Ministry of Food, Agriculture and Fisheries
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DROUGHT IMPAIRS SUGAR UTILIZATION IN YOUNG ABORTION-SENSITIVE MAIZE OVARIES

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Introduction

Drought sensitivity of maize is at maximum during flowering and yield losses exceed those which would be expected from inhibition of photosynthesis alone (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 are known to affect developmental processes in several ways including altered expression patterns of genes and activities of enzymes (4, 5, 6, 7). In order to study how drought interferes with ovary carbohydrate metabolisms we conducted an experiment in 18 lysimeter-tanks under field conditions.

Stress levels and effects on yield and yield structure

Irrigation was successively terminated in 14 of the lysimeters, starting 45 days before flowering, and leaving 4 as irrigated controls. Thus, 5 treatments ranging from fully watered [t_c], medium stress [t_1] - [t_2] to severe drought stress [t_3] - [t_4] were obtained (Fig. 1). All plots were rewatered to field capacity 7 days after pollination

Treatment	Grain Yield (g m^{-2})	Dry-matter Yield (g m^{-2})	Harvest Index	Kernel Number per Ear	Predawn ψ_w (MPa)
[t_c]	723 a	1415 b	0.51 a	299 a	-0.10 a
[t_1]	844 a	1509 b	0.56 a	295 a	-0.11 ab
[t_2]	953 a	1865 a	0.51 a	177 ab	-0.14 b
[t_3]	426 b	899 c	0.48 a	191 ab	-0.23 c
[t_4]	218 b	815 c	0.27 b	101 b	-0.39 d

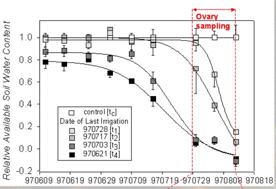
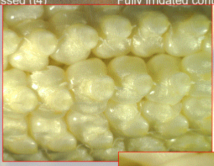
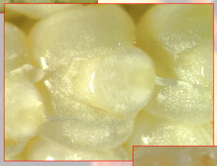
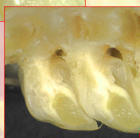


Fig. 1. Relative available soil water content as a function of time. Error bars denote ± 1 standard error of the mean.

Ovaries were sampled on -6, -4, 0, 3 and 7 days from pollination (DFP) and analysed for concentrations of ABA, sucrose, reducing sugars, activity of vacuolar and cell wall bound acid invertases, and expression levels of two genes *Ivr2* (4) and *Incw2* (8) encoding a vacuolar invertase and a cell wall invertase, respectively. Further, *Ivr2* mRNA and invertase activity were detected in situ.







Drought increases ABA levels early

ABA transiently rose in the youngest ovaries -6 DFP in response to severe drought stress but differences were no longer evident from –4 DFP (Fig. 2).

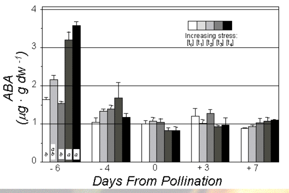


Fig. 2. Drought effects on ovary ABA concentration at 5 stages of development.

Drought-limited acid invertase activity is implicated by increased sucrose levels

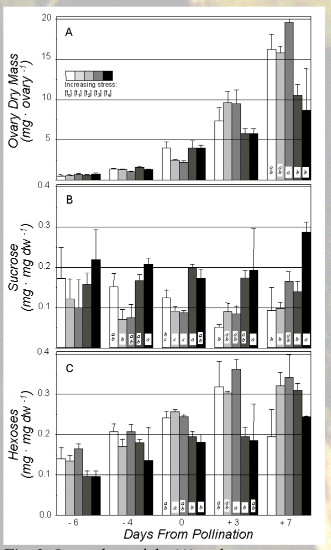


Fig. 3. Ovary dry weight (A) and concentration of sucrose (B) and reducing sugars (C) at five developmental stages: -6, -4, 0, +3 and +7 DFP in the treatments [t_c] - [t_4].

Drought stress is expected to reduce photosynthate supply from leaves to ovaries. Accordingly, sucrose concentration (Fig. 3B) was initially slightly lower at medium stress levels [t_1]-[t_2] but was unexpectedly, increased (P > 95%) at severe stress levels [t_3]-[t_4] from 0 DFP onwards. However, the increase in sucrose was not transformed into an increased growth of ovaries. On the contrary average ovary weight was halved in severely stressed plants at +7 DFP (Fig. 3A), pointing to an impaired capacity for sucrose use. This impairment was further substantiated by decreased concentration of hexoses (Fig 3C). Together, the observations indicate a limitation in the first step of sucrose utilization, the hydrolysis to glucose and fructose. In ovaries and young kernels this conversion is predominantly catalyzed by acid invertases and reduced activity of this group of enzymes in response to drought has been reported earlier (3, 9).

Acid invertases are localized to vacuoles and cell walls, and each of these two broad groups of enzymes are encoded by a number of maize-genes. We therefore went on to study their individual roles in early grain development and their marked sensitivity to drought stress.

Vacuolar rather than cell wall invertase seems to control sucrose to hexose conversion

Activity of vacuolar acid invertase was higher than activity of cell wall bound acid invertase from -6 DFP to 3 DFP (Fig. 4), while at 7 DFP the cell wall form preceded. Severe drought reduced the activity of vacuolar invertase (