INVERSE EFFECTS OF GIBBERELLIN ON PEROXIDASE ACTIVITY AND GROWTH IN DWARF STRAINS OF PEAS AND CORN [1]

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Several investigations have indicated that plants possessing a dwarf habit of growth show a greater peroxidase activity than their normal counterparts (4, 8). This seems to be true even where the plants are otherwise genetically identical. Thus, in a cross of Phaseolus yielding both dwarf and giant progeny, the dwarfs had a greater and the giants a lesser peroxidase activity than the normal parent types (4). In a series of hybrids in Epilobium the increase in peroxidase activity paralleled the reduction in height (6). In view of the fact that gibberellic acid (GA) is known to change the phenotype of certain strains of dwarf peas and corn to the normal (1, 5), it was of interest to see whether such dwarfs treated with GA would respond by manifesting a lower peroxidase activity as well as an increased height. Such inverse effects of GA on peroxidase activity and growth were obtained in these experiments.

METHODS AND MATERIALS

The pea varieties used were obtained from Associated Seed Growers, Inc., New Haven, Connecticut. The dwarf pea, Progress # 9, responds well to GA, but its genetic differences from the tall type, Alaska, are partially unknown and probably complex. The corn used was a segregating population (kindly provided by Dr. Bernard O. Phinney of the University of California at Los Angeles) which yielded 25 % dwarf-1 mutants, which differ from the tall plants by a single gene (5). The seeds were sown in vermiculite in 4 × 4-inch plastic containers and automatically subirrigated at 12-hour intervals with a solution of Hyponex (a complete commercial salt mixture for plant nutrition sold by the Hydroponics Chemical Company, Copley, Ohio) 120 g/100 l. The plants received a photoperiod of 16 hours and a light intensity of 1200 ft-c provided by mixed fluorescent and incandescent lights. The peas were grown at 17° C and the corn at 23° C.

Fourteen days after planting the peas were treated with gibberellic acid. One microgram GA (kindly supplied by Dr. P. W. Brian, of Imperial Chemical Industries) in 0.003 ml ethanol was applied to the stipules enclosing the 5th internode. At various times after treatment the plants were harvested and the 5th internodes excised and ground with a chilled mortar and pestle in 0.025 M, pH 6.1 KH_2PO_4–Na_2HPO_4 buffer (1 g fresh weight converted to 10 ml of brei). The homogenates were then stored in the frozen condition in Lusteroid centrifuge tubes until further use. The peroxidase activity was unchanged by such storage.

Seven days after planting the corn was treated with 1 μg of GA. This was applied in 0.003 ml of ethanol to the tip of the 1st leaf as it emerged from the coleoptile. Two and four days after treatment the plants were harvested and sections of the basal third of the 1st leaf sheath excised and homogenized as above. In each experiment segments from 20 to 30 plants were combined into 1 homogenate.

The tissue homogenate was centrifuged at 2000 G for 10 minutes and the supernatant made up to standard volume and used for peroxidase and protein nitrogen determinations. Duplicate determinations of the specific activity were made on each homogenate. Peroxidase determinations were made by the technique of Siegel and Galston (7). An aliquot of the homogenate was placed in a colorimeter tube; 0.1 ml of 0.5 M pyrogallol and sufficient buffer were added to bring the volume to 9.0 ml; at time zero 1.0 ml of 5×10^{-3} M H_2O_2 was added and the tube rapidly inverted. The formation of purpurogallin was followed in the colorimeter with readings at 20, 40 and 60 seconds and the activity taken as the rate of color formation over 1 minute since the rate was linear within this time. The protein nitrogen determination was carried out after the method of Galston and Dalberg (2). The protein was precipitated with trichloroacetic acid and subjected to a micro-Kjeldahl digestion followed by Nesslerization.

RESULTS

The effects of GA on growth and peroxidase activities of the dwarf pea internode and dwarf corn sheath are shown in table I. All activities are expressed as micromoles of purpurogallin formed per minute per microgram protein nitrogen. In the dwarf pea, Progress # 9, plants treated with GA showed a peroxidase activity depressed to 70 % that of the untreated controls. This difference is significant at the 1 % level 2 days after GA application. This difference between controls and GA-treated plants was evident 24 hours after treatment and persisted through the 4 days of the experiment, although the peroxidase level of the internode changed as it elongated and matured. In the tall pea, Alaska,

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plants treated with GA showed neither an increased internode length nor a lower peroxidase level. These peroxidase activities were not changed by dialysis, indicating that the differences in activity were not due to dialyzable inhibitors or cofactors.

The peroxidase activity of dwarf-1 corn plants is higher than that of normal plants, the difference being greatest in the mature tissues of the leaf sheath. Treatment with GA lowers the peroxidase activity of the dwarf to that of the normal. A comparison of treated and control dwarf leaf sheaths 2 and 4 days after GA application shows that the peroxidase level rises in both, but that the GA-induced difference becomes greater with time. After 4 days, the differences are significant at the 1 % level (table I). As with the peas, dialysis of the homogenate did not change the peroxidase activity.

Although treatment with GA increased the length of the normal leaf sheath, it produced no significant effect on peroxidase level. GA was also without effect on the peroxidase level of the unexpanded sheath of the 2nd leaf of dwarf plants 4 days after treatment, implying that GA effects on peroxidase activity are exerted, during active growth of dwarf cells.

