Theoretical and Applied Genetics 47, 231-242 (1976)

Seed Protein Traits of Fasciated Pea Recombinants and the Role of the Mutant Genes Involved

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Summary. The protein traits of some fasciated and non-fasciated pea recombinants have been studied in the light of mutant gene actions and interactions. The results indicate that combinations of different mutant genes affecting morphological characters have indirect influences on the quantitative composition of seed flour proteins of the genotypes. At the level of single specific amino acids, there were drastic quantitative alterations. The results also indicate that differences in the mutated background genotypes may have a role in the protein quantitative alterations. Correlations among protein and yield traits have been calculated and discussed. Percent crude protein was found to be negatively correlated with different protein yield traits at the plant level.

Introduction

The potential value of a given genotype in breeding programmes depends on a variety of factors. Among them, protein traits are a major component, especially in leguminous plants. A number of single gene protein-rich mutants have been obtained in *Pisum sativum* by induced mutations. Unfortunately, many show negative features, such as low fertility and reduced yield components, which reduce their potential value (Gottschalk and Müller 1970). Restoration of fertility and improvement of yield characters can be achieved by crossing these mutants with other fertile ones and selecting new recombinants with desirable qualifications (Gottschalk 1972). During the radiation experiments carried out by Gottschalk (1966), a number of fasciated pea mutants were obtained, of which the X-ray induced mutant 489C showed an extraordinarily high yielding capacity with regard to the character, "number of seeds per plant". Its seed protein content was very variable during successive generations. It arose as a result of a group of simultaneously mutated genes (Gottschalk 1972 and 1975). Because of its unusual genetic background, mutant 489C was used as a recurrent parent in a series of crosses with other fertile and less-fertile mutants of the same collection. The main goal behind this programme was to eliminate the negative features of mutant 489C, such as tallness, lateness and reduced seed size. It was also hoped that its potential value would be improved by transferring to its genome some desirable genes existing in other mutants. In the segregating generations of these crosses, a great number of different recombinants were selected. The phenotypic description and the yield qualifications of some of these recombinants have been given elsewhere (Gottschalk and Hussein 1975).

The present paper provides results on protein traits of the seed flour of some fasciated and non-fasciated recombinants selected during this programme. The results are discussed in relation to the yielding capacity of these recombinants and the interactions of the mutant genes involved are also considered.

Material and Methods

For the present investigations, 10 recombinant lines, mutant 489C and the initial line 'Dippes gelbe Viktoria' were used. The recombinants showed homozygosity in their morphological characters in F_3 or F_4 generation. The following genotypes were used as parental lines in the crossing programme from which the recombinants had been selected:

- IL: 'Dippes gelbe Viktoria' medium internode length with plant height of about 65 cm; medium late with flowering time of about 50 days from sowing; non-fasciated with protein content of about 22-23%. It is the standard line with which all genotypes are compared.
- Mutant 489C: Apical part of the stem strongly fasciated; tall; flowering time about 10 days later than the IL; small seed size of strongly variable protein content in successive generations; seed production greatly increased.
- Mutant 46C: Early flowering due to the very low position of the first flowers on the stem; starts flowering about 10-15 days earlier than the initial line; reduced seed production.
- Mutant 122: Narrow leaflets; seed production slightly reduced.

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- Mutant 176A: Narrow leaves; with the exception of plant height all plant organs reduced; normal fertility; not identical with mutant 122.
- Mutant 1201A: Dichotomous stem bifurcation; somewhat increased seed production; unstable penetrance of the mutant gene (Gottschalk and Chen 1969).
- *Cochleata* mutant 5137: obtained by Blixt (1967); showing spoonlike stipules and certain flower anomalies; greatly reduced fertility.

Seed flour of the following recombinants was used for biochemical analysis:

- R 850: very short internodes, fasciated, late and normal leaves (selected in F_3 of the cross 489C × 122);
- R 852: long internodes, non-fasciated, not late and normal leaves (selected in F_3 of the cross 489C × 5137 *coch);*
- R 853: internode length = IL, linearly fasciated, not late and normal leaves (selected in F_3 of the cross 489C × 5137 *coch);*
- R 854: long internodes, non-fasciated, not late and narrow leaves (selected in F_3 of the cross 489C × 122);
- R 162: medium short internodes, slightly shorter than the initial line, fasciated and late (selected in F_4 of the cross 489C × 1201A);
- R 25: long internodes, non-fasciated and not late (selected in F_3 of the cross 489C × 46C);
- R 26: long internodes, non-fasciated and not late (selected from the same cross as R 25, but from a different F_3 family);
- R 620: long internodes, non-fasciated, late and narrow leaves (selected in F_3 of the cross 489C × 122);
- R 170: long internodes, linearly fasciated and flowering time late = mutant 489C (selected in F_4 of the cross 489C × 1201A);
- R 80: very short internodes, fasciated, not late and normal leaves (selected in F_4 of the cross 489C × 176A).

For biochemical analyses, seeds from all plants available of a given genotype were mixed together, and random samples, each of 40 seeds were used. Observations on yield components were recorded in the season 1974.

Crude protein was estimated in the seed flour samples using the Kjeldahl method. The total nitrogen content of the seed flour (on dry weight basis) was determined. The crude protein % was then calculated by multiplying the nitrogen values by 6.25 (Keil and Sormova 1965). All the amino acids, except those containing sulfur, were analysed by an amino acid analyser (Aminolyser, Optica) using the procedures given by Mondino (1967 and 1969a,b).

The yield of crude protein, total amino acids (only 15 acids), essential amino acids and lysine per plant were calculated by interpolation between "seed weight per plant" and the percentages of the different protein traits. Correlation coefficients were calculated for the different characters studied. It should be noted that two expressions have been used frequently in this text: "protein quality traits" for the percentages of protein traits in the seed flour unit weight; and "protein quantity traits" for the amounts of protein traits at the plant level.

