucine, isoleucine, phenylalanine, threonine, methionine and lysine); methionine and lysine were separately evaluated. The mean values for all these criteria are graphically illustrated in figure 13 considering three mutants and two recombinants in two subsequent growth seasons. All the means are related to the corresponding values of the initial line.

Let us first regard the total protein production of the five genotypes. As expected, the values of the initial line for the characters "number of seeds per plant", "seed size" and "protein content of the seed flour" do not fully resemble in different growth seasons. If we calculate the mean values for the character "protein production per plant" from these traits

the following values were obtained:

— 1970: 1.095 g,
— 1971: 1.562 g.

The difference is extraordinarily high. In 1971, the plants of the variety 'Dippes gelbe Viktoria' produced about 43 percent more seed proteins than in 1970. The mutants and recombinants investigated show likewise clear differences in both these years, but in the opposite sense; all the values for 1971 are lower than those for 1970.

Mutant 68C has been used for crosses with other beneficial mutants of our collection because of its genetically conditioned increase of the ovule number per carpel. The protein content of its seed flour is similar to that of the initial line (101.9 and 101.0% in 1970 and 1971). However, the protein production per plant varied considerably in both the growth seasons as a consequence of strong differences of the seed size (protein production = 131.1 and 92.4% of the initial line in 1970 and 1971, respectively). A similar situation was observed for the early flowering mutant 46C. The negative factor in this case is the reduced number of seeds per plant (79 and 72% of the control values, respectively). Therefore, its total protein production was far below the control values of the initial line (77 and 76% in 1970 and 1971, respectively).

In contrast to the two genotypes just mentioned the situation of the bifurcated mutant 1201A is more favourable. In 1970, the protein content of its seed meal was about equivalent to that of the initial line, but in 1971, it was 12% lower. However, the stem bifurcation results in an increase of the seed production per plant. Considering 14 generations, the mean values for the character "seed number per plant" varied between 92 and 121% of the control values. With regard to the seed size, only minute deviations from the initial line were observed. Therefore, favourable values for the character "seed weight per plant" were obtained exceeding the control by about 30% in 1970 and 1971. Considering the thousand grain weight and the protein content of the seed meal the total production of seed proteins per plant was 31 and 12% higher than that of the initial line in both the generations tested.

All the data mentioned refer to the mutants. By combining the mutant genes it was our aim to produce recombinants which could be of interest in pea breeding. The low seed production of the early flowering mutant 46C should have been improved by an increase of the seed number that is inherited through gene 68C. However, this was not possible because of certain negative interactions between the respective genes (Gottschalk, 1972). Considering five subsequent generations the recombinant R300 (= 68C/46C) reached values between 72 and 85% of the corresponding values of the initial line only for the character "number of seeds per plant". The protein content of its seed meal is equivalent to that of the control (104.3 and 103.9%, respectively). However, a decrease of the protein production per plant may occur as a consequence of varying seed sizes in subsequent years. In 1970, the protein yield of the recombinant was 98.6% of the control value, in 1971, it was only 81%. Thus, the differences between the recombinant and the initial line are considerably lower if we not only regard the seed but also the protein production. Therefore, the breeding value of the recombinant is improved by its favourable protein situation.

The recombinant R 350 (= 68C/1201A) shows a different initial situation which, however, does not result in an improvement of the protein production. The characters "increased seed number per pod" and "increased pod number per plant" are combined in the plants of this genotype. There is no negative interaction between the two genes involved; nevertheless, their combination does not result in the effect expected. With regard to the number of seeds per plant the mean values are similar to those of the initial line considering 5 generations, but the seeds are smaller. In this way, the seed weight per plant decreases to about 90% of the corresponding control values. The protein content of the seed flour was 102.4% of the values of the initial line in 1970, but in 1971 only 87.6%. Furthermore, if we consider the reduced seed size, even stronger reduction of the protein yield per plant is reached (92 and 78% of the control values for 1970 and 1971, respectively).

More important than the total protein content determined by means of the Kjeldahl method are the essential amino acids because they exclusively are of importance for the protein quality and the nutritive value of the seed proteins. Let us first consider the total amount of the seven essential amino acids mentioned above. The mean values of the initial line were 0.509 (1970) and 0.726 g per plant (1971), this is a proportion of 46.5% of the total seed proteins in both the years. The three mutants investigated show a very different behaviour in this respect. Mutant 68C reached the control value in 1970; in 1971, however, the total amount of the seven amino acids was only 81% of the corresponding amount of the initial line. Mutant 46C showed in both the growing seasons equally negative values (69 and 68% of the control, respectively). In contrast to this, the bifurcated mutant 1201A exceeded the initial line not only in 1970 but also in 1971 when all the mutants and recombinants tested showed considerably lower values than in 1970. The total amount of the essential amino acids produced from this mutant was 24 (1970) and 7 (1971) percent higher as compared to the initial line.

