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DROUGHT REGULATES INVERTASE EXPRESSION AND CARBOHYDRATE LEVELS IN MAIZE OVARIES

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Introduction

Drought sensitivity of maize is at maximum during flowering (e.g. 1). Analyses of mature crops that have been stressed during this developmental stage reveal large reductions in seed number per cob and in harvest index (2). Drought damage to ovaries that leads to abortion of seeds is confined to early stages of ovary development (3), which coincide with cell differentiation. Carbohydrate levels affect developmental processes by altering gene expression patterns and enzyme activities (4, 5, 6) but are also controlled by the same processes. In order to study how drought interferes with ovary carbohydrate metabolisms we conducted a lysimeter experiment under field conditions.

Materials and Methods

The experiment comprised 18 lysimeters that were covered by a mobile roof during rainfall only. Water was supplied with drip irrigation and soil moisture content measured with a neutron-probe. Irrigation was successively terminated with 3-4 day intervals in 14 of the lysimeters, starting 45 days before flowering, and leaving 4 as fully irrigated controls. According to the time course of soil moisture depletion (Fig. 1), treatments were grouped in 5 stress levels [1] - [5]. Ovaries were sampled on 970729, 970801, 970805, 970808 and 970812 corresponding to -6 DAP, -4 DAP, 0 DAP, 3 DAP and 7 DAP (Days After Pollination) and analysed for concentrations of sucrose, reducing sugars and starch, activity of vacuolar and cell wall bound acid invertase, and expression levels of two genes IVR-2 (4) and CWI-2 (7) encoding a vacuolar invertase and a cell wall invertase, respectively.

Results and discussion

Contrasting stress levels were obtained in the treatments (Fig. 1) ranging from fully watered [1], medium stress [2] - [3] to severe drought stress [4] - [5].

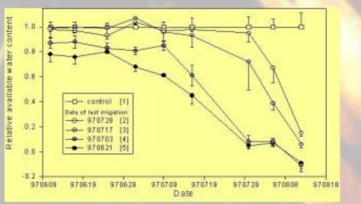


Fig. 1. Relative available soil water content as a function of time for the 5 treatments [1] - [5] ranging from fully watered to severely drought stressed. Error bars denote ± 1 standard error of the mean.

Drought stress is expected to reduce photosynthate supply from leaves to ovaries. Accordingly, sucrose concentration (Fig. 2) was initially slightly lower at medium stress levels [2]-[3] but was unexpectedly, significantly increased (P > 95%) at severe stress levels [4]-[5] from 0 DAP onwards. At the same time conc. of reducing sugars was significantly decreased at severe stress levels. Starch conc. was significantly increased at all dates, while the decrease in ovary dry weight was significant only at the last date. Similar effects of drought on soluble carbohydrates has been reported earlier (3, 8) and indicate that sucrose utilization under severe drought stress is enzymatically down-regulated in ovaries. Abortion of ovaries has been found to be preceded by depletion of starch deposits in the ovary wall and basis at time of pollination (3) and it was proposed that limitations in the first step of sucrose utilization, the hydrolysis to glucose and fructose by acid invertase, was rate limiting. However, we found that drought increased starch conc. It is possible that non-aborting ovaries accumulated more starch to give an increase on average. In older seeds low hexose to sucrose ratio induce starch deposition and growth via a sucrose synthase pathway, while a high ratio tends to maintain cell division and prolong the prestorage phase of seed development (5).

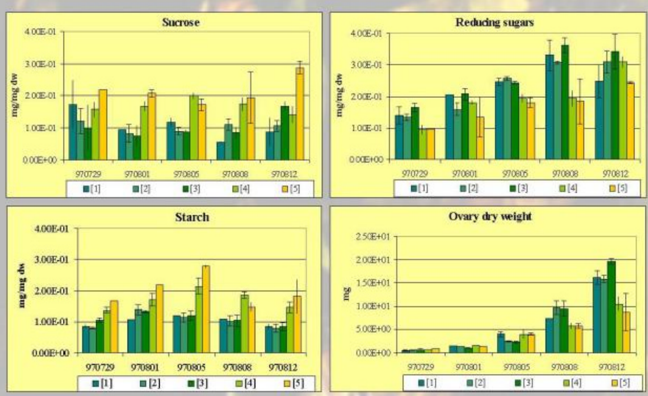


Fig. 2. Concentration of sucrose, reducing sugars and starch in ovaries, and ovary dry weight at different developmental stages: -6 DAP, -4 DAP, 0 DAP, 3 DAP and 7 DAP in the 5 treatments [1] - [5]. Error bars denote ± 1 standard error of the mean. (n = 2-6 per group).