Results

Because of the unusual genotypic background of the recurrent parent mutant 489C, a wide range of phenotypic variation occurred in F_2 and later generations of the different crosses (Gottschalk and Milutinović 1973). This genetically conditioned variation allowed selection of a great number of recombinant lines differing from one another in many morphological characters. This is attributed to the redistribution of the many mutant genes existing in 489C (Gottschalk and Hussein 1975). The 10 recombinants included in the present investigation showed homozygosity for different morphological characters in F_3 or in F_4 generations.

To facilitate presentation of the data, the genotypes studied were classified into two major groups:

- non-fasciated genotypes, and
- fasciated genotypes.

Further classifications were made within each group according to internode length, leaf shape and time of flowering. The values of the initial line (Dippes gelbe Viktoria) were taken as the standard (= 100 %, assigned by a horizontal broken line in the graphs) with which the values of all other genotypes are compared.

1. Non-Fasciated Recombinants

This group contains five recombinants each selected from a different F_3 family. These recombinants are: R 852, R 25, R 26, R 854 and R 620.

The three recombinants R 852, R 25 and R 26 are phenotypically similar to each other. They have long internodes and normal leaves (like mutant 489C) and their flowering time is medium late which is equal to that of the initial line. These three recombinants are very similar in protein quality traits (Fig. 1). Only R 26 has higher percent crude protein than the initial line. The percent total amino acids and the percent essential amino acids of the three recombinants are slightly less than in the initial line, but they show a considerable increase in lysine percentage. If these recombinants are compared on the basis of productivity per plant (Fig. 2), it will be seen that R 852 is slightly higher in crude protein yield, but almost equal

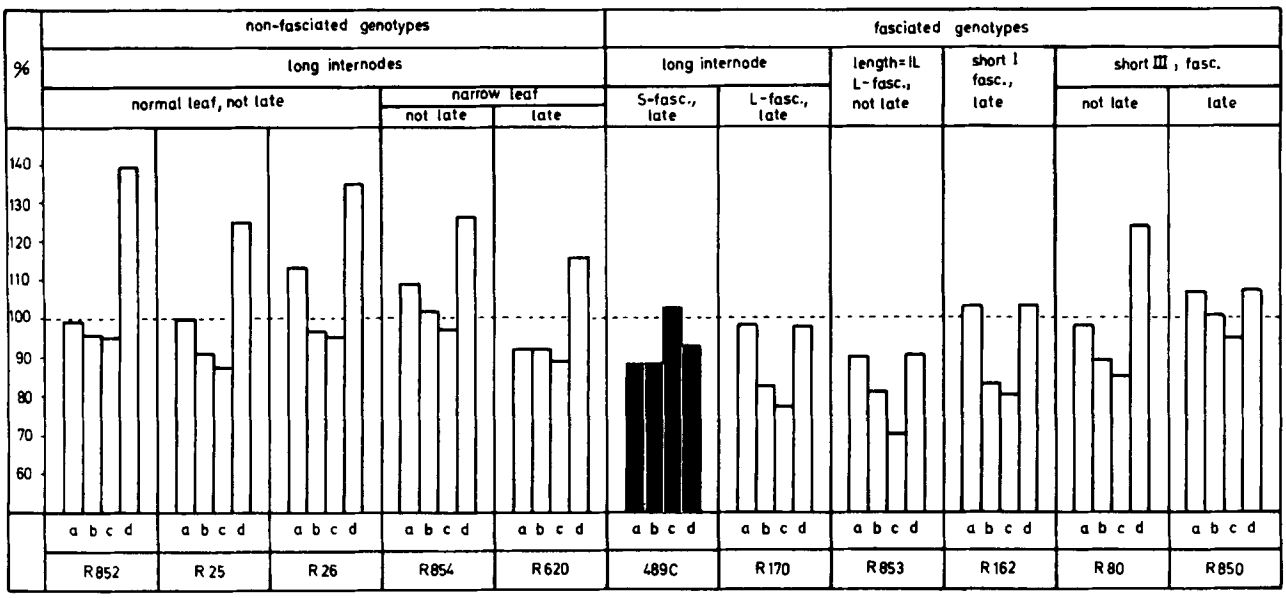

Fig. 1. Criteria of different protein quality traits of the seed flour unit weight (100 gr) in mutant 489C and the recombinants selected from crossing 489C with other mutants. The values are related to the corresponding values of the initial line = 100% (broken line). a: percent crude protein; b: percent total amino acids; c: percent essential amino acids; d: percent lysine. S- and L-fasc.: denote strong and linear type of stem fasciation, respectively

