# ALTERATIONS IN BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERS IN RADIATION-INDUCED MUTANTS OF GRAIN LEGUMES

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SUMMARY

On the basis of selected examples from different grain legumes the biochemically and physiologically detectable alterations in distinct characters as caused by the action of mutant genes are presented comparatively. Emphasis is directed towards the complicated interactions between different mutant genes in order to evaluate the influence of the genotypic constitution on the expression of mutated genes.

## Introduction.

Considering the point of impact of the application of nuclear techniques in higher plants with the aim of improving their productivity, the geneticist preferentially regards the induction of mutations. The enthusiasm in earlier periods of mutation breeding steered towards objectives which could be achieved in the few cases only by chance. This was partly due to the complexicity of the respective characters chosen which should be improved. Numerous investigations during the last years mainly on the molecular basis have revealed now a better understanding on the genetic components which are responsible for plant traits. They have at the same time shown, that the phenotypic variation observed in mutants are not only due to genetic events, either in structural or regulatory genes, but also from epigenetic events which may reflect altered levels of gene activity. For elucidating the numerous interactions between genetic and epigenetic factors, responsible for the phenotypic variation the availability of the extensive collections of radiation-induced mutants of different crop plants nowadays offer numerous opportunities for further improvements in crop plant production, in quality and quantity. Efforts must be started in order to learn how desired traits are controlled genetically and for solving practical and economic problems.

The cardinal point of all improvement of higher plants is to be aware that we have to introduce genetic variability into the crop plants for selecting plants with genes for desired traits. This should be the first step towards higher levels of improved adaptation and effectiveness of agricultural production.

Therefore, genetic programs for improvement of plant production should finally be base on the fact to learn how desired traits are controlled genetically in order to manipulate their interactions in the desired manner.

## Results and Discussion.

Reviewing the international literature with regard to induced mutations, including gene-, chromosome- and genome mutations, it becomes obvious that the number of experimentally produced mutants of different crop plants is still increasing (1). Mutants constitute on one side the material for increasing the frequency of desirable genes, that means the variability, which can be enlarged in future, and, on the other side the material for investigating fundamental biological processes and their interactions which is basic for their understanding. In this way many mutants are preferentially of interest for basic research.

This will be illustrated in the following schematical drawing, indicating some qualitative and quantitative traits which are influenced by mutant genes. A "standardized legume plant" is shown in order to characterize the effects concerning general traits affected by mutational events and their mutual antagonistic effects (Figure 1 ).
Roughly, we can discriminate four main areas of interest, namely
- the flower structures and the reproductive processes associated with these structures and ultimately the development of the seeds,
- the external morphology of the foliage

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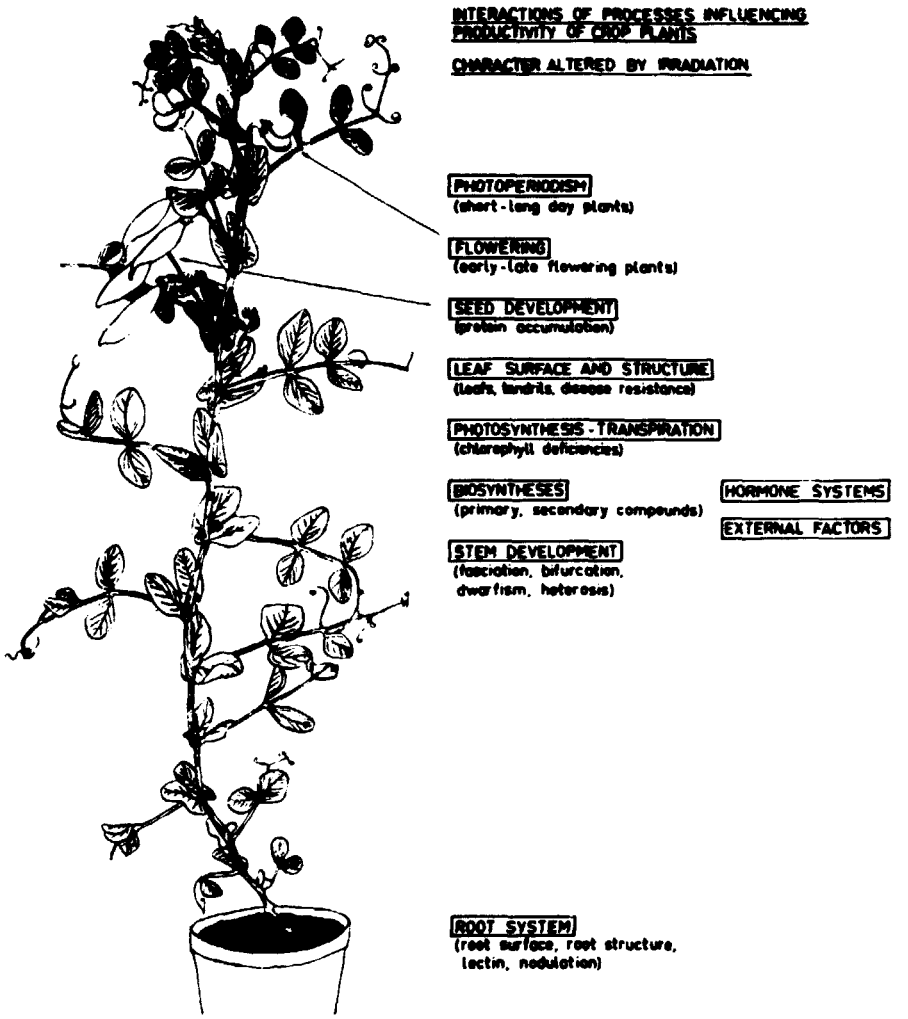