Lysine and methionine have been separately evaluated from the pool of the essential amino acids for the reasons already mentioned. With regard to

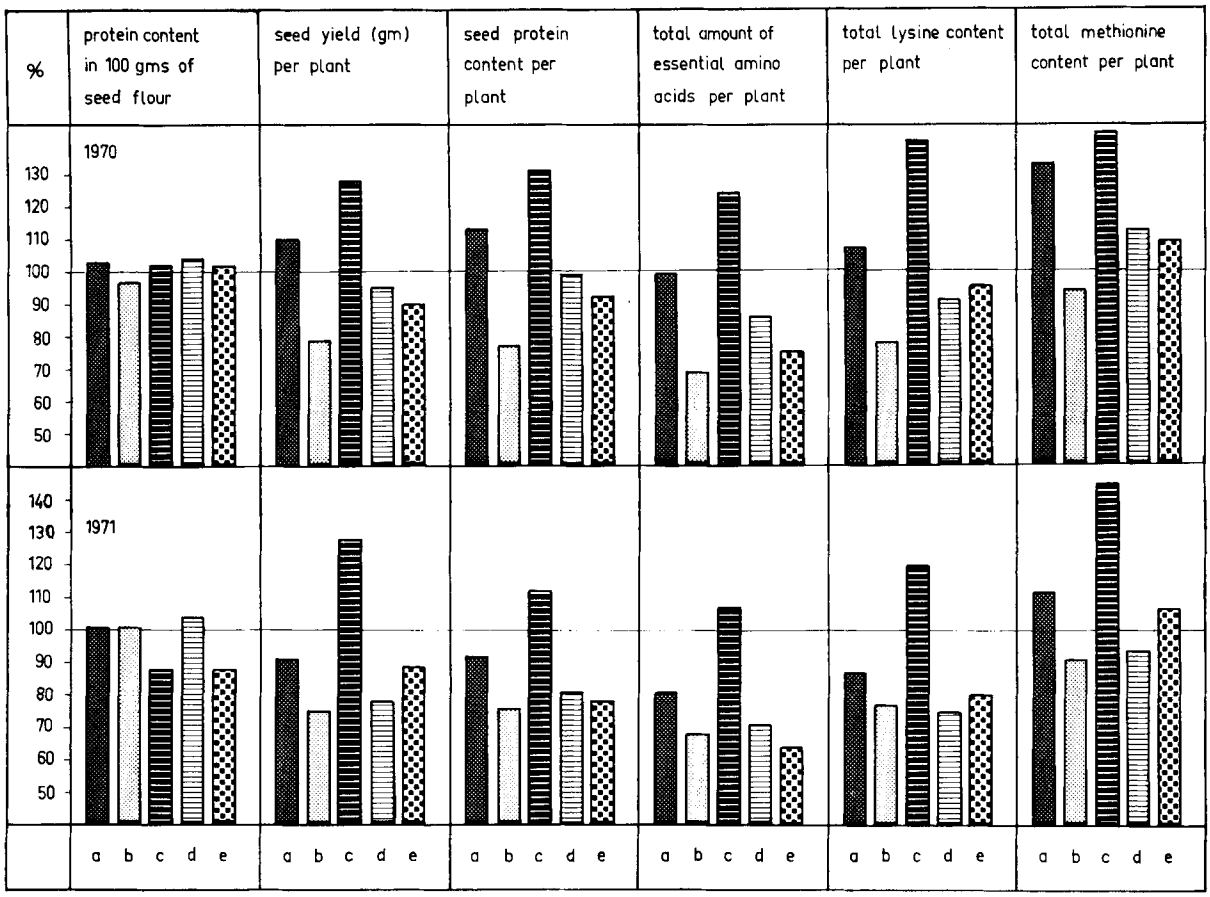

Fig. 13. Comparison of different criteria of the protein yield in some pea mutants and recombinants in two subsequent generations. All the values are related to the corresponding values of the initial line = 100%.
a: mutant 68C; b: mutant 46C; c: mutant 1201A; d: recombinant R300 (= 68C/46C); e: recombinant R350 (= 68C/1201A)
upper part: 1970, lower part: 1971

the lysine production mutant 68C is equivalent with the initial line while both the values of mutant 46C are very low (middle part of fig. 13). Mutant 1201A shows a very favourable situation in this concern. Its lysine production was 40% (1970) and 20% (1971) higher. The methionine production is in all the three mutants in comparison to the initial line higher than the lysine production; the mutants show a similar relation. Mutants 68C and 1201A exceeded the control values considerably. The situation in the two recombinants R 300 (= 68C/46C) and R 350 (= 68C/1201A) is less favourable. The total amount of the essential amino acids and the lysine production lies below to that of the initial line, the methionine production is approximately similar. Their behaviour however cannot be generalised for each of them shows a specific situation in relation to the respective parental mutants.

Discussion

The empirical data given in the present paper show that each of the genes studied influences the synthesis of the seed proteins in a distinguishing way. An interesting insight in the relations between the composition of the genome and the biosynthesis of metabolic products serving as storage substances in the earliest ontogenetic stages is already obtained from the analysis of a small number of mutants. These results are of interest for mutation breeding since important substances required for animal and human nutrition are involved. Moreover, the comparison of mutants and recombinants enables us to discuss the problem of the combined action of mutant genes.

1. *The Genetically Conditioned Variability of the Seed Proteins*

An interesting result of our investigations consists in the fact that nearly each mutant analysed has a specific pattern of seed proteins differing not only from the initial line but also from the other mutants. As shown by Adriaanse *et al.* (1969) in different varieties of *Phaseolus vulgaris* these patterns are characteristic and constant for each genotype and are not influenced by environmental factors. The genes in

question do certainly not control the biosynthesis of the seed proteins directly, they influence these processes indirectly. Thus, the genes studied are not comparable with the voluminous group of genes controlling the course of meiosis and being directly responsible for the formation of functionable germ cells (Gottschalk 1968, 1973). It cannot be expected that it is possible to find the gene groups responsible for the synthesis of the seed proteins in radiation genetic experiments. Mutants of this type would represent biochemically deficient genotypes which are not viable because of the lack of single amino acids (Hess 1968). The findings given in the present paper could be interpreted by means of the concept of the "biochemical pleiotropy". A distinct gene not concerning directly with the protein synthesis gets mutated. The gene products resulting from the mutated gene induce changes in the biosynthesis of other substances during the ontogenetic development. In this way, the gene indirectly can influence the composition of these substances from which the storage proteins of the seeds are studied in the present paper. Not only the changes of the quantitative situation of certain protein components but preferably the striking differences of the protein patterns demonstrate that the respective genes influence the protein metabolism on a broad base. It can be assumed, that not only the proteins but also other groups of substances are altered under the influence of the mutated genes. This could already be demonstrated for the five main sugars of the seeds of some of these mutants (Thierfeldt 1972). It is our opinion that the pleiotropy of the gene action involves different biosyntheses of the organism the clarification of which requires comprehensive biochemical analyses. Another possibility for interpreting our findings would be the assumption of the action of modifying genes (Johnson *et al.*, 1970).

The co-operation of the genes studied which indirectly influence the protein synthesis is of particular interest. This can be demonstrated by comparing the recombinants with their parental mutants. The findings available from the genotypes analysed show, that the total protein amount and the lysine production of the recombinants agree well with those of the low parental mutant. In contrast to this, the methionine content of the recombinant resembles to that of the high parent (fig. 11). These findings obtained by means of the amino acid analyser were fully confirmed after having studied the same genotypes by microbiological methods (Henke 1972). However, these clear correlations could not be confirmed when we consider not only the protein content of equal amount of seed meal but also the seed production of the genotypes. It could be shown, that the combined action of specific genes has negative consequences with regard to the seed protein synthesis quantitatively as well as qualitatively. It is obvious, that the relations found in the small group of mutants and recombinants analysed in the present investigations cannot be generalized. On the contrary, each gene combination will result in a specific biochemical situation.

2. The Breeding Value of Mutants and Recombinants under Consideration of the Seed Proteins

The findings of the present paper show that there are clear differences between the genotypes studied with regard to the quantitative and qualitative situation of the seed proteins. These data represent an essential supplement for the estimation of the breeding value of the respective strains considering their seed yield as well. Two examples are demonstrated to show how the biochemical situation of mutants can influence their qualification for breeding purposes.

The useful character of mutant 46C is its considerable earliness. In 1973, it was the only mutant of a group of different genotypes which was able to seed in the arid areas of India, while other mutants of our collection as well as some Indian local varieties dried prematurely as a consequence of the lack of the monsoon rainfall. Thus, it has demonstrated its positive selection value under extreme climatic conditions. In West Germany it is not of interest for pea breeding because of its low seed yield. In the protein content it does not differ from the initial line but it shows a negative shift between the two main fractions; the proportion of the globulins is increased, that of the albumins is decreased. This shift means a qualitative degradation of the proteins as the albumins represent the nutritionally more valuable fraction because of their amino acid composition (Bajaj *et al.* 1971, Boulter and Derbyshire 1971). This shift appears also with regard to the total amount of the essential amino acids; it lies below the corresponding values of the initial line. The mutant shows a more favourable situation than the initial line with regard to specific amino acids such as lysine and methionine. However, this increase does not represent a positive yield trait because the seed production of the mutant is too low. Considering this criterion protein yields of only 75 to 80% of the control values are reached. The same relations are valid for the total content of the essential amino acids. Thus, the unfavourable yielding capacity is not compensated by a favourable quantity or quality of the seed proteins.

Mutant 1201A shows an increased seed production because of its stem bifurcation. The protein content of its seed flour does not essentially differ from that of the initial line. The albumin fraction is somewhat reduced but the total amount of the essential amino acids is clearly increased than in the control. With the exception of phenylalanine, threonine and valine all the other essential amino acids are present in higher quantities. The protein yield per plant of this mutant is 10—30% higher because of the high seed production. Also a qualitative improvement can be stated in addition to the quantitative one because

the production of essential amino acids per plant is likewise increased. Thus, the favourable seed yield of mutant 1201A is supplemented by an advantageous quantitative and qualitative alteration of the seed proteins.

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