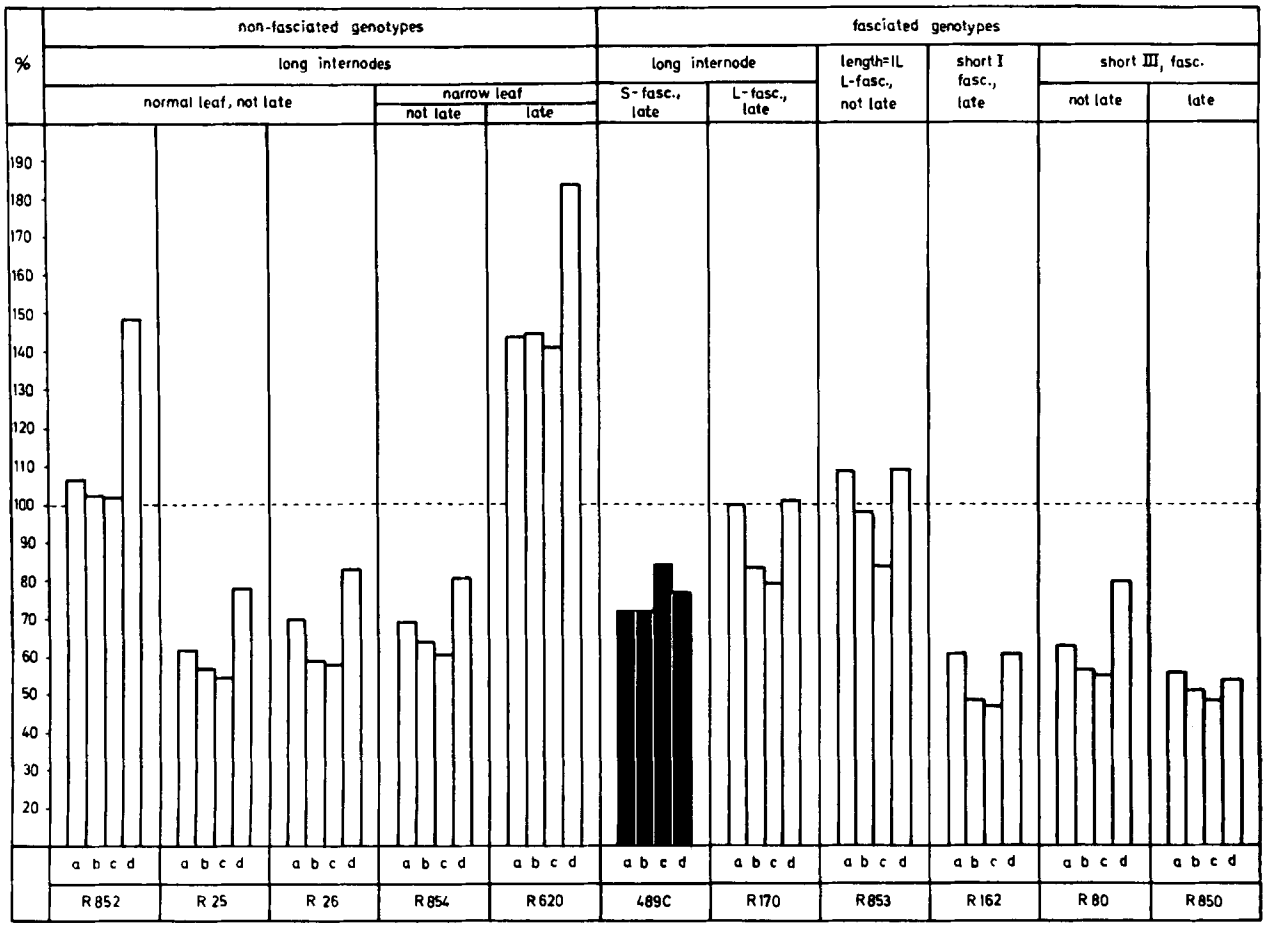

Fig. 2. Criteria of different protein yield traits per plant of mutant 489C and the recombinants selected from crossing 489C with other mutants. The values are related to the corresponding values of the initial line = 100% (broken line). a: crude protein; b: total amino acids; c: essential amino acids; d: lysine. S- and L-fasc.: denote strong and linear type of stem fasciation, respectively

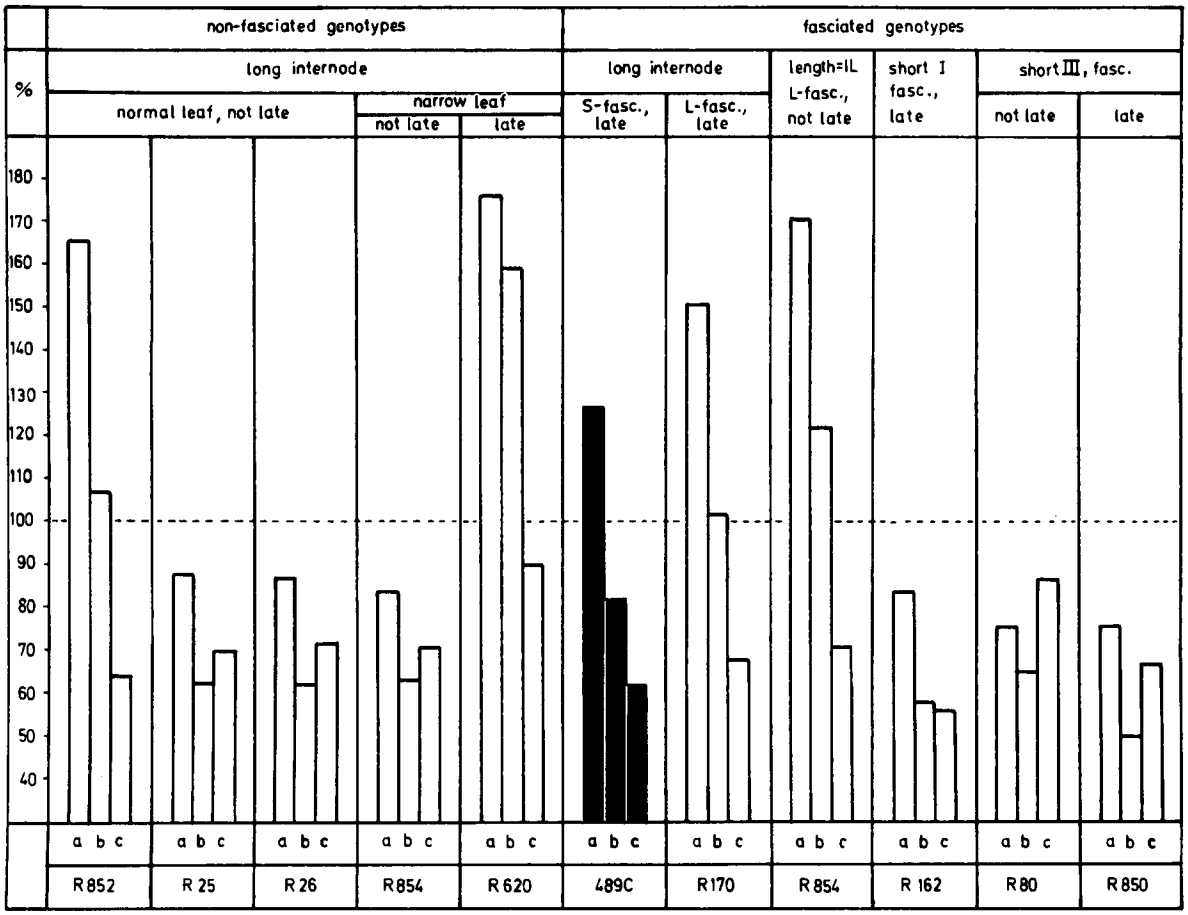

Fig.3. Criteria of seed yield traits of mutant 489C and the recombinants selected from crossing 489C with other mutants. The values are related to the corresponding values of the initial line = 100 % (broken line). a: number of seeds per plant; b: weight of seeds per plant (gr); c: weight of 100 seeds (gr). S- and L-fasc.: denote strong and linear type of stem fasciation, respectively