Figure 1 : Interaction of processes influencing productivity of crop plants.

leaves responsible for the regulation of entrance and exit of gases during daytime and night-time,
- the photosynthetic apparatus which is influenced by a number of internal factors inherent in the plant itself, like structure and chlorophyll content, accumulation of products of photosynthesis within the chlorophyll-bearing cells and influences of the protoplasm. There is a considerable variation with regard to the interactions of these internal factors between mutants and recombinants even when grown under the same environmental conditions. Furthermore, external factors like temperature, light, carbon dioxide and water are effective,
- the type of stems with different external and internal structure,
- the kinds of root systems, their physiological and dimensional balance with the shoot systems as well as factors responsible for establishing the building up of the symbiontic association between bacteria and roots.

Furthermore, the whole system is regulated by the combined action of hormone systems and environmental factors.

All the factors mentioned above show an enormous variation of phenotypic expression between mutants of one variety on a great diversity of sites of even one cultivar so that one can assume that there exist considerable opportunities for their improvement.

These aspects are not entirely new ones, but it must emphasized that there is a shortage in investigations for

learning "how" desired traits are controlled by mutant genes.

The difficulties for exploring the genetical background of a "desired" trait can be demonstrated, for instance, in the case of the storage proteins of legume seeds.

Considering the amount of seed proteins in grain legumes, numerous attempts have been made for improving the seed protein production by inducing mutants in different cultivars in order to select for that trait. Summarizing the results of numerous research groups one can state that, although for the expression of that trait a considerable variation could be achieved, the latter did not exceed essentially the boundaries of the respective genera.
This can be demonstrated for a large collection of pea mutants, as shown in figure 2.

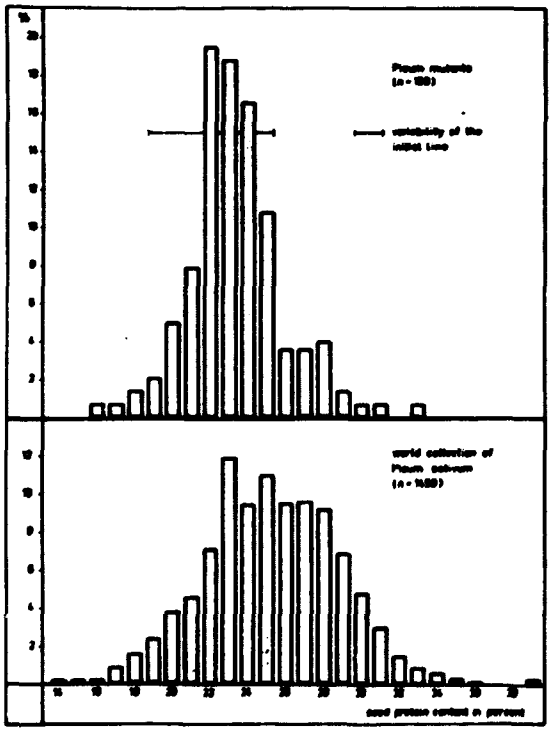

Figure 2: Variation in the mean values for the character "protein content of the seeds" in 138 mutants of Pisum sativum as compared with 1450 genotypes of the world collection.

As can be seen, there exists a large increase in variation by inducing mutations for protein content but it remains within the limits of the genus Pisum.(2). The same is valid for other grain legumes as shown in figure 3.
The ranges of the character seed protein as shown by horizontal lines indicate at the same time the variabiliy depending

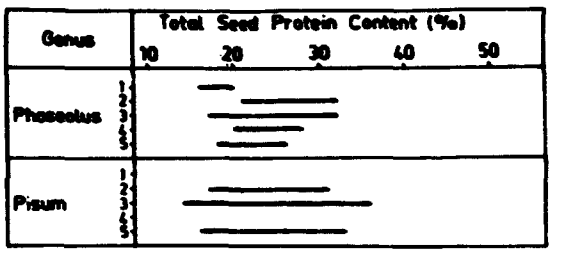


Figure 3: Variability in seed protein content in Phaseolus and Pisum.

on the genotypes analysed, environmental factors and the intensive management of the crop plants(3).Thinking about these findings, we have only reached the surface of the problems. The seed protein content and composition is a typical quantitative character with a continuous variation and controlled by multigen families. Therefore, we cannot expect that single mutational events will contribute great effects in total seed proteinproduction. Important traits like these are extremely complex. They are the result of many interacting components (4). We need without doubt much more time before we understand these characters well enough for employing them successfully at the cellular and molecular level. Even many less complex aspects of plant function are still not well understood. As little is known about the molecular structure and organization of the genome or factors regulating individual gene expression in plants, the first aim should be to identify and understand rate limiting steps in metabolism and growth for crop plants. A basic knowledge in genome structure and gene expression must be developed in order to realize the potential for transferring genes from one species to another, altering DNA-sequences and developing metabolic and growth regulatory products that promote or arrest the plant system. Therefore, it is necessary to use techniques for mutant selection generating new genetic variants within a crop species , characterizing them at different levels in order to overcome many obstacles before genetic engineering can become useful for crop improvement.The practical value will,furthermore, vary from crop to crop.

