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ENZYME DISTRIBUTION IN SOME RADIATION-INDUCED MUTANTS OF *PISUM SATIVUM* WITH DIFFERENT INTERNODE LENGTHS

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Abstract—The activities of peroxidase, catalase, laccase, and tyrosinase in radiation-induced homozygous *Pisum* mutants have been investigated. The mutants differ from the parental stock only with respect to internode length. A direct relationship could be found between internode length and enzyme activity: the shorter the internodes the higher the activities of all four enzymes. An exception from this pattern in enzyme activity is a mutant with extremely long internodes; it shows a lower peroxidase activity while the activities of all other enzymes is greater than in the original line. The anomalous results are interpreted as being due to metabolic imbalance.

INTRODUCTION

THE IMPORTANCE of changes in plant proteins, particularly in enzymes involved in differentiation, has been stressed by several authors.[1-7] Mathan and Cole,[4] working on a leaf shape mutant in the tomato, found significant differences between normal and mutant plants with respect to the activities of four oxidative enzymes: peroxidase, catalase, tyrosinase, and laccase. The mutants exhibited higher activity than the normal type in all four enzymes. The same authors showed a dosage effect of the mutant gene with respect to morphology as well as to enzyme activity, in that the heterozygote was intermediate between the initial line and the homozygous mutant.

Similar results were obtained in studies on the peroxidase activity in homozygous dwarf tomato mutants[6] as well as in dwarf *Zea mays* plants.[2] It has been shown that, generally in all dwarf plants, there is a clear increase in the activity of peroxidase. There thus exists a relationship between genetically controlled morphological changes in higher plants and the activities of several enzymes.

Therefore, it was of particular interest to study the enzyme activities in some radiation-induced mutants of a pea line. These mutants differ from the original line only in one trait, namely, with respect to their internode lengths. If there is a correlation between mutant genes affecting this character and enzyme activity, the examination of various parts of such plants should provide some further data on the function of the genes in question.

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RESULTS

No significant differences in enzyme activity in the stem could be found within the same genotype and the results are given as the average of seven determinations. In order to have a better comparison of our results we have taken the mean values of the normal type as 100 per cent, per 1 g dry weight, and calculated the corresponding values obtained from the

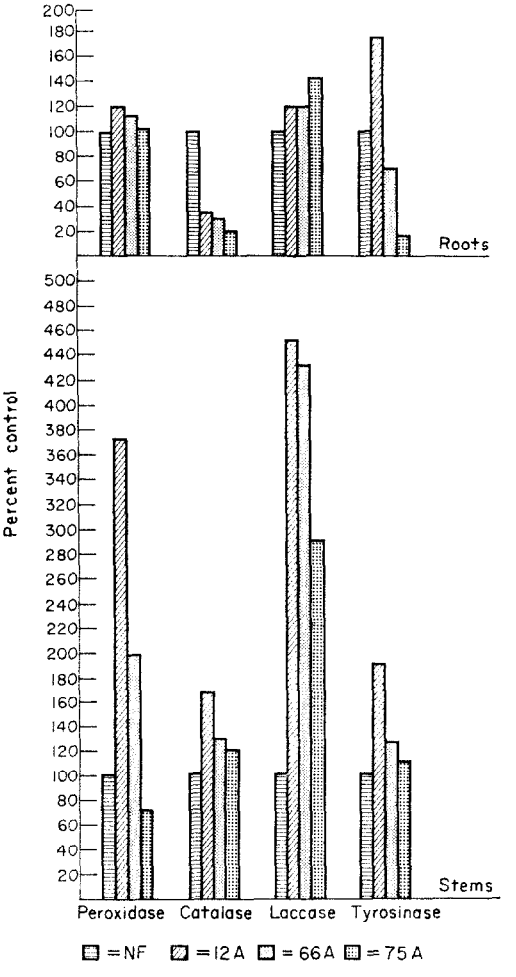

FIG. 1. DISTRIBUTION OF PEROXIDASE, CATALASE, LACCASE AND TYROSINASE IN THE STEMS AND THE ROOTS OF DIFFERENT PEA LINES.

mutants as a percentage of those of the initial line. The characteristic changes in the activities of four oxidative enzymes in the stems and the roots of the normal type (NF) and the mutant lines (12A, 66A, 75A) are illustrated in Fig. 1.

In the stems of all mutants there are distinct differences in the activity of any one of the oxidative enzymes compared with the initial line. The activity of peroxidase is greatly increased in the dwarf mutant, No. 12A, with the shortest internodes. The activity is increased by a factor of 3·7. The semi-dwarf mutant, No. 66A, shows a twofold increase. That means

that there is a direct relationship between gene-controlled internode length and peroxidase activity. The shorter the internode, the higher the activity. The dominant mutant, 75A, whose internode length is doubled compared with the initial line, on the other hand shows a 30 per cent lower peroxidase activity than the normal type.

The activities of the other three enzymes, catalase, laccase, and tyrosinase, are increased in all mutant lines. The activity of laccase, which exceeds that of peroxidase is remarkably high. On comparing the enzyme levels in the mutants, there is a "descending order" in enzyme activity depending on internode length. With increasing internode length there is a steady decrease in enzyme activities. One may conclude that there exists a dosage effect between the mutant genes in question and oxidase activities, as already noted for tomato mutants.[4]

The clear-cut relationship between internode length and activity of oxidative enzymes found in the stems does not extend to the roots. There is only a small increase in peroxidase activity in the dwarf mutants, but no change could be detected in mutant No. 75A. Catalase activity is reduced in all three mutants, that of laccase raised, whereas tyrosinase has only a higher activity in the roots of mutant No. 12A; the two others show a reduction. These data reveal that the mutant genes influence the enzyme activities in the roots but not as drastically as in the stems.