to the initial line in total amino acids and essential amino acids; its lysine yield is about 50 % greater than that of the initial line. On the other hand, the two recombinants R 25 and R 26 are much lower than the initial line in all protein quantity traits per plant. The superiority of R 852 over R 25 and R 26 in protein yield traits is due to its higher seed productivity (viz. number of seeds and weight of seeds per plant). However, the increase in the weight of seeds is not equivalent to the number of seeds, because of the smaller seed size characterizing R 852 (Fig.3). As mentioned earlier, the three recombinants are phenotypically similar to each other, but the present results indicate differences in their genotypic background. Thus, the gene for long internodes expressed its potentiality in increasing the seed yield per plant and, consequently, in increasing protein yield traits in the background of R 852, but not in R 25 and R 26. It should be remembered that the two latter recombinants have been selected from the F_3 of the same cross, but from two different families (see Material and Methods).

Recombinants R 854 and R 620 both have long internodes and narrow leaves. The former character is inherited through parent mutant 489C, and the latter through parent mutant 122. The main difference between these two recombinants is in flowering time. While R 854 is medium late (like the initial line), R 620 is much later (like parent mutant 489C). The protein quality traits of the two recombinants show differences from each other. The percent crude protein and the percent lysine of the seed flour of R 854 are higher than the corresponding values for the initial line, while the percent total amino acids and the percent essential amino acids are very close to the values of the initial line. In contrast, the protein quality traits in R 620 are lower than in the initial line, except for lysine which is higher (Fig.1).

Table 1. Coefficients of correlations (r) between protein quality traits and protein and seed yield traits

	protein quality traits of the seed flour			Protein quantity traits per plant				seed yield traits			
	1	2	3	4	5	6	7	8	9	10	
	total amino acids %	essential amino acids %	lysine %	weight of crude protein (gr)	weight of total amino acids (gr)	weight of essential amino acids (gr)	weight of lysine (gr)	number of seeds per plant	weight of seeds per plant (gr)	100 seed weight (gr)	Array
% crude protein	0.57	0.28	0.56	-0.53	-0.55	-0.58*	-0.44	-0.67*	-0.65*	-0.09	A
% total amino acids	-	0.80**	0.51	-0.16	-0.04	-0.01	-0.03	-0.47	-0.25	0.38	B
% essential amino acids		-	0.31	-0.18	-0.15	0.11	-0.06	-0.36	-0.22	0.20	C
% lysine			-	-0.12	-0.10	-0.10	0.19	-0.25	-0.22	0.02	D

* Correlation coefficients greater than 0.58 and 0.71 are significant and highly significant at 5% and 1% levels of significance, respectively. Degrees of freedom = 10.

The decrease in percent crude protein and percent total amino acids of the seed flour of R 620, which is parallel to that in mutant 489C, may indicate that the gene for late flowering, present in the background of both genotypes, has a negative effect on protein content. The gene for narrow leaves seems to have a positive effect on protein content of the seed flour. This statement finds its justification when comparing R 854 with R 852 (Fig. 1). Both genotypes are phenotypically similar, except that R 854 has narrow leaves. As mentioned earlier, the percent crude protein of R 852 is about equal to that of the initial line, while that of R 854 is about 9% greater. This positive effect of the gene for narrow leaves on protein content is diminished or even suppressed in the presence of the gene for late flowering (compare R 620 with R 854 and R 852, Fig. 1). Comparing protein quantity traits at plant level, R 620 is greatly superior to the initial line, while R 854 is much lower (Fig. 2). The superiority of R 620 over the initial line and R 854 in protein yield traits at the plant level is due to its higher seed productivity (Fig. 3), resulting from the action of the gene for late flowering. Thus, in R 620 the unfavourable effect of the late flowering gene on protein quality traits has been compensated by better seed yield productivity, and consequently by favourable protein yield traits at plant level. This conclusion is supported by the significant or almost significant negative correlations found between the percent crude protein and some protein and yield traits per plant (Table 1, array A).

2. Fasciated Recombinants

The present data indicate that the recurrent parent mutant 489C (strongly fasciated, long internodes and late flowering) has less crude protein in the seed flour compared with its initial line (Fig. 1). According to Gottschalk et al. (1975), this is an exceptional result for this mutant, as its protein content during most of the previous generations was higher than that of the initial line 'Dippes gelbe Viktoria'. Our results also indicate that the seed flour of this genotype contains a lower percentage of total amino acids and of lysine; however, the essential amino acids remained almost the same as in the initial line (Fig. 1). At plant level (Fig. 2) all protein quantity traits are greatly reduced because of the smaller seed size characterizing this genotype (Fig. 3).

As pointed out elsewhere (Gottschalk 1975; Gottschalk and Hussein 1975), the phenomenon of stem fasciation in the present material occurs in two dif-

ferent forms, "strong fasciation" and "linear fasciation". When fasciation is combined with short or very short internodes, it is often difficult to distinguish the two types. The genes for "strong" and "linear" fasciation belong to a series of at least 4 multiple alleles (Gottschalk 1975). In the present group of recombinants, the gene for fasciation is combined with several other genes affecting internode length and flowering time. The results of the actions and interactions of these genes on protein traits of the seed flour will be presented and discussed.

This group of recombinants contains five different lines differing in the type of fasciation, internode length and flowering time. The recombinants are denoted R 170, R 853, R 162, R 80 and R 850.