For elucidating the induced variability in crop plants electrophoresis, especially isozyme technology is appearing as a powerful analytical tool which shows aspects of characters not yet explored within and between species of crop plants. (5). In this connection we are working mainly with seeds as differentiated organs which constitute the endpoint of an integrated developmental process. The genetic information for e.g. storage proteins appears to be repressed in all tissues except for certain developmental stages in the cotyledons when it is actively expressed. Studies concerning the kinetics of protein incorporation in the

cotyledons suggest that numerous biological processes contribute to differences in protein content and quality among genotypes within species and higher taxonomic groups(6). The analysis of the genetic structure of different mutants requires a discrimination as close as possible to the DNA level. Since plants possess large amounts of variation in genes specifying isozymes, the isozyme-technique has proven to be a suitable measure for genetic variation. In order to find out suitable genotypes it is always necessary to screen large numbers of mutants for collecting data. For studying larger samples of seed material we have developed a rapid method for identification and characterization of even minor differences in isoelectric points of soluble seed proteins.It is based on the physiologically conditioned leakage of proteins from seeds within the first minutes after seed imbibition. For analysing these soluble proteins according to their isoelectric points, small tissue sections of dry cotyledons are directly extracted on the gel during isoelectric focusing. The insertion of the tissue into the electric field is accomplished by placing a quarter of one cotyledon on an agarose gel. The gel is then placed on the precooled plate of a flat bed apparatus.Isoelectric focusing is performed for 2o min. with 5 mA. At the end of this time the tissue sections are removed. Afterwards the electrophoretic separations run with 5 W for 7o-9o minutes. The detection of the protein bands as well as the isozymes is performed by the usual reaction mixtures(7). The viability of the seeds analysed is not affected. This is especially of great value if there are only small amounts of seeds available. The variations, furthermore, are not due to the extraction procedures.

Investigations were performed on 16 genotypes of the genus Pisum, including "species" and mutants(8).The results are shown in figure 4.

(1: Pisum abyssinicum; 2: Pisum elatius; 3: Pisum Pulvum; 4: Pisum sativum ssp. transcaucasicum; 5:Pisum sativum ssp.sat. con.sat.,var.episcopi; 6:Pisum sativum ssp.sat.con.sat.var.episcopi, opal;7: Pisum sativum,ssp.sat.con.sat.var.fabiformae; 8: Pisum sativum ssp.sat.con. speciosum var.capucin; 9:Pisum syriacum; 1o: Pisum sativum ,cv. Paloma, initial line for mutations; 11: Pisum sativum cv. Kaliski, initial line for mutations; 12: Pisum sativum, mutant orange pod; 13: Pisum sativum, mutant orange cotyledon; 14: Pisum sativum, mutant butterfly stipules; 15: Pisum sativum, mutant early flowering; 16: Pisum sativum, mutant brown leaf spots).

The schematical drawings of the banding patterns for the non-specific esterases show a distinct variation with regard to the enzyme polymorphism in different genotypes.In the case of non-specific esterases it is known that differences in the patterns can be caused by post-translational modifications either of genetical or environmental factors. In this way

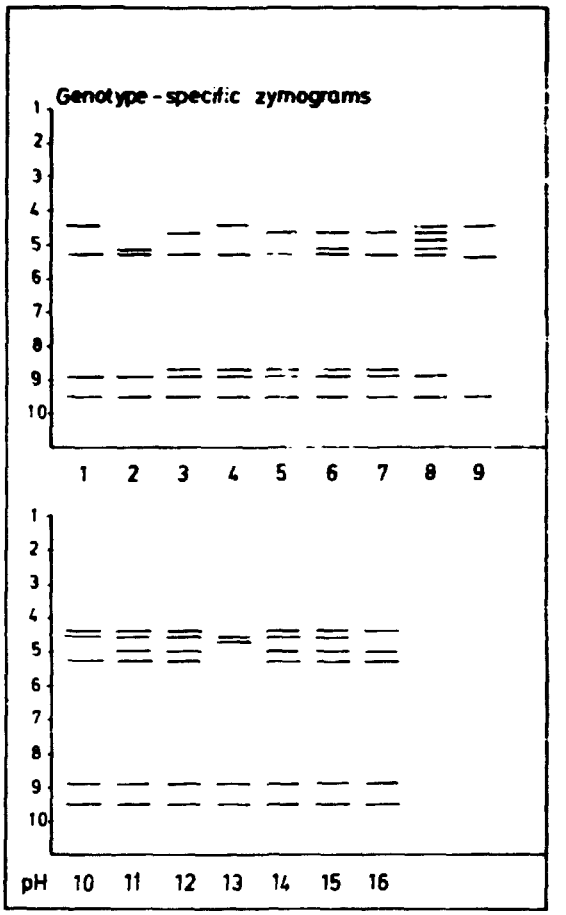


Figure 4: Genotype-specific zymograms after direct isoelectric focusing.

we can look for further parameters concerning the biochemical makeup of the respective genotypes. The broad variability in isozyme patterns within the genus Pisum is astonishing since it is known to be monospecific. Pisum species can be regarded more or less as ecotypes of one ancestral species. Even in crosses of Pisum sativum with Pisum fulvum showing greater genetic divergence than other races, with other peas, the progenies are at least partially fertile and all characters studied were transferred without difficulty. In this connection the question concerning morphological-biochemical relationships within the genus Pisum become interesting. This genus represents another example that strong biochemical variation is not coupled with corresponding morphological differences.

