Phytochemistry, 1974, Vol. 13, pp. 543 to 546. Pergamon Press. Printed in England.

ENZYME ACTIVITIES IN *PENNISETUM* SEEDLINGS GERMINATED IN THE PRESENCE OF ABSCISIC AND GIBBERELLIC ACIDS

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(*Revised Received* 16 *July* 1973. *Accepted* 3 *October* 1973)

Key Word Index—*Pennisetum typhoides*; Gramineae; growth and enzyme activity; abscisic acid; gibberellic acid; alanine aminotransferase; aspartate aminotransferase; glutamate dehydrogenase.

Abstract—Effects of abscisic acid (ABA) and gibberellic acid (GA_3), alone and in combination, on growth and activity of alanine aminotransferase (GPT), aspartate aminotransferase (GOT), and glutamate dehydrogenase (GLDH) were studied in aerial parts of *Pennisetum typhoides* seedlings. ABA inhibited growth and activity of GLDH, but stimulated the activity of GPT and weakly that of GOT. GA_3, on the other hand, did not affect the activity of any of the enzymes tested, but in combination with ABA tended to antagonise the effect of the latter.

INTRODUCTION

WHILE the information relating to the influence of ABA on plant growth abounds in published literature,[1,2] its effect on the activity and synthesis of various enzymes, which play a key role in the growth and metabolism of higher plants, is still fragmentary.[1]

The present investigation was designed to study the effect of ABA on the activity of some of the key enzymes of amino-acid metabolism.

RESULTS

Growth

As in other test systems, ABA proved to be a powerful inhibitor of growth in *Pennisetum* (Table 1). Even at a concentration of $3{\cdot}8 \times 10^{-6}$ M, ABA retarded elongation growth, both in coleoptiles and in the first foliage leaf. In the presence of $7{\cdot}4 \times 10^{-6}$ M ABA, col-

TABLE 1. EFFECTS OF GIBBERELLIC ACID AND ABSCISIC ACID ON SEEDLING GROWTH OF *Pennisetum typhoides*

Conc. (M)	Seedling growth (mm) after 4 days			Conc. (M)	Seedling growth (mm) after 4 days		
	Root	Coleoptile	Leaf		Root	Coleoptile	Leaf
Control	50	11	47	GA_3 $2{\cdot}89 \times 10^{-4}$	50	16	55
ABA $3{\cdot}8 \times 10^{-6}$	55	8	20	ABA $3{\cdot}8 \times 10^{-6}$ +			
ABA $7{\cdot}4 \times 10^{-6}$	40	6	20	GA_3 $2{\cdot}89 \times 10^{-5}$	35	12	48
ABA $1{\cdot}48 \times 10^{-5}$	25	6	15	ABA $7{\cdot}4 \times 10^{-6}$ +			
GA_3 $2{\cdot}89 \times 10^{-5}$	50	15	60	GA_3 $2{\cdot}89 \times 10^{-5}$	40	15	36

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[1] ADDICOTT, F. T. (1972) in: *Plant Growth Substances* (CARR, D. J., ed.), p. 272. Springer, New York.
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coptiles and first foliage leaves showed up to 50% inhibition in their longitudinal extension. At still higher concentration ($1{\cdot}48 \times 10^{-5}$ M), root growth was inhibited up to 50%. In contrast, GA_3 promoted elongation of both coleoptiles and leaves, but the two concentrations of GA_3 employed (Table 1) hardly differed in their growth promoting activity. Root growth, however, was unaffected by GA_3. When GA_3 and ABA were administered simultaneously, they appeared to antagonise each other's effects on extension growth.

TABLE 2. EFFECTS OF GIBBERELLIC ACID AND ABSCISIC ACID ON THE ACTIVITY OF ALANINE AMINOTRANSFERASE (GPT)

Conc. (M)	Activity in % of control	Conc. (M)	Activity in % of control
Control	100	GA_3 $2{\cdot}89 \times 10^{-4}$	109
ABA $3{\cdot}8 \times 10^{-6}$	227	ABA $3{\cdot}8 \times 10^{-6}$ + GA_3 $2{\cdot}89 \times 10^{-5}$	245
ABA $7{\cdot}4 \times 10^{-6}$	340		
ABA $1{\cdot}48 \times 10^{-5}$	334	ABA $7{\cdot}4 \times 10^{-6}$ + GA_3 $2{\cdot}89 \times 10^{-5}$	201
GA_3 $2{\cdot}89 \times 10^{-5}$	98		

Alanine aminotransferase (GPT)

In contrast to growth, the activity of GPT in extracts of seedlings grown in the presence of ABA was higher than that of control plants (Table 2). In comparison to the control, the mean activity of this enzyme increased to about 127, 240 and 234% in the extracts of the seedlings grown in presence of $3{\cdot}8 \times 10^{-6}$ M, $7{\cdot}4 \times 10^{-6}$ M and $1{\cdot}48 \times 10^{-5}$ M ABA respectively. GA_3 at both concentrations tested did not elicit any effect on the activity of GPT. However, when added with ABA (especially $7{\cdot}4 \times 10^{-6}$ M ABA), GA_3 counteracted the stimulation in GPT activity brought about by growth of the seedlings in ABA solutions.

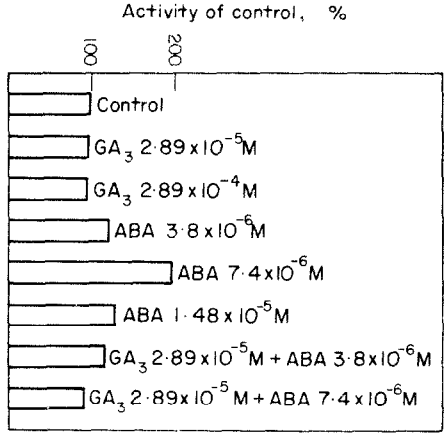

FIG. 1. EFFECTS OF GA_3 AND ABA ON THE ACTIVITY OF ASPARTATE AMINOTRANSFERASE (GOT).

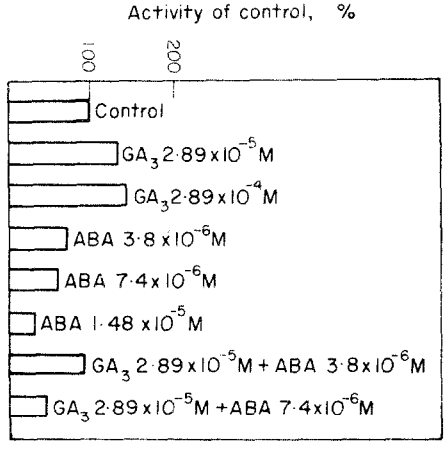

FIG. 2. EFFECTS OF GA_3 AND ABA ON THE ACTIVITY OF GLUTAMATE DEHYDROGENASE (GLDH).

Aspartate aminotransferase (GOT)

ABA also increased the activity of GOT, although the response was much less than with GPT (Fig. 1). A significant stimulation of activity could be observed only in plants grown

in the presence of $7{\cdot}4 \times 10^{-6}$ M ABA. GA_3 was found to be without effect on the activity of GOT but, as in the case of GPT, GA_3 appeared to counteract the effect of ABA on the activity of this enzyme.

Glutamate dehydrogenase (GLDH)

In contrast to its effect on GPT and GOT, ABA inhibited the activity of GLDH. With increase in concentration of ABA employed (Fig. 2) a corresponding decrease in the activity of this enzyme was clearly evident. GA_3 slightly enhanced activity, but the values were not significant. However, when administered in the presence of ABA, GA_3 appeared to neutralize the effects of the former growth regulator.

DISCUSSION

When seeds of *Pennisetum* were germinated in the presence of ABA, the activity of GPT was greater than that of plants germinated in water, and to a lesser extent, that of GOT was similar. That GPT is activated more than GOT could be due to the fact that the former enzyme has been shown to be an important regulatory enzyme.[3–5] In contrast to its effect on transaminases, ABA strongly inhibited the activity of GLDH. Thus, one can observe differential effects of ABA in influencing the activities of various enzymes of amino acid metabolism. These results are compatible with the suggestion that promotion or inhibition of enzyme activity by ABA is probably a reflection of its physiological role in a given process.[1]

Essentially, ABA is a potent inhibitor of numerous growth processes.[1,2] Its several physiological effects can be partially or wholly counteracted by growth promotors. The results of the present investigation also clearly indicate that GA_3 can overcome the inhibition of growth caused by ABA in *Pennisetum* also. GA_3, ineffective itself, tended to counteract the action of ABA on the amount of enzyme activity developed during germination.

In excised leaf discs, ABA is known to accelerate senescence (assessed by the decrease in chlorophylls, proteins and nucleic acids), while in several other test objects the hormone effectively inhibits protein synthesis.[1,2,6–10] The inhibition of GLDH activity in *Pennisetum* by ABA is in agreement with these reports. In the absence of active protein synthesis little assimilation of ammonia through GLDH would be expected.

It is possible that under conditions of stress, when protein synthesis is impaired, profound alterations take place in amino acid metabolism in order to maintain an available pool of amino-groups to resume protein synthesis as soon as the stress is over. Since ABA is probably present in all higher plants[11] and is known to be a major factor facilitating the adaptive response of plants to root stresses[12] the findings reported here, although they remain to be tested thoroughly, may well represent one of the important adaptive mechanisms in plants to deal with conditions of stress.

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EXPERIMENTAL

Plant material. Seedlings of *Pennisetum typhoides* (Burm. f.) Stapf & Hubbard, a C_4-plant,[13] were raised from seeds in 9 cm Petri dishes lined with a single layer of filter paper moistened with 5 ml dist. H_2O or the test soln. The test soln consisted of desired concentrations of racemic ABA (Fluka) and gibberellic acid (Merck) solns. During the experimental period, seedlings were maintained at 28° in a growth chamber and received illumination (*ca* 3000 lx) from a light bank consisting of fluorescent lamps.

Extraction. About 1 g plant material, consisting of the first foliage leaves and coleoptiles, obtained from 4-day-old seedlings was homogenized at 4°, as uniformly as possible, in 4 ml Tris-HCl-buffer (pH 7·0, with 0·001 M EDTA, 0·003 M $MgCl_2$ and 0·02% mercaptoethanol) for 2 min in a Potter–Elvehjem homogenizer (Potter S-Braun, Melsungen, 1200 rpm). The extract so obtained was immediately centrifuged in a Sorvall SS-1 centrifuge maintained at 4° for 15 min at 20000 *g*. The supernatant was used as the enzyme source. All enzyme determinations were initiated immediately after extraction.

Enzyme assays. All the enzymes were assayed essentially after Bergmeyer.[14] Activity of aminotransferases as well as glutamate dehydrogenase was measured at room temp. following the loss of absorption at 366 nm due to oxidation of NADH using a Zeiss DMR 21 double beam spectrophotometer coupled to an automatic recorder. The enzyme activity has been expressed in μmol NADH reduced/mg protein. The test mixture was as follows:

Alanine aminotransferase (*E.C.* 2.6.1.2) *or glutamate-pyruvatetransaminase* (*GPT*). 80 μM phosphate buffer pH 7·4, 88 μM L-alanine, 0·5 μM NADH, 0·3 units LDH, 20 μM ketoglutarate and 0·2 ml enzyme in a total vol. of 1·2 ml.

Aspartate aminotransferase (*E.C.* 2.6.1.1) *or glutamate-oxalacetatetransaminase* (*GOT*). 70 μM phosphate buffer pH 7·0, 175 μM aspartic acid, 0·5 μM NADH, 0·5 units MDH, 20 μM ketoglutarate and 0·05 ml enzyme in a total vol. of 1·2 ml.

Glutamate-dehydrogenase (*GLDH*, *E.C.* 1.4.1.2). 25 μM Triethanolammonium-HCl-buffer pH 8·0, 600 μM ammonium acetate, 0·5 μM NADH, 20 μM ketoglutarate and 0·2 ml enzyme in a total vol. of 1·2 ml. As recommended by Bergmeyer[14] before starting the reaction by ketoglutarate the enzymes were preincubated in presence of the reaction mixture for 5 min.

Soluble protein determination. The protein content of the extracts was measured by the method of Lowry *et al.*,[15] using bovine serum albumin as standard.

Acknowledgements—Grateful thanks are due to Professor Ziegler for critical evaluation of the manuscript, and for helpful suggestions. This research was supported by grants from Alexander von Humboldt-Foundation and Deutsche Forschungsgemeinschaft.

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