Recombinant R 170 has long internodes and late flowering (like mutant parent 489C) and linear fasciation. The latter character had been observed to recover frequently in segregating generations of all crosses in which mutant 489C was one of the parents. The genetic mechanism behind this recovery is not yet known. The protein quality traits of this recombinant are variable (Fig.1). The percentages of crude protein and lysine in the seed flour are very close to the corresponding values of the initial line, while the total amino acids and the essential amino acids are much less and even lower than those of mutant 489C. At plant level (Fig.2) similar results were found, but with some improvement over mutant 489C. This improvement relative to 489C is due to the extraordinarily high number of seed per plant in this genotype: in R 170, the character "number of seeds per plant" reached a value of about 150 %, while in mutant 489C it reached about 127% of the corresponding value of the initial line (Fig.3). It is possible that this difference in favour of R 170 arises from the substitution of the allele for linear fasciation instead of the allele for strong fasciation. However, the increase in the number of seeds per plant resulting from the action of the gene for linear fasciation is not followed by an equivalent increase in seed weight, and consequently in protein traits per plant, because of the reduced seed size of R 170. Fortunately, this latter character is not correlated with any other character, whether of yield or protein (Table 1). Even within the fasciated genotypes this correlation is absent. The lack of correlation between seed size and number of seeds or weight of seeds per plant, especially within the fasciated genotypes, is a direct argument for the small seed size not being a part of the pleiotropic spectrum of the gene for stem fasciation. This is additional evidence for the same conclusion reported by Gottschalk and Hussein (1975). It should be possible to eliminate this negative character of R 170 by further hybridization and selection.

R 853 is medium late and has medium internode length like the initial line. It is also linearly fasciated. All protein quality traits of the seed flour are much more reduced compared with those of the initial line (Fig.1). At plant level (Fig.2), R 853 is higher in crude protein and lysine production, but lower in essential amino acids, than the initial line. There is no clear change in the yield of total amino acids. The situation becomes pronounced if one compares this recombinant with mutant 489C. As in the case of R 170, the improvement in protein and amino acid yield per plant in R 853 compared with 489C is due to its extraordinarily high number of seeds and the weight of seeds per plant highly correlated to it. In R 853, the number of seeds per plant reached a value of about 171 %, while the weight of seeds reached a value of about 122 %, of the corresponding values for the initial line (Fig.3). On the basis of the present information, one may conclude that the gene causing the slight reduction in internode length has no direct negative effect upon the plant yield, and consequently no indirect negative effect on protein yield traits. The same gene also does not negatively affect the potentialities for plant yield exhibited by the gene for linear fasciation. Comparing R 853 with R 170 (Figs.1, 2 and 3), the former recombinant surpasses the latter in all aspects. The only morphological difference is that R 853 has shorter internodes than R 170. The explanation for the superiority of R 853 over R 170 lies in the differences in their background genotypes. Additional support for this claim comes from the fact that R 853 was derived from two parental mutants completely different in origin: mutant 489C was derived from the German variety 'Dippes gelbe Viktoria' as a result of a group of simultaneously mutated genes (Gottschalk 1972 and 1975); the *cochleata* mutant 5137 was derived from the Swedish variety 'Parvus' (Blixt 1967).

The three remaining recombinatns which belong to this group are shorter in internode length than the initial line and also differ from one another in the degree

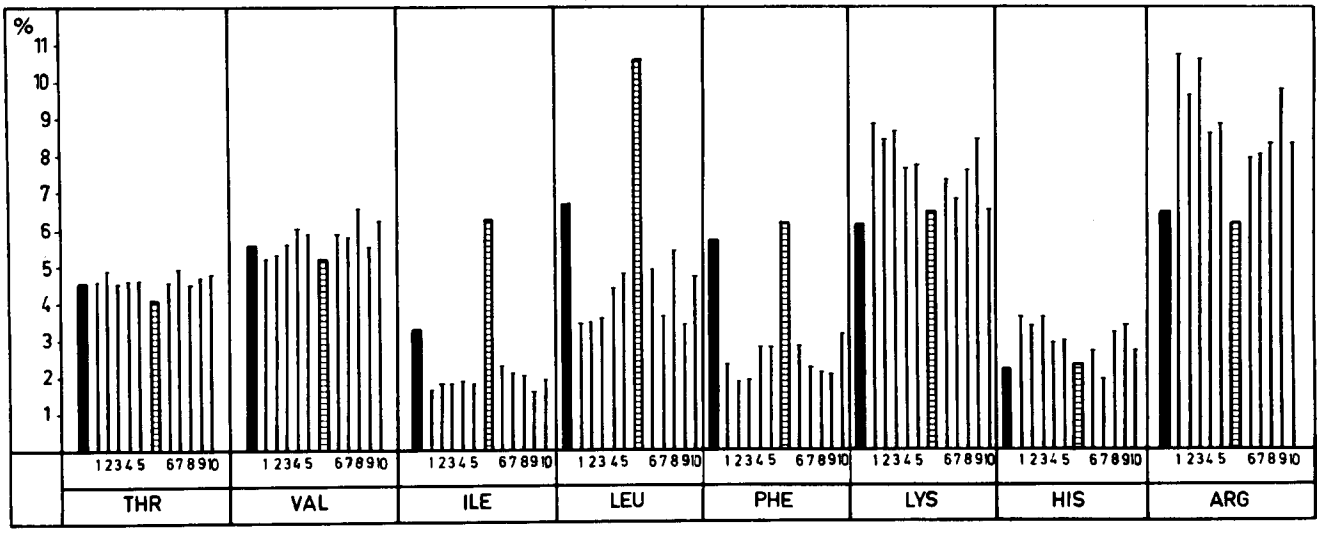

Fig.4. Percentages of the essential amino acids individually as related to the total amount of the amino acids of the hydrolysate in the initial line = black column, mutant 489C = striped column and the 10 recombinants. 1 to 5: the non-fasciated recombinants R 852, R 25, R 26, R 854 and R 620, respectively; 6 to 10: the fasciated recombinants R 170, R 853, R 162, R 80 and R 850, respectively

of shortening (for details, see Gottschalk and Hussein 1975). These recombinants are R 162, R 80 and R 850.

The mean plant height of R 162 is about 77% of that of the initial line. It has been classified to a degree of shortness denoted as "short I". As well as being fasciated, it is late in flowering time like parent 489C. The crude protein and lysine percentages of R 162 are about equal to the corresponding values for the initial line, while the total amino acids and the essential amino acids are highly reduced (Fig.1). At plant level (Fig.2), this genotype shows a drastic reduction in all protein quantity traits, compared with both the initial line and mutant 489C.