The zymograms can also be used for elucidating climatic influences on plant development , as shown in figure 5.

Plants of the initial line of our mutant collection and one early-flowering mutant (46) were grown in the experimental fields in Brazil and Germany. The harvested seeds were analysed as described above. The

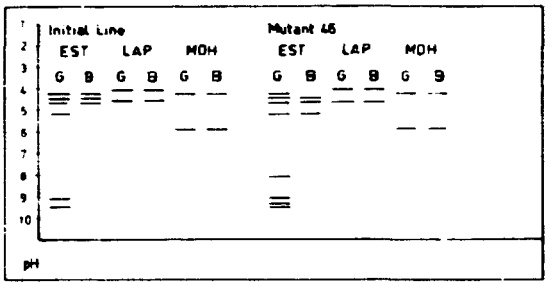


Figure 5: Comparison of the zymograms of EST,LAP,MDH of two pea genotypes(IL,mutant 46) grown under different climatic conditions, (B = Brazil, G = Germany).

study of the multiple forms of enzymes provides in this way a source of information not only for the genetic basis of isozymes but also for the matter of regulation of protein incorporation into seeds under different environmental conditions.
The characterization of genotypes also can be useful for discerning between genotypes showing distinct physiological properties. This can be shown in the case of accessions of Phaseolus vulgaris exhibiting differences in the agglutinating activity of their lectins against red blood cells(7).This is demonstrated in figure 6.
The banding patterns for proteins and for the isozymes of the ADH and EST show clear differences between distinct genotypes of a large collection of Brazilian selected lines, adapted to marginal environments, for disease resistance, wild types and primitive forms, mutants and

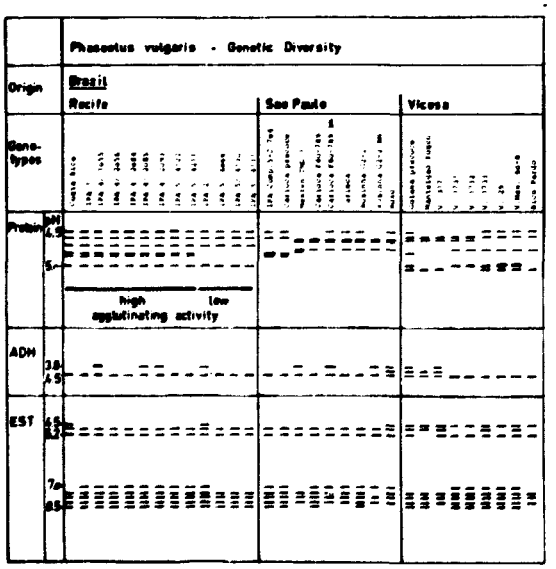


Figure 6: Genetic diversity in Phaseolus vulgaris.

high yielding varieties. It must be emphasized that in the drawings different staining intensities are not considered in terms of quantitative aspects of seed protein genetics. It is only referred to numerical bandings.
But electrophoretic methods are not only convenient for characterizing gene mutations but also for chromosomal mutations (9). This will be shown in figure 7.
The genotypes are characterized by the following traits: Initial line, mutants 17 A and 488 are homozygous for a reciprocal translocation, as indicated in the karyogram(left side). These genotypes should help to get insights into the mutual influences of genes as a consequence of position effects as well as gene-dosage effects. The SDS- protein patterns show distinct differences. They become clearer in considering the isozyme patterns of the same genotypes, as shown in figure 8.
Considering the patterns of the aminopeptidase, an enzyme which is closely involved in the mobilization of the seed storage proteins during germination, we can state differences with regard to the number of isozymes and the staining intensities of comparable bands. More drastic changes in isozyme patterns can be found for MDH while the other systems are more or less unchanged. MDH is an enzyme which seems to be involved in reactions concerning the enzymatic adaptation.
The results show that by the action of mutated genes a considerable heterogeneity is induced which can be exploited for characterizing distinct genotypes since polymorphic forms of enzymes may have adaptive significance.Furthermore, the knowledge of the presence of enzymatically active proteins found in seeds should constitute a useful basis for studying the metabolic events during seed germination.
Provided with selected suitable genotypes the electrophoretic analysis by direct isoelectric focusing of tissue sections allows a study of the inheritance of distinct proteins, as shown in figure 9.
It is an example for the possibility of evaluating genetical behaviour of single proteins in the F2 generation of distinct genotypes. The example concerns the inheritance of proteins in seeds of a F2 progeny of Pisum sativum from a cross between parents having normally coloured yellow and orange cotyledons, showing five different coloured segregants.
In order to test the genetic control of the photoperiodic and thermoperiodic reactions, pea mutants and recombinants which are homozygous for different mutant genes were grown in the phytotron. In general, pea plants are day-neutral so that the conditions of long-day, short-day can be analysed with regard to the expression of distinct mutant genes. Independently from the expression of genes responsible for growth and flowering, ripe seeds from numerous genotypes were analysed electrophoretically with regard to their protein- and isozyme patterns. We

