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Short Communication

Low Temperature Induction of Hormonal Sensitivity in Genotypically Gibberellic Acid-Insensitive Aleurone Tissue

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ABSTRACT

An exposure of genetically gibberellic acid-insensitive isolated wheat aleurone tissue/deembryonated seeds to low temperature for 20 hours prior to addition of exogenous gibberellic acid results in a significant increase in sensitivity to gibberellic acid. The results may reflect a low temperature-induced increase in hormone receptor sites and could have important implications for elucidating the nature of the primary site of hormone action.

The gibberellin-induced initiation and control of hydrolytic enzyme activity in the cereal aleurone tissue, first reported in 1960 (7, 8), is one of the better known hormonal responses in plants. The phenomenon of insensitivity to GA_3 in the response of aleurone tissue has been most thoroughly explored in wheat seeds with the Rht3 dwarfing genotype (1–4, 6). The present study was undertaken to see whether sensitivity to GA_3 could be induced in genetically insensitive aleurone tissues.

MATERIALS AND METHODS

Seed samples of GA_3-insensitive Rht3 dwarf wheats were obtained from two sources. Minister Dwarf and Tom Thumb were obtained from Dr. Rod King, Division of Plant Industry CSIRO, Canberra, while Tordo was obtained from Prof. C. J. Driscoll, Department of Agronomy, Waite Agricultural Research Institute, Adelaide.

All seeds were deembryonated by dissecting the embryo prior to the start of experimentation. The deembryonated seed were surface sterilized by soaking for 7 min in 50% solution of NaOCl (4% w/v available chlorine). After copious washings with distilled water, the deembryonated seeds were transferred aseptically to a large sterile Petri dish containing two sheets of filter paper and 10 ml of sterile distilled water, and the deembryonated seeds were allowed to imbibe for 12 h at 30°C unless stated otherwise. All subsequent operations were carried out in a laminar flow cabinet with all equipment sterilized by autoclaving for 20 min at 15 p.s.i. All solutions were sterilized by passing through a Millipore filter (0.22 μm). Ten deembryonated seeds were preincubated in 125-ml conical flasks containing 5 ml of distilled water and 10 mg/l streptomycin. Preincubation for 20 h was carried out at 5°C and 30°C in a water bath shaking at 50 rpm and, at the end of this period, the ambient solution was poured off and replaced with 5 ml of 20 mM $Ca(NO_3)_2$ with different dosages of GA_3. The flasks were then returned to the water bath and the tissue was further incubated for a period of 24 h at 30°C. At the end of this time, the ambient fluid was decanted and the caryopses were homogenized with an Ultraturrax in 7 ml of grinding medium containing 0.1 M NaCl and 0.02 M calcium acetate. The homogenate was centrifuged at 3,000*g* for 10 min, and the supernatant was added to the flask containing the incubation medium. This extract was assayed for α-amylase activity according to the method of Gibson and Paleg (5).

RESULTS AND DISCUSSION

Sensitivity of the Rht3 aleurone tissue/deembryonated seeds to GA_3 was monitored by determining the amount of α-amylase produced as a result of 24-h incubation with different concentrations of exogenous GA_3. GA_3-insensitive seeds of Tom Thumb, Tordo, and Minister Dwarf, all of which are Rht3 dwarf wheats (1, 2, 4), were examined. A 20-h preincubation at 5°C, as compared with 30°C, of deembryonated seeds of Tom Thumb caused a 2.5-fold increase in the amount of α-amylase detected after a subsequent 24 h of incubation with GA_3, while the increase was

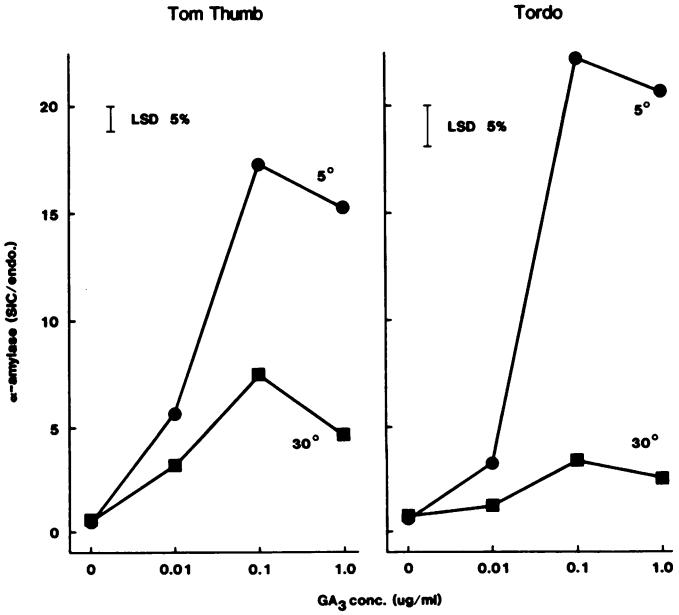

FIG. 1. Low temperature-induced sensitivity to GA_3 in deembryonated seeds of the Rht3-containing varieties Tom Thumb and Tordo. Each value represents the mean of three replicates.

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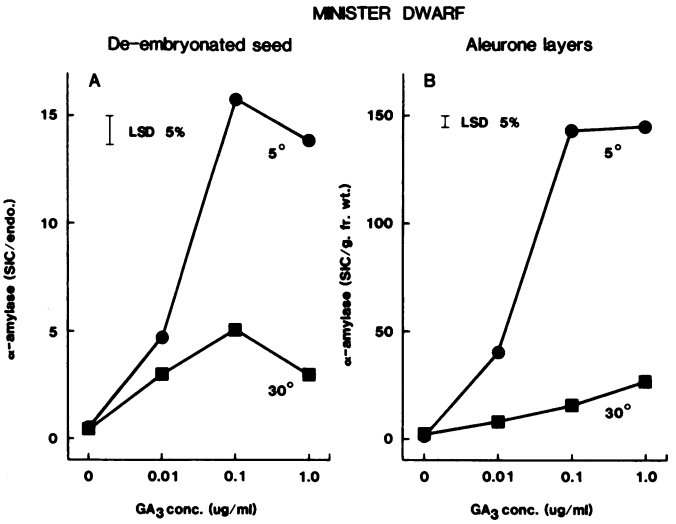

FIG. 2. Comparison of α-amylase produced by tissues of Rht3 containing variety Minister Dwarf preincubated for 20 h at different temperatures. The deembryonated seeds (A) were imbibed for 24 h at 30°C prior to preincubation. Aleurone layers (B) were dissected from seeds which had been imbibed for 24 h at 30°C, and subsequent procedures were as detailed in "Materials and Methods." Each value represents the mean of three replicates.

7-fold for deembryonated seeds of Tordo (Fig. 1). A similar 3-fold increase was detected for deembryonated seeds of Minister Dwarf (Fig. 2A). The levels of α-amylase produced by the deembryonated seeds of Tom Thumb and Minister Dwarf preincubated for 20 h at 30°C (Figs. 1 and 2A) are essentially the same as those reported in the literature when the two varieties were described as insensitive to GA_3 in terms of their α-amylase response (4). Further, it is pertinent that, in other experiments with isogenic lines from the same initial cross, the magnitude of the response to GA_3 in low temperature-treated aleurone layers/deembryonated seeds of an insensitive (F_6Rht3) selection was fully comparable with the aleurone layer/deembryonated seed responses of a sensitive (F_6rht3) selection (unpublished results).

Low temperature treatment of isolated aleurone tissue produced an equally dramatic increase in GA_3 sensitivity (Fig. 2B). The aleurone tissue was isolated from grain of Minister Dwarf and, clearly, involvement of the endosperm in perceiving or responding to the low temperature is unnecessary (Fig. 2, A and B). Absence of any significant difference in α-amylase produced by the minus-GA_3 controls at both 5°C and 30°C preincubation (Figs. 1 and 2) rules out the possibility of the low temperature treatment being effective via an increase in the levels of endogenous gibberellins.

Whereas the presence of the Rht3 gene was found to result in a reduction in the amount of GA_3-induced α-amylase, the time course of α-amylase production, the relative amounts of isozyme produced by the α-amylase structural genes, and the starch liquefaction capacity of the enzyme were all unaffected (2). Furthermore, the presence of the Rht3 gene did not alter the uptake and metabolism of GA_3, nor the levels of endogenous inhibitors, nor cellular metabolism of the aleurone tissue (6). Last, lag-times of GA_3-induced enzyme production were similar regardless of the preincubation temperature (unpublished data). Thus, it seems possible that the low temperature treatment which "cures" or circumvents the genetic lesion manifest in aleurone tissue of fully matured seeds containing the Rht3 gene results in an increase of effective hormone receptor sites.

The ability to vary the response of the aleurone tissue to GA_3 without the addition of any chemical agents will prove invaluable in elucidating the nature of the primary site of hormone action. The correlation between low temperature-induced sensitivity to GA_3 and low temperature-induced changes in certain components of the aleurone tissue will be published soon.

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