Recombinants R 80 and R 850 are phenotypically similar in fasciation and internode length, but differ in flowering time. R 80 is equal to the initial line in flowering time, while R 850 is later (= 489C). Both recombinants have very short internodes. They have been classified to a degree of shortness denoted as "short III". The mean plant height of both genotypes is about 54% of that of the initial line (Gottschalk and Hussein 1975). The two recombinants have been selected from the segregating generations of two different crosses, but they have one parent as common ancestor, i.e. mutant 489C (see Material and Methods). The crude protein percentage of R 80 is about equal to that of the initial line, while the lysine percentage is much higher, showing an increase of over 20% in the seed flour. It is the only fasciated genotype among the group which can be considered as lysine-rich. The protein quality traits of R 850 are almost equal to those of the initial line, but with a slight improvement in crude protein and lysine (Fig.1). At plant level, both recombinants showed greatly depressed protein quantity traits compared with the initial line as well as mutant 489C (Fig.2). This negative productivity found in the three short internode and fasciated recombinants (R 162, R 80 and R 850) is to be expected, since the components of seed yield per plant are reduced under the effect of the genes for short and very short internodes (Fig.3).

One may conclude that, in the presence of the genes for "short" and "very short" internodes, the potentialities of the genes for fasciation and late flowering in giving high seed productivity, with a consequent reflection on protein yield per plant, are negatively affected.

The Amino Acid Quantitative Composition of the Genotypes

Only 15 amino acids could be quantitatively determined by the amino acid analyser of the Institute using acid hydrolysate. The amino acid spectra of the initial line, mutant 489C and the 10 recombinants are graphically illustrated in Figs.4 and 5. The acids are arranged in the same order as registered by the apparatus.

As expected, no qualitative differences were found between different genotypes, but the analytical data

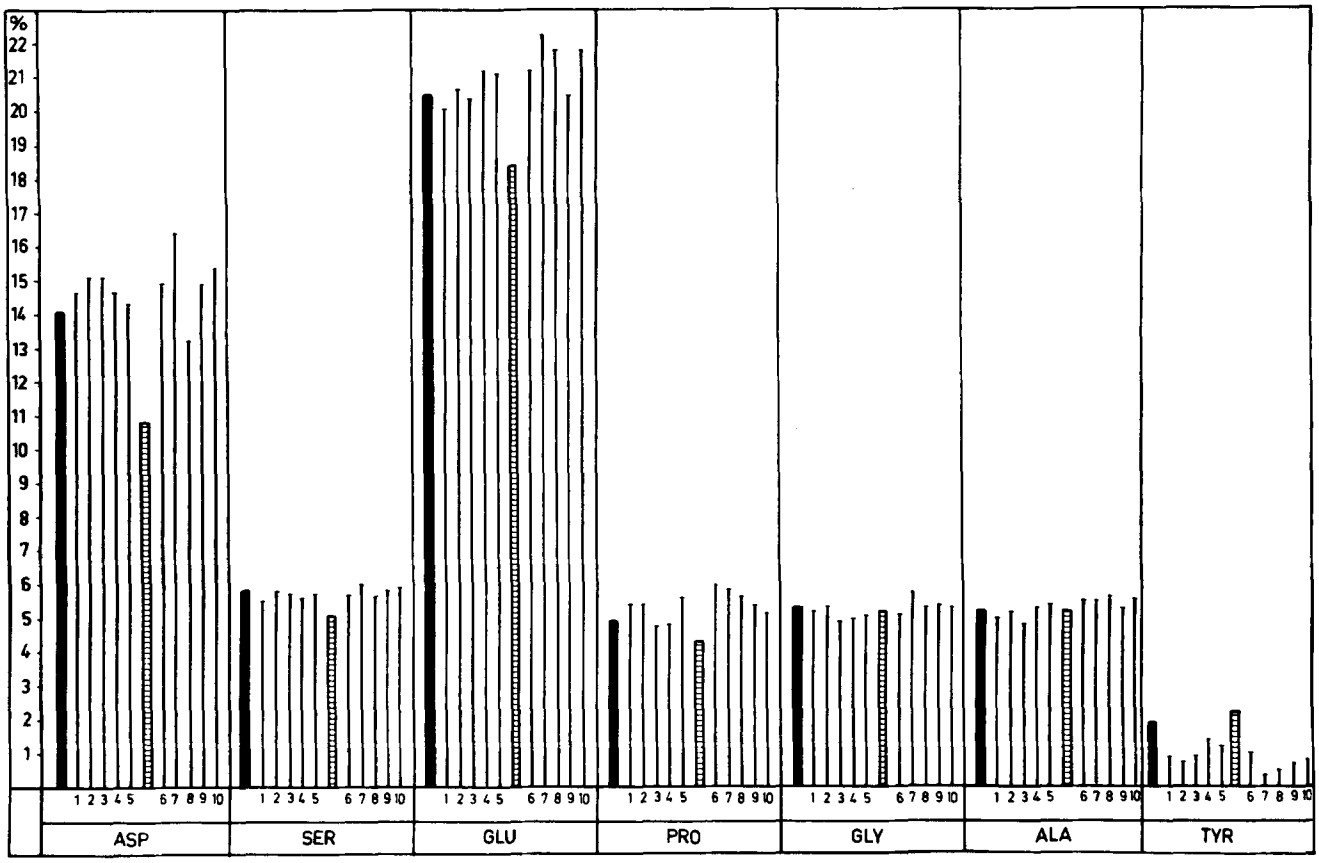

Fig.5. Percentages of the non-essential amino acids individually as related to the total amount of amino acids in the hydrolysate in the initial line = black column, mutant 489C = striped column and the 10 recombinants (arrangement as in Fig.4)

indicate some genetically conditioned quantitative differences in specific amino acids. Similar results were reported by Gottschalk and Müller (1974) in mutants and recombinants of the same ecotype. Figure 4 shows the quantitative proportions of the various essential amino acids of the total hydrolysate. Lysine and arginine occur in the highest proportions. The values for both acids ranging between 6 and 11%. It is interesting to note that most of the recombinants mentioned in the present investigation show some increase in the proportions of both acids compared with the initial line as well as mutant 489C. The histidine proportion is also higher in all non-fasciated, and in some fasciated recombinants than in the initial line and mutant 489C. Only the fasciated recombinant R 853 is similar to the initial line in the proportion of histidine. The analytical data show clear differences in the proportions of isoleucine, leucine and phenylalanine, with all recombinants showing much reduced proportions of these three amino acids compared with the initial line and mutant 489C. The latter genotype is the most isoleucine- and leucine-rich of all the lines. For phenylalanine, both the initial line and mutant 489C are about equal in their proportions; for threonine and valine no clear changes in their proportions were found.