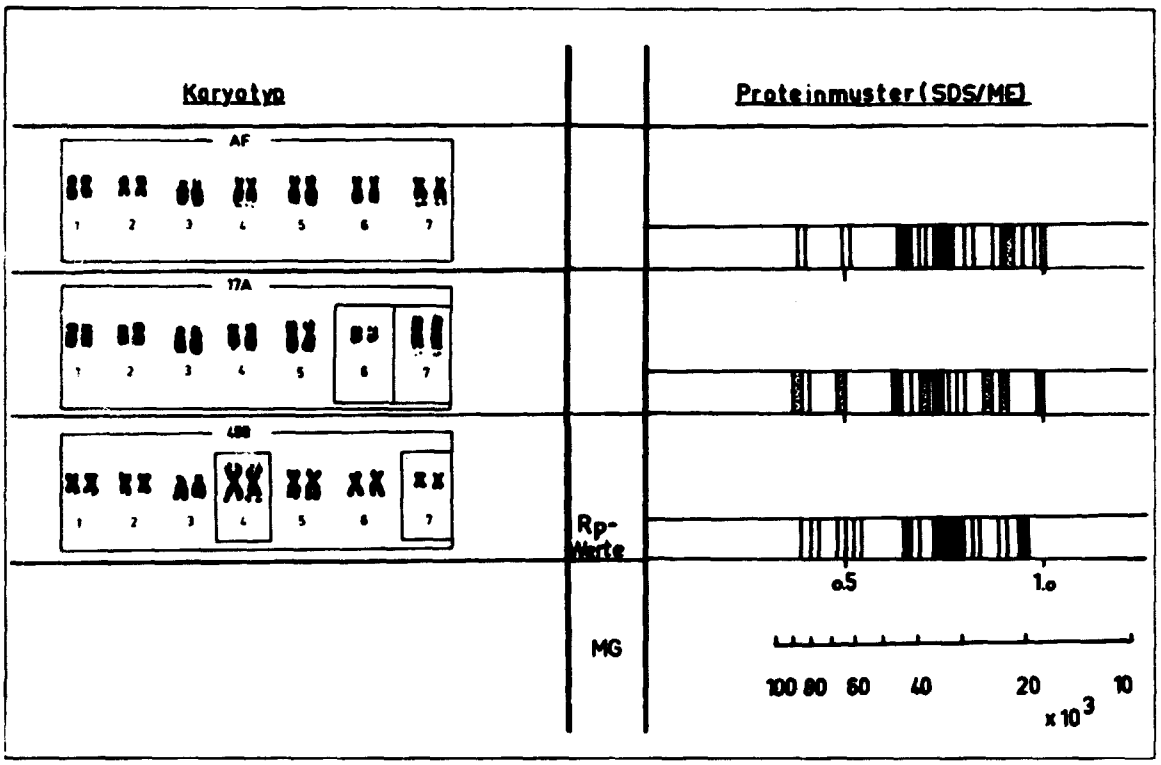


Figure 7: SDS-protein patterns from seeds of mutants homozygous for reciprocal translocations.

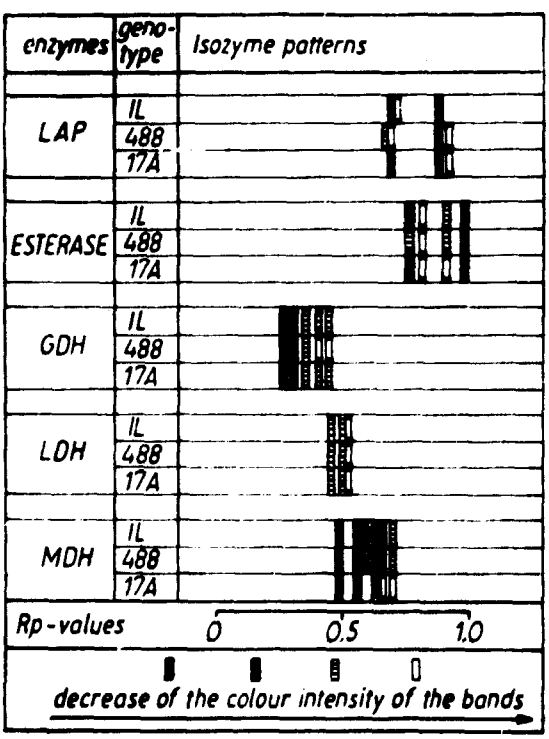


Figure 8: Isozyme banding patterns in seeds of different pea genotypes.

