

BRIEF PAPERS

THE EFFECT OF GIBBERELIC ACID ON ENZYME ACTIVITY AND OXYGEN UPTAKE IN BEAN PLANTS (PHASEOLUS VULGARIS) ^{1, 2, 3}

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Gibberellic acid, a tetracyclic dihydroxylactonic acid, C₁₉H₂₂O₆, produces marked shoot elongation in many plants (2, 3, 11, 12, 13). Unlike other auxins, its stimulation of growth of intact plants often results in substantial increases in height, and in fresh and dry weights (2). Brian (2) noted that the ash, nitrogen, phosphorus, potassium, total soluble carbohydrates, and carbon increased in the shoots and decreased in the roots of gibberellic acid treated wheat and pea plants. For the entire plant there was a net increase in these constituents. In rice seedlings Yabuta et al (13) found no difference in the ash, reducing sugar, total nitrogen, or total weight, but the treated plants contained less chlorophyll and total sugar. Wittwer et al (11) recently demonstrated that gibberellin will induce parthenocarpic fruit development in the tomato and earlier flowering in several crops. Kato (5) has reported significant increases in oxygen and water uptake of pea stem sections treated with gibberellin. Little information has been reported on the effect of gibberellic acid on plant metabolism; therefore, certain enzyme systems and rates of oxygen uptake of intact bean plants treated with gibberellic acid were studied.

Bean seeds var. Blue Lake (Stock no. 42335, Roger Bros. Seed Co., Inc., Idaho Falls, Idaho) were sown in quartz sand in a greenhouse. After 8 days

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the newly emerged seedlings were transferred to aerated solution cultures (standard Hoagland). Plants were grown during the long days (15- to 16-hour photoperiod) of early summer; temperature variations from night to day were 10 to 15° C. Ten microliters of an aqueous solution containing 10 micrograms of gibberellic acid was delivered from a pipette to the apex of the epicotyl at the time the primary leaves were fully expanded. Three replications of both treated and non-treated plants were utilized for all studies.

For enzyme determinations, the plants were harvested 96 hours after gibberellic acid was applied, separated into leaves, stems, and roots, frozen immediately, and maintained at -20° C until needed. Extracts (triplicate) of the leaves (10 plants), roots (10 plants), were prepared by macerating in a Waring Blendor for 5 minutes with 40 ml of water, and for the stems (20 plants) 20 ml of water was used. The solutions were permitted to stand for ½ hour. The solids were then removed by centrifugation and the supernatant liquid filtered. An aliquot of the filtrate was used for the enzyme determination.

The methods employed for the determination of the enzymes were those of Sandstedt et al (1, 7, 8) for α -amylase and β -amylase; Sumner et al (9) for phosphorylase; Ignatieff and Wastenays (4) for phosphatase; and Kertesz (6) for pectin methyl-esterase.

Oxygen uptake of stem segments from the same lot was determined immediately following harvest. The oxygen uptake of the freshly prepared stem sections was measured in a Warburg respirometer (10) which was shaken at 80 rev/min at 30° C. Each

TABLE I
EFFECT OF GIBBERELIC ACID ON CERTAIN ENZYMES IN VARIOUS TISSUES OF BEAN PLANTS *
(FRESH WT BASIS)

ENZYMES	LEAVES		STEMS		ROOTS	
	TREATED	NOT TREATED	TREATED	NOT TREATED	TREATED	NOT TREATED
α -Amylase	Trace	Trace	Trace	Trace	Trace	Trace
β -Amylase **	81.3	89.4	43.5	54.0	98.7	72.1
Phosphorylase	Trace	Trace	Trace	Trace	Trace	Trace
Phosphatase †	4.5 × 10 ²	3.36 × 10 ²	5.13 × 10 ²	4.69 × 10 ²	Trace	Trace
Pectin methyl-esterase ††	0.52	0.48	0.16	0.22	26.4	59.4

* Values represent averages of 3 replications with the exception of pectin methyl-esterase which are the averages of 2 replications.

** Mg soluble starch converted to maltose by the enzyme per gm tissue in 1 hr at 25° C and pH 4.5.

† Mg phosphorus liberated from β -glycerophosphate by the enzyme per gm tissue in 1 hr at 25° C and pH 5.8.

†† Mg methoxyl group liberated from pectin by the enzyme per gm tissue in 1 hr at 25° C at pH 7.0.

flask contained approximately 200 mg of stems cut in 5-mm lengths and suspended in 2 ml of 0.1 M phosphate buffer solution at pH 5.7. The experiments were run in an air phase 1 hour after equilibrium had been established.

At the time of harvest the overall shoot length of the treated plants was more than double that of the non-treated plants, while the roots of the former were about 2/3 the length of the roots of the control plants. Most of the observed elongation of the treated plants resulted chiefly from the extension of the internodal regions. No difference was observed in the total fresh and dry weights between the treated and non-treated plants.

The data from the enzyme determinations expressed on a fresh weight basis indicated the following trends: Only traces of α -amylase and phosphorylase activities (table I) were detected, and no differences could be ascribed to treatment with gibberellic acid. β -Amylase activity was higher in the extracts of leaves and stems of the non-treated plants; however, plants treated with gibberellic acid showed higher β -amylase activity in the roots. Significantly higher phosphatase activity was present in the extracts of leaves and stems of the treated plants. There was less pectin methyl-esterase activity in the roots of the treated plants with no difference in the stems and leaves.

On a plant part basis (table II) the uptake of oxygen by the stems (the internode between the primary leaves and the first trifoliate leaf) was increased sevenfold following treatment with gibberellic acid, while with the epicotyl segment between the primary leaves and the cotyledons, the microliters of oxygen absorption was doubled. Expressed on a fresh weight basis the oxygen uptake by the first internode of the treated plants was less than the non-treated plants, while no difference was observed with the internode between the cotyledons and the primary leaves. Kato (5), using pea stem sections suspended in gibberellic acid solutions (10 mg/liter) was unable to detect any difference (dry weight basis) in respiration in sections prepared from the first and second internode; however, oxygen uptake was increased in sections prepared from the third internode.

TABLE II

EFFECT OF GIBBERELIC ACID ON OXYGEN UPTAKE BY THE INTERNODES OF BEAN PLANTS

PLANT TISSUE	O ₂ UPTAKE, μ L/HR AT 30° C			
	PLANT PART		TISSUE, 100 MG FRESH WT	
	TREATED	NOT TREATED	TREATED	NOT TREATED
First internode	121	18	40	71
Internode (epicotyl below primary leaves)	44	22	12	14

SUMMARY

Young bean plants 96 hours after the application of 10 micrograms of gibberellic acid to the apex of the epicotyl showed on a fresh weight basis that phosphatase activity was increased in the leaves and stems of the treated plants. No differences could be detected for α -amylase and phosphorylase. Treatment with gibberellic acid resulted in a decrease in activity for β -amylase in the leaves and stems, for pectin methyl-esterase in the roots, for oxygen uptake in the first internode, and an increase for β -amylase in the roots. On a plant part basis, however, the oxygen absorption was greater in the internodes of the treated plants.

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