Discussion

The pea internode and corn leaf sheath both show a reduced length in the dwarf varieties used here. Upon treatment with GA both these tissues show a marked elongation and decreased peroxidase. Since peroxidase activities were based upon the amount of protein nitrogen present and dialysis did not change the activities, it is assumed that the differences found in activity reflect differences in enzyme concentration and not the presence of dialyzable cofactors or inhibitors.

In the dwarf-1 mutant of corn a single genetic factor results in both decreased size and increased peroxidase content. This is in agreement with previous investigations which found that dwarf mutants of Phaseolus, Zea, Nicotiana, and Tropaeolum contained greater amounts of peroxidase than the taller plants (4, 8). In the present investigation of the peroxidase levels of 7 pea varieties, only in the variety Progress # 9, was there a correlation between increased peroxidase level and decreased size. Genetic control of internode length in peas is complex, and since the precise genetic characterization of the varieties used was not available, it is not possible to relate either dwarfism or peroxidase activity in these peas to a single gene.

The application of gibberellic acid to the dwarf corn made the dwarf phenotypically normal not only with respect to height but also with respect to peroxidase level. During the elongation and maturation of the leaf sheath the peroxidase level rises. The lower peroxidase level of the GA treated plants appears, in this instance, to be due to an inhibition of this increase.

The question may legitimately be raised whether the GA induced depression (or prevention of increase) of peroxidase activity is causally connected with the increased growth, or whether it is merely one of many biochemical consequences of the altered growth rate. This question cannot be answered at the moment. All that can be said is that the observed depression of peroxidase activity relative to the untreated controls occurs concurrently with the observed increases in growth.

In the normal corn, GA produced some increase in length but did not affect the peroxidase level. This indicates that growth rate may be controlled by a multiplicity of biochemical systems, only one of which is related to the peroxidase changes noted, and affected in the dwarf by treatment with GA.

Table I

Effect of GA Application on the Growth and Peroxidase Activities of Peas and Corn *

Plant	GA treatment	Length of corn leaf sheath or pea internode in mm		Peroxidase activity in mμM purpurogallin/μg prot N/min	
		2 days	4 days	2 days	4 days
Dwarf corn	−	14**	20	52†	73
"	+	32	44	46	56
Tall corn	−	42	53	47	57
"	+	54	64	44	54
Dwarf pea (Progress # 9)	−	5.3‡		38††	
"	+	9.3		27	
Tall pea (Alaska)	−	21.3		21	
"	+	21.8		19	

* Each value is the mean of at least 3 experiments.

** Confidence limits for corn leaf sheath lengths ± 8.0 % (P=0.05), ± 11 % (P=0.01)

† Confidence limits for corn peroxidase activity ± 8.5 % (P=0.05), ± 12 % (P=0.01)

‡ Confidence limits for pea internode lengths ± 9.7 % (P=0.05), ± 12 % (P=0.01)

†† Confidence limits for pea peroxidase activity ± 12 % (P=0.05), ± 21 % (P=0.01)

One other group of investigators has reported that treatment with gibberellin increases the peroxidase level (3). These investigators used guaiacol as a substrate. In repeating our work with this substrate we were able to confirm their results. Thus, it appears that the effect of GA on peroxidases involves a change in substrate specificity such that there is lower activity toward pyrogallol and higher activity toward guaiacol.

Summary

In studies on the growing zone of the sheath of the 1st leaf of corn, it was found that the dwarf-1 mutant has a greater level of peroxidase activity than the normal wild type. Treatment with GA increases the growth rate and decreases the peroxidase activity per unit protein nitrogen of the dwarf. Although GA increases the growth rate of the normal, it has no effect upon the peroxidase level.

Using the 5th internode of the pea it was found that the dwarf variety Progress # 9 has a greater level of peroxidase activity than the tall variety, Alaska. Treatment with GA increases the growth rate and decreases the peroxidase level of this dwarf. In Alaska, a tall variety of pea, GA affected neither the growth rate nor the peroxidase level.

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EFFECT OF LIGHT UPON THE BEHAVIOR OF CITRIC ACID IN LEAVES OF BRYOPHYLLUM CALYCINUM SALISB.[1]

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Although there is an extensive literature on the diurnal variation of acidity in leaves of plants of the family Crassulaceae, most discussions of the problem have been limited to a consideration of the phenomenal rhythmic changes in the amounts of malic acid present. Only occasionally has attention been paid to the closely similar behavior of citric acid, although Guthrie (2) pointed out in 1936 that citric acid may increase 5-fold in leaves of *Bryophyllum calycinum* Salisb. during the night, and that this change accounts for about one fourth of the diurnal change in titratable acidity. Guthrie made use of the gravimetric pentabromoacetone method of Hartmann and Hillig (4, 5) to determine citric acid, but it was not until rapid small-scale volumetric or colorimetric modifications of this fundamental method (3, 8) were developed that the metabolism of citric acid in plant tissues could be conveniently studied.

Guthrie's observations on the behavior of citric acid in Bryophyllum leaves have been confirmed in general outline by subsequent workers (1, 9), and no instance has been found in the experience of this laboratory of a failure of citric acid to increase when the leaves of this plant are maintained in darkness. However, as was pointed out in a recent paper (10), the converse experiment in which leaves picked at sunrise, when they contain a high level of citric acid, are exposed to light has revealed at least 2 instances in which citric acid failed to diminish in the anticipated manner. Notwithstanding this, malic acid in the same samples behaved in the normal way. A distinction could apparently be drawn between the effect of artificial illumination under fluorescent lights and the

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