DISCUSSION

Since the mutants under study differed from the parental line only in internode length, it was to be expected that the differences in enzyme activities would be found mainly in the stems. An analysis of the distribution of the four oxidases shows indeed that there are significant differences in enzyme levels (Fig. 1). The increase in enzyme activity in the stems shows that there is a correlation between the mutant genes and oxidase activities.

Large differences in peroxidase activity are found between the two dwarf mutants and the initial line. In each case a single recessive gene leads to a gradually decreased internode length and a corresponding increase in peroxidase activity. These findings are in agreement with earlier results, where dwarf mutants of different plant species have been found to exhibit higher activities of peroxidase than tall plants.[2, 4, 6, 8] The physiological role of peroxidase in plants is not yet fully understood, because of its broad hydrogen-donor specificity[9] and its occurrence as multiple forms.[10] Furthermore, the enzyme is implicated in respiration,[11] in fatty acid oxidation,[12] in lignification,[13] and in the synthesis and breakdown of indole-3-acetic acid.[14, 15] Ray[16] has shown that peroxidase oxidizes IAA *in vitro*, thus rendering it inactive. But whether the peroxidase plays this role also *in vivo* is unknown. Furthermore, the studies of Galston *et al.*[17] show that the IAA–oxidase system may be inhibited by catalase.

In the light of these results, one can argue that in these pea mutants the high peroxidase

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activities lead to an increased destruction of IAA. The amount of catalase, which would inhibit the IAA–oxidase system is, obviously, not available in the mutants studied. On the other hand it is possible to normalize not only the growth but also the high peroxidase activity in dwarf peas by application of gibberellic acid.[2] That means that the observed changes in peroxidase activity in our one-gene-mutants are not the primary consequences of the effect of the mutant gene in question. The results obtained with the other enzymes studied support this. The significance of these enzymes in plants is not yet clear. A growth-regulatory role[4] for tyrosinase, phenoloxidase, and peroxidase is supported by results obtained by studying isoenzyme patterns of peroxidase in these mutants (Müller, unpublished). Marked differences in isoenzymes in roots, stems, and apex are apparent, but there are no great differences between the mutants (cf. Ref. 6). In contrast to the findings of Mathan,[7] we have found laccase and tyrosinase in peas.

While in the stems there are clear increases in enzyme activities, in the roots the situation is more complex (Fig. 1). We find only small differences in peroxidase and laccase activities compared with the normal type, the catalase activity is much smaller and the tyrosinase activity only a little higher. Evans and Alldridge[6] found similar results in dwarf peas. Obviously, the mutant genes exert only little influence on the enzyme activities in the roots.

The mutant No. 75A, which has extremely long internodes, is an exception to the above-discussed pattern of enzyme activities. The relationship between peroxidase and internode length is confused by the relatively higher activity of catalase (Fig. 1). If the catalase is able to inactivate the IAA–oxidase system[17] the higher activity of calatase in this mutant and the lower activity of peroxidase may be the cause of the extreme elongation of the internodes. But it is difficult to understand why this mutant exhibits, like the dwarf mutants, significantly higher activities in all the other oxidative enzymes. Differences in the proportions of the four enzymes may possibly lead to some kind of metabolic imbalance, causing the observed growth anomalies. Further studies on the unusual mutant are in progress.

MATERIAL AND METHODS

1. *Plant Material*

The pea lines analysed in this study came from the mutant collection of Prof. Dr. Gottschalk. We used the parental line, a pea cultivar 'Dippes gelbe Viktoria', and three homozygous mutants differing only in internode length. The mean values of total plant heights and the internode lengths are given in Table 1.[18]

TABLE 1. MEAN VALUES OF TOTAL PLANT HEIGHTS AND INTERNODE LENGTHS (ACCORDING TO GOTTSCHALK[18])

Mutant no.	Trait	Total height (cm)	Internode length (cm)
Initial line	Normal	88·5 ± 1·17	4·5
12A	Dwarf	31·8 ± 0·88	1·9
66A	Semi-dwarf	55·0 ± 2·10	2·6
75A	Extremely long	117·8 ± 3·21	7·9

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2. *Growing Conditions*

The seeds were sterilized by soaking them for about 30 min in 1% NaClO. They were grown in a soil–sand mixture (4:1) in beakers (1000 ml) under controlled conditions: day 12 hr, 22°, 65 per cent relative humidity, light intensity 4000–7000 lux at plant height. 14 days after sowing, the plants were harvested. At that time they had reached the following heights: NF = 20–22 cm, 12A = 10–13 cm, 66A = 13–16 cm, 75A = 33–38 cm.

3. *Preparation of Homogenates*

The experimental techniques for preparing homogenates from stems and roots were almost identical to those described by Mathan and Cole.[4] In contrast to these authors, we related the readings of the enzyme activities not to the fresh, but to the dry weight of comparable plant material, oven dried at 85° for 24 hr. The activities of the following enzymes were studied photometrically, according to known methods: peroxidase,[19] catalase,[20] laccase,[21] tyrosinase,[22] with Dopa as substrate. For each biochemical assay eight plants were used, the experiments were repeated seven times.

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