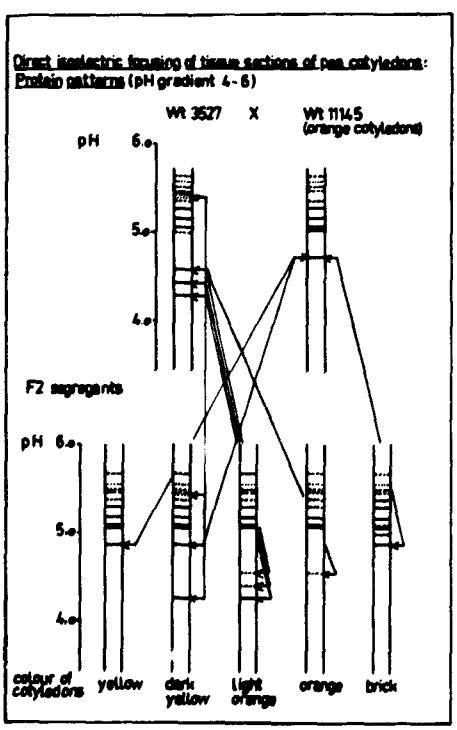


Figure 9: Direct isoelectric focusing of tissue sections of pea cotyledons: protein pattern (pH-gradient 4-6).

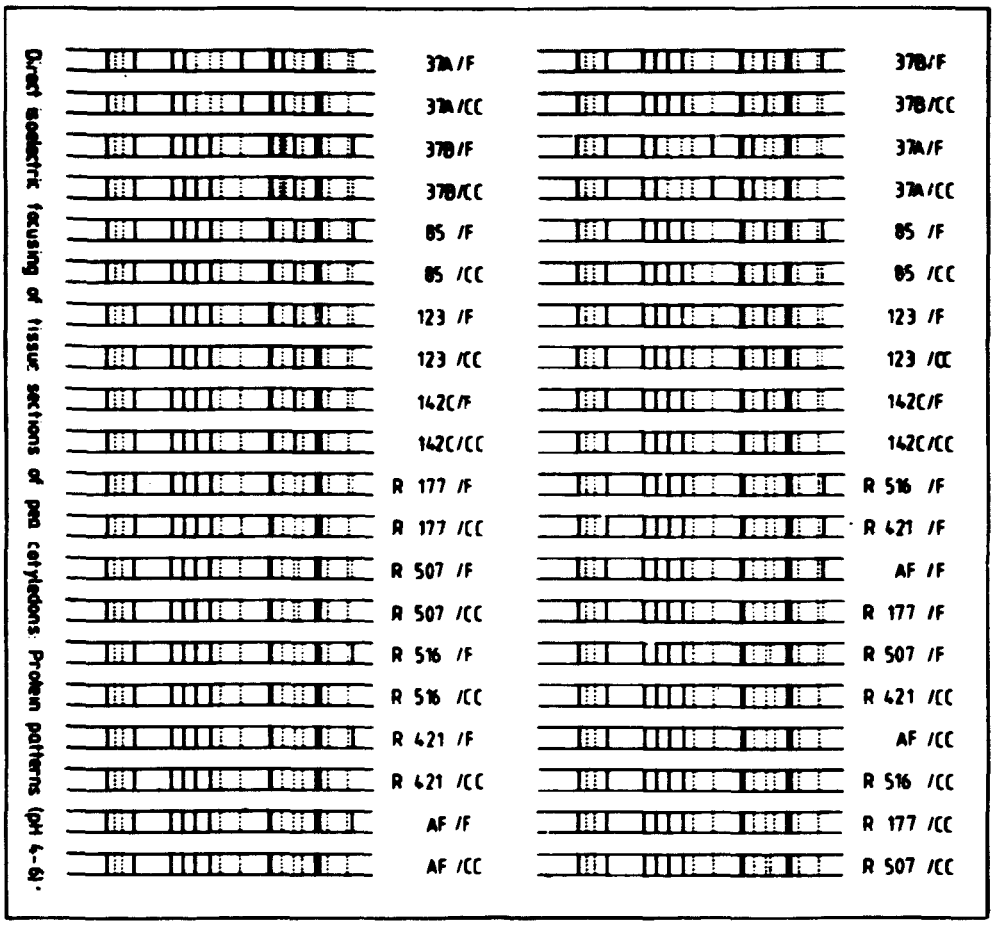


Figure 1o: Direct isoelectric focusing of tissue sections of pea cotyledons: protein patterns (pH gradient 4-6).

used seed material from plants grown under long-day conditions (12h light, temperature during day 25°C, during night 15°C) and compared the material with that grown in the experimental field. The results are shown in figures 1o and 11.

In the upper part of both figures the patterns are drawn schematically according to criteria of increasing staining intensities of the respective bands, in the lower parts the banding patterns are grouped for comparison according to the genotypes grown in the climate chamber and the experimental field.

It can be seen that, although all genotypes shown uniformity in many characteristic bands - indicating the common genetic basis - distinct quantitative differences can be seen in the whole spectrum of patterns (from left to right). The comparison of the protein- and isozyme patterns from seeds deriving from experimental field and from the phytotron reveal also distinct quantitative differences with regard to numerous bands, indicating a different expression of the respective genes which code for them. In this way the influence of environmental factors on the regulation of protein incorporation can be analysed. Of great interest are genotypes which show qualitative differences, indicating drastic changes in gene expression. In this way some sort of "selection filter" is available for the rapid selection of mutant genotypes which can be analysed in detail for the understanding of regulatory processes.

