Response of aleurone layers from *Triticum dicoccum* genotypes to gibberellic acid (GA$_3$): Time course of amylase stimulation

Chun Mei Chang and S.G. Bhagwat
Nuclear Agriculture and Biotechnology Division

This Paper received the Best Poster Award (Third Place) at the Zonal Seminar on Physiological and Molecular Interventions for Yield and Quality Improvement in Crop Plants, held at S.V. Patel University of Agriculture & Technology, Meerut in 2010

Abstract

The height of a wheat plant is a trait which is related to gibberellin metabolism. The cells of GA insensitive semi-dwarfs have a defect in GA$_3$ utilisation. In the present study, the GA$_3$ response characteristics of aleurone layers from a *Triticum dicoccum* tall parent and its semi-dwarf mutant have been studied and compared to a variety containing *RhtB1b* semi-dwarfing gene. The results showed significant differences in the pattern of amylase secretion between the tall and semi-dwarf varieties. After 24h of incubation in 10$^{-4}$M GA$_3$, the semi-dwarf mutant showed about half the amount of amylase activity as compared to the parent. The *RhtB1b* semi-dwarf showed intermediate amylase activity as compared to the parent and mutant.

Introduction

Gibberellic acids (GAs) form a large family of diterpenoid compounds, some of which are bioactive growth regulators, that control diverse developmental processes such as seed germination, stem elongation, leaf expansion, trichome development and flower and fruit development (Olszewski *et al*., 2002). Introduction of the Norin-10 gene(s) resulted in semi-dwarf varieties of wheat that were lodging-resistant, and could accumulate biologically active gibberellic acids to higher levels than are found in wild-type controls (Sun, 2000), and these mutations behave as dominant altered-function mutations (Gale *et al*., 1983). The wheat aleurone layer consists of a single layer of uniform, highly differentiated cells and provides an ideal system to study gibberellic acid (GA) response, with GAs specifically promoting the rapid expression of genes encoding hydrolases such as $\alpha$-amylases and its secretion occurring in a time bound manner. Therefore, any mutation/block in the GA signaling pathway or its utilisation would affect the production and secretion of amylases. Thus measurements of amylase stimulation/activity can be used to study any change in the GA action. The present study was carried out to understand the effect of GA on amylase production from aleurone layers of wheat plants with difference in height, using a tall parent and its semi-dwarf mutant.

Materials and Methods

The *Triticum dicoccum* varieties used were NP200 (tall parent), HW1095 (semi-dwarf mutant) and DDK1029 (*RhtB1b* carrier).

Chemicals used were procured from standard sources and were of analytical grade.
Aleurone incubation

Seeds were surface sterilized, the edges of the seeds including the embryo were cut off with a blade and the cylindrical portions were incubated in sterile distilled water for 24h. The starchy endosperm of each seed was removed and the aleurone layers were washed thoroughly with sterile distilled water and incubated in the incubation buffer containing 0.001M sodium succinate, 0.02M CaCl₂ (Bhagwat and Bhatia, 1994) and 1mg/mL Cefotaxime, for up to 80h. The GA treated samples contained 10⁻⁴M GA₃. The aleurones were incubated in a rotary shaker at 28°C and 85rpm. Aliquots were collected every 12h in sterile environment and assayed for total protein content and amylase activity.

Amylase assay and protein estimation

Amylase activity was measured by incubating an aliquot at 37°C in acetate buffer (pH 4.8) along with starch for 30min. The enzyme reaction was stopped by adding 0.1M NaOH and placed in a boiling water bath for 5min with 1% DNSA. The resulting colour was measured at 540nm. Total protein was measured by Lowry’s method.

Results:

which was secreted into the medium. There was no stimulation in the absence of GA₃ (Fig. 1) throughout the incubation time. The total secreted protein in the medium was comparable in the GA treated and the control (Fig. 2).

Comparison of the varieties

Amylase stimulation in the varieties under identical conditions showed that stimulation began at 18h incubation in the variety NP200, and at 24h in HW1095 and DDK1029, following which, the amylase was secreted at a steady rate up to 54h. Subsequently, there was decrease in the rate (Fig. 1).

Total amylase activity

Total amylase activity over 72h showed that the variety NP200 produced more amylase as compared to HW1095 and DDK1029 was intermediate.

Discussion

The dicoccum wheat is less explored as compared to the bread wheat and macaroni wheat and information on the response of aleurones of dicoccum has not been reported. In this study, a tall variety NP200 and its gamma ray-induced semi-dwarf mutant have been compared for their amylase stimulation in response to externally applied gibberellin. The time course study revealed that the response of dicoccums is comparable to that of other wheats, however, with its own characteristic time for stimulation under the experimental conditions used. Comparison of the parent and the mutant revealed that the total amount of amylase synthesized and secreted in the medium up to 48h, was highest for the parent, lowest for the mutant and intermediate for the RhtB1b carrier semi-dwarf and the differences were significant. The results indicated that the T. dicoccum
mutant HW1095 has altered GA metabolism as a result of which, the plant height is reduced and stimulation of amylase in aleurone is altered. The mutation appears to be different than the earlier known semi-dwarf gene RhtB1b since the stimulation pattern is different for the two semi-dwarfs. The activation of GA-stimulated amylase is also relevant to grain quality. Grains exposed to untimely rain at harvest would undergo amylase stimulation in pre-harvest sprouting. Lower stimulation of amylase may result in lower damage to the grain quality.

Conclusions
Aleurone layers of tall dicoccum variety showed amylase stimulation in vitro by externally applied GA$_3$. A semi-dwarf mutant showed reduced stimulation. A Norin10 (RhtB1b) carrying semi-dwarf showed different time course, which indicated that the mutation in HW1095 may be different in the RhtB1b mutation. The aleurone layers provide a useful system to study the effect of mutation or GA metabolism.

References