Activity of vacuolar acid invertase was higher than activity of cell wall bound acid invertase from -6 DAP to 3 DAP (Fig. 3), while at 7 DAP the cell wall form preceded. Severe drought reduced the activity of vacuolar invertase significantly (P > 95 %) from -4 DAP onwards while cell wall invertase activity was reduced nearly significantly on 3 and 7 DAP.

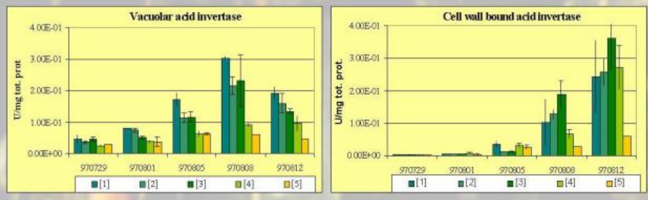


Fig. 3. Specific activity of vacuolar and cell wall bound acid invertase (µmol sucrose converted per minute per mg total protein) at 30 °C and pH=5.0 at different developmental stages: -6 DAP, -4 DAP, 0 DAP, 3 DAP and 7 DAP in the 5 treatments [1] - [5]. Error bars denote ± 1 standard error of the mean. (n = 2-6 per group).

Previous work (e.g. 5; 9) has emphasised the role of cell wall invertases in controlling sucrose unloading in the ovary and young seed, the maintenance of a high hexose to sucrose ratio and thereby normal ovary differentiation. However, in our study (Fig. 4) the hexose to sucrose ratio was better correlated to activity of vacuolar invertase than to activity of cell wall invertase or to the sum of activities ($R^2 = 0.43$***, not shown).

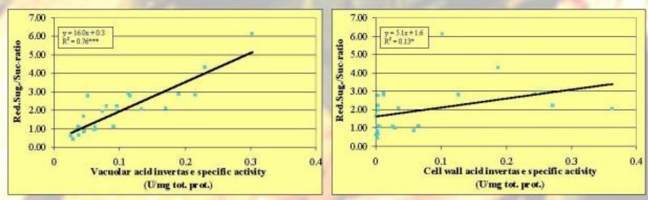
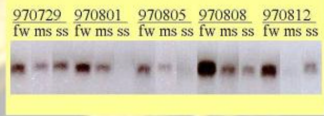


Fig. 4. Ratio between reducing sugars and sucrose as function of vacuolar invertase and cell wall bound invertase activity . *, **, *** = significant at 95, 99, or 99.9 % level, respectively.

Fig. 5. Northern blot of vacuolar invertase (IVR-2) at different developmental stages: -6 DAP, -4 DAP, 0 DAP, 3 DAP and 7 DAP in fully watered (fw), medium stressed (ms) or severely stressed (ss) treatments. ^{32}P-probe hybridised to a mRNA about 2000 bases long.



Abundance of mRNA transcribed from IVR-2 (Fig. 5) was significantly (P > 99 %) decreased by drought at 3 DAP and over all dates. Vacuolar invertase activity correlated with IVR-2 expression (Fig. 6) except for the early stages (-6 and -4 DAP) of development, where IVR-2 was highly expressed but activity low. Also cell wall invertase activity correlated with CWI-2 expression, but mRNA-level was generally to low for quantification in a sufficient number of samples to permit a robust statistical analysis.

IVR-2 expression has in itself been found to be up-regulated by both mono- and di-saccharides (4). Although, in the present experiment, IVR-2 expression and vacuolar invertase activity were positively correlated with the level of reducing sugars, they were negatively correlated with the level of sucrose, indicating that the measured effects of drought were not merely a reflection of altered sugar concentrations in the tissue.

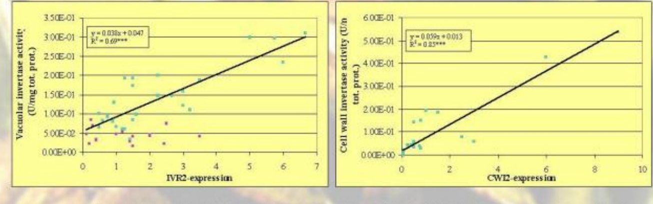


Fig. 6. Vacuolar invertase activity as function of relative abundance of mRNA for IVR-2 and cell wall bound invertase activity against relative abundance of mRNA for CWI-2. Only data for 0 - 7 DAP were included in the regression on IVR-2 expression.

Conclusion

Expression of the IVR-2 gene encoding a vacuolar invertase was down-regulated by drought. Vacuolar invertase activity was accordingly decreased by drought, and seemed to be the main determinant of the ratio between reducing sugars and sucrose in young ovaries.

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