The results are manifold and shall not be discussed more in detail. It remains to state that we have the possibility to use these techniques for collecting desired genotypes, for exploiting their genetical potential and elucidating the basic principles e.g. of stress injury and resistance, since environmental stresses like heat, drought, cold, salt, toxic ions are the main limiting factors restricting crop production. But we are in the early beginning of such investigations.

The quantitative changes observed in the seed material deriving from different

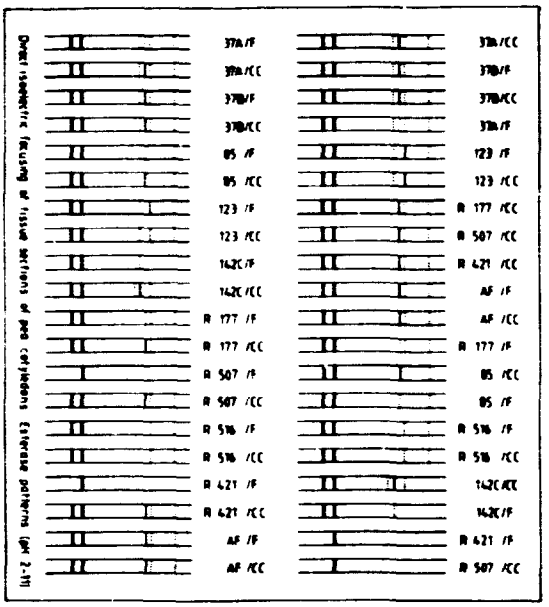

Figure 11: Direct isoelectric focusing of tissue sections of pea cotyledons: esterase patterns (pH gradient 2-11).

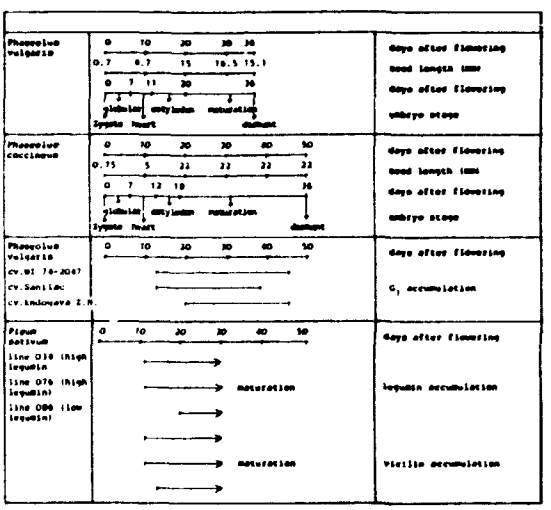

Figure 12: Developmental time table and protein accumulation in seeds of Phaseolus and Pisum.

growing conditions may be due to genetically conditioned regulatory mechanisms involved in seed development that are responsible for the final protein composition. Morphogenetic studies on the embryogeny of Phaseolus show that seed development passes through nine distinct stages from fertilization to maturation, as shown in figure 12.
This schematical drawing reveals the different species-specific time-courses of seed ripening as well as genotype-dependent accumulation of distinct storage proteins in the seeds.The elucidation of the many interactions between flowering time, duration of seed development after flowering which are genetically controlled and therefore modifiable characters as well as the onset of protein synthesis, its kinetics and its termination are a further field for application of induced mutants(6). The last plant system to be regarded in this review is the root system which also shows a great variability with regard to the extent in different mutants, as shown in figure 13.

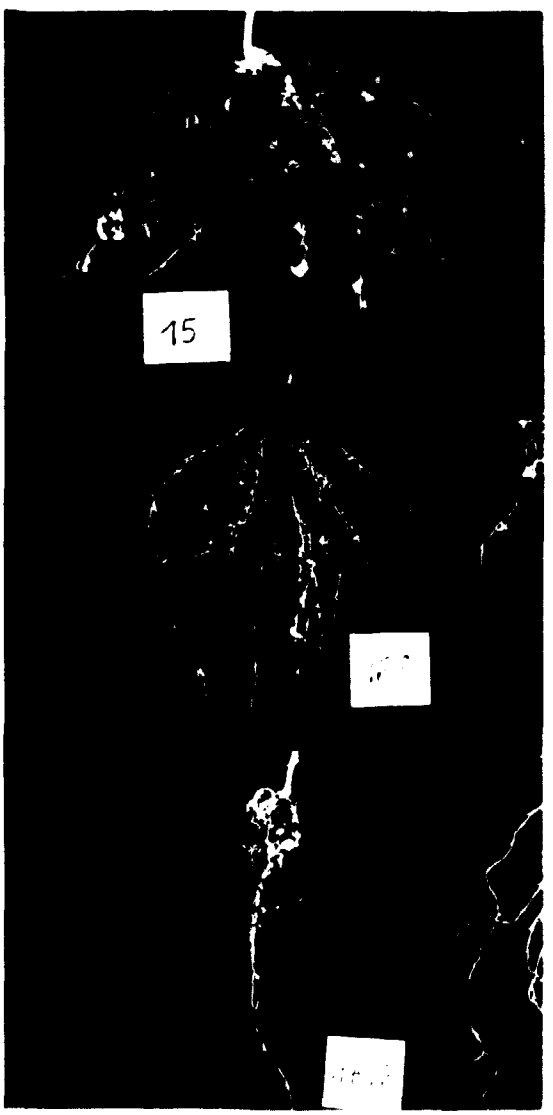


Figure 13: Root systems of mutants of Pisum.

But a more important point in legumes is the symbiontic nitrogen fixation within highly differentiated root nodules. They are formed by interactions

of Rhizobium with legumes. Considering the nodule formation in numerous mutants of Pisum a considerable variation could be observed.This will be demonstrated in figure 14.
The formation of the nodules that are effective in nitrogen-fixation depends on the side of the plant from genetic informations within the respective plants. It is known that one host protein is responsible for protection of the oxygen-sensitive nitrogenase, but other host proteins specific for the symbiosis are scarcely known(1o). But the understanding of the functioning of until now speculative "symbiosis genes" would help in solving the problem of an enhanced nitrogen fixation efficiency. The other partner of the symbiontic association , the bacteria, can more easily be manipulated in the laboratorium.
The potential for improving nitrogen-fixation through genetic manipulation

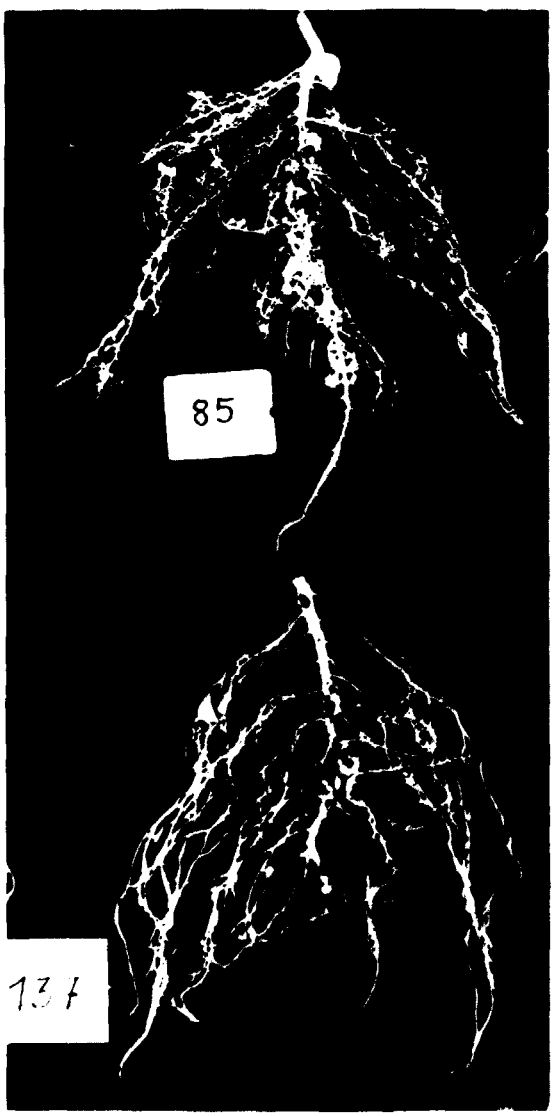


Figure 14: Nodulation in mutants of Pisum.

of host plants is not well-defined because of the fact that the processes and the genes involved are not known. In this case, perhaps, the reactions of mutants of legumes lead to a better understanding, avoiding further speculations on the feasibility of the projects mentioned and the extension of symbiontic nitrogen-fixation in plants which do not benefit from the processes described.
In this connection another group of proteins occurring in in the seeds and the roots of legumes need consideration: the lectins. They seem to be important determinants of host range specifity(6) in Rhizobium-legume symbiosis.But the hypothesis that they are responsible for recognizing the symbiontic Rhizobia is too simple to explain the relationships between bacteria and legume roots. A screening of the occurrence of lectins and their electrophoretically separable subunits is performed in cooperation with colleagues in Piracicaba in Phaseolus , in Bonn with other legumes. The first results have shown a genotype-specific dependent large heterogeneity. Also that variation must be analysed in convenient genotypes more in detail in order to be able to connect occurrence and biological function of th lectins more reliably.

Concluding remarks.

Summarizing the various aspects of work performed on radiation-induced mutants in legumes, one can state that, historically, the strategies used for screening and generating strains producing large amounts od e.g. seed proteins have included, primarily, random mutation and selection. This method, although successful in many cases has a moderate likelihood of general success.It is necessary to replace these methods by more directed and rational techniques in exploiting the mutant material available. These procedures will be more effective than random screening for selection of desired characters. Studying the parameters which affect the regulation of biosynthetic pathways in distinct genotypes, mutants or recombinants, will enlarge our present limited knowledge and understanding of the genetic components responsible for plant traits, the presupposition for a variety of practical applications, including plant genetic engineering. But this work requires combined efforts, persistence and skill.

ALTERAÇÕES NOS CARACTERES BIOQUÍMICOS E FISIOLÓGICOS POR MUTAÇÃO INDUZIDA POR RADIAÇÃO EM GRÃOS DE LEGUMINOSAS.

SUMÁRIO

Com base em exemplos selecionados de diversos grãos de leguminosas, faz-se uma comparação das alterações passíveis de determinação bioquímica e fisiológica, causadas pela ação de genes mutantes, em caracteres distintos.

Dá-se ênfase às complicadas interações entre genes mutantes diferentes a fim de se avaliar a influência da constituição genotípica na expressão de genes mutantes.

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