The non-essential amino acids spectra are reported in Fig.5. Glutamic acid is present in the highest proportion (between 18-22%), while tyrosine shows the lowest proportion (between 0.4-2.2%) of the total amino acids. No clear changes in the proportions of serine, proline, glycine and alanine were found. The fasciated and non-fasciated genotypes generally retain the same values of these amino acids present in the initial line as well as mutant 489C. For glutamic acid, all the non-fasciated recombinants are equal in proportion to the initial line, while all the fasciated recombinants show higher proportions than mutant 489C, with some even surpassing the initial line.

A wide range of variation among different recombinants, the initial line and mutant 489C occurred for the asparagine proportion. Some of the recombinants

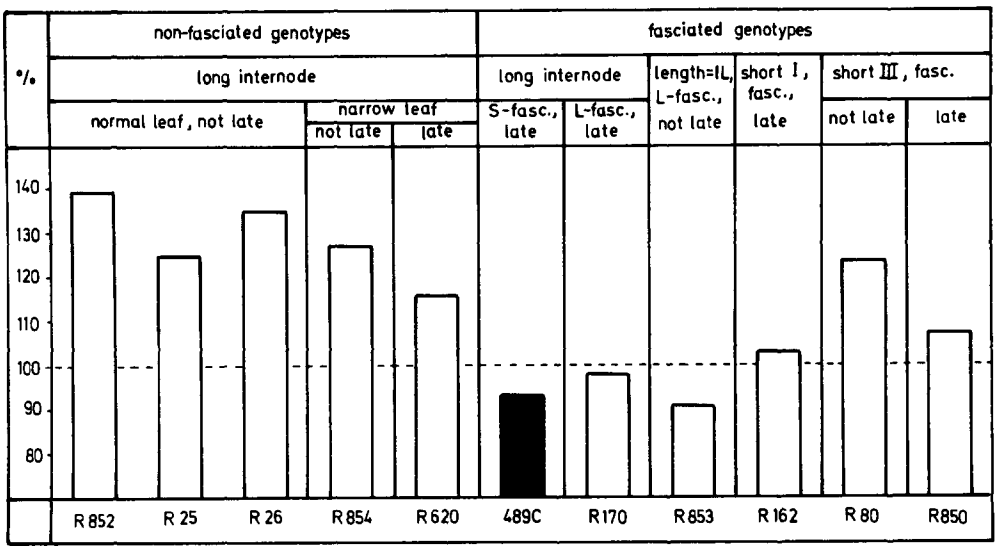

Fig.6. Percentage of lysine in 100 gr seed flour of mutant 489C and the 10 recombinants. The values are related to the corresponding value of the initial line = 100 % (broken line)

surpass the initial line, while mutant 489C is the only genotype containing the lowest proportion. The non-fasciated recombinants mostly retain the same proportion as in the initial line. On the other hand, all the fasciated recombinants contain higher proportions of asparagine than does mutant 489C. Some of them are even equal to the initial line, while only R 853 appears to have a higher proportion.

For tyrosine, the initial line and mutant 489C are about equal in proportion. In general all fasciated and non-fasciated recombinants have a much lower proportion of tyrosine than do the two parental lines.

From the foregoing debate, it seems that the mutant genes involved in the present recombinants have some influence on quantitative alterations in the proportions of specific amino acids. This will be demonstrated in Fig.6: all the non-fasciated, long internode recombinants produce lysine in higher quantities than the initial line. Thus, the combination of the "non-*fasciata* " gene with the long internode gene seems to have a positive effect on lysine production. The gene for narrow leaves seems to have no influence at all on lysine content (compare R 854 with R 852, R 25 and R 26). The gene for late flowering seems to have a negative effect on lysine production. This can be demonstrated by comparing R 854 and R 620: the latter recombinant has a much lower lysine content while the only difference between the two recombinants is that R 620 flowers later than R 854. In contrast, most of the fasciated genotypes are lysine-poor. In general, it seems that the *"fasciata"* gene has a negative influence on lysine production. Combination of the *"fasciata"* gene with long or medium internode genes influences lysine production in a negative manner (compare the genotypes mutant 489C, R 170 and R 853). Combination of the *"fasciata"* gene with a gene for short internodes (short I) in R 162 gives a compromise, as the lysine content of this recombinant is about equal to that of the initial line. Combination of the *"fasciata"* gene with a gene for very short internodes (short III) has a positive influence on lysine production (compare R 80 and R 850 with other fasciated genotypes). However, incorporation of a gene for late flowering with *"fasciata"* and very short internodes affects lysine production negatively (compare R 80 with R 850). The latter recombinant contains about 15% less lysine than the former, while the only difference between these two recombinants is that R 850 flowers much later. This result is in accordance with that reported earlier for the non-fasciated genotypes.

Correlations Between Protein Quality Traits and Protein and Seed Yield Traits:

The coefficients of correlations between the protein quality traits (as percentages of the seed flour) and the protein and seed yield traits have been calculated and presented in Table 1. It will be seen that no sig-

nificant correlations between seed size and any of the protein quality traits are found (see column 10, Table 1). This means that seed size is not an indicator for predicting quantitative changes in the protein quality of the genotypes. The character percent crude protein shows negative correlations with all protein quantity traits and seed yield traits at the plant level. However, some of these coefficients of correlation are significant and some are not (see array A, Table 1). The weakness of these negative correlations may indicate the possibility of selecting lines rich in protein quality and quantity traits. In this context, Gottschalk et al. (1975) and Müller and Gottschalk (1973) working with *Pisum sativum*, and Hussein and Disouki (1976) working with *Phaseolus vulgaris*, selected mutant lines simultaneously rich in protein and yield traits. The character, percent crude protein, shows positive correlations (though insignificant) with other quality traits. It will further be seen in Table 1 (array B) that the character, percent total amino acids, shows highly significant positive correlation with the character, percent essential amino acids. The percent total amino acids is also positively (though insignificantly) correlated with the percent lysine. Other protein quality traits do not show any specific correlation with other protein and seed yield traits.

Discussion

In *Pisum sativum*, it has been stated that striking differences in protein traits result from the action of single mutant genes (Gottschalk and Müller 1970). Moreover, the combination of two or more mutant genes in one genotype may also influence the quantitative and qualitative protein composition of the seed flour in an indirect way. In the present investigation, a number of different homozygous recombinants have been selected in the F_3 or F_4 generations, after crossing the multiple gene mutant 489C with other mutants. The actions and interactions of the recombined mutant genes on protein quality and quantity traits of the seed flour of the recombinants have been studied. The value of a given genotype for breeding purposes is a complex attribute. The protein and amino acid traits are essential for the estimation of this value, also taking into account the seed yield (Gottschalk and Müller 1974). These authors gave two examples to show how the biochemical situation of the genotypes can influence their qualifications for breeding purposes: the first was an unfavourable yielding capacity not compensated by favourable quantity or quality of the seed proteins; and the second was a favourable seed yield supplemented by an advantageous quantitative and qualitative alteration of the seed proteins. The present investigations demonstrate an additional situation. For example, recombinant R 620 shows unfavourable protein quality traits of the seed flour, which are balanced by a favourable seed yielding capacity and, consequently, a favourable protein and amino acid yield per plant.

Genes controlling the morphological characters of the plant may influence its seed protein quality and quantity traits in an indirect way. Recombinants R 854 and R 620 are phenotypically similar, but they differ in flowering time. R 854 is not late (= initial line), while R 620 is much later (= mutant 489C). The gene for late flowering in the background of R 620 caused a clear reduction in all protein quality traits of its seed flour compared with R 854. However, there was an inverse situation for the protein quantity traits at plant level, due to the increased seed production caused by the same late flowering gene. In contrast, the gene for narrow leaf present in the background of R 854 seems to have a positive influence on protein quality traits. This has been demonstrated by comparing R 854 with R 852. Both genotypes are phenotypically similar, except that R 854 has narrow leaves, but the percent crude protein and the percent amino acids of R 854 are more favourable than those of R 852. This positive effect of the gene for narrow leaves is diminished in the presence of the gene for late flowering (compare R 620 with R 854 and R 852).

Genes for stem fasciation seem to have negative influences on protein quality traits but they are known to have positive effects on seed yield and consequently on protein yield per plant. Apart from some cases where different mutant gene combinations may modify these influences, it was found that the majority of the fasciated genotypes (including mutant 489C) have lower protein quality traits than do the initial line and other non-fasciated genotypes. Support for the conclusion that the *"fasciata"* gene has a negative influence on protein quality and a positive influence on protein quantity is provided by the negative correlations found in this study between the trait percent protein and other protein and yield quantity traits (Table 1).

This situation becomes more obvious when the situation is considered with regard to specific amino acids, such as lysine. The positive influence of the *"fasciata"* gene on protein quantity traits per plant comes from the fact that stem fasciation causes highly increased seed production. This is the main reason for the high protein production of the fasciated mutants and recombinants reported by Müller and Gottschalk (1973).

Combination of different mutant genes in one genotype influences the protein quality and quantity traits in an indirect way (Gottschalk and Müller 1974). Our results support this claim by providing several examples. When the gene for stem fasciation is combined with genes for long or medium internodes, the protein quality traits are reduced, while the protein quantity traits at the plant level are improved. On the other hand, when the *"fasciata"* gene is combined with genes for short or very short internodes, the protein quality traits are improved, but at the expense of protein quantity produced by the plant. The combination "*fasciata* - very short internodes-late" produces a marked improvement in the protein quality traits, but the potentiality of the *"fasciata"* gene in increasing seed and protein yield of the plant is suppressed in the presence of the genes for short or very short internodes (compare R 850 with R 853). The influence of the interaction between the recombined genes becomes obvious at the level of specific amino acids, such as lysine. It is known that methionine is the limiting amino acid for the nutritive value of the proteins of legumes. Unfortunately, it was not possible to estimate this acid by the present method of analysis, so lysine was chosen because of its nutritive value. The combination "non-*fasciata* -long internodes" gives a higher percentage of lysine, while the combination "*fasciata*-long or medium internodes" gives a much lower percentage of the same acid (compare the non-fasciated genotypes with mutant 489C, R 170 and R 853; Fig. 6).

The possibility can not be excluded that the mutated background genotypes provided by the parental mutants to the recombinants play a certain role in their potential value in breeding programmes (Emery et al. 1964). This can be demonstrated by comparing the recombinants R 852, R 25 and R 26. The three genotypes are phenotypically similar (non-fasciated, long internodes, normal leaves and not late) but the gene for long internodes expresses its potentiality in increasing the seed yield of the plant, and consequently the protein yield traits, in the background of R 852, but not in R 25 and R 26. Thus, the background of R 852 seems to be different from that of R 25 or R 26. Another example demonstrating the role of the mutated background genotype is provided by comparing the fasciated recombinants R 853 and R 170. The former recombinant surpassed the latter in many aspects. The only morphological difference is that R 853 is shorter than 170. Additional evidence of the role of differences in the background genotype is that R 852 and R 853 had been selected from a cross in which the second partner was the *cochleata* mutant (4137) derived from the Swedish variety 'Parvus' by X-rays (Blixt 1967).

Acknowledgements

The author wishes to express gratitude to Professor Dr. W. Gottschalk for providing his material and the facilities of his institute for this study. His constructive advice and criticism during preparation of the manuscript are also acknowledged. Thanks are also due to Mrs. Ursula Laux for typing the manuscript and drawing the figures.

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Received October 28, 1975
Communicated by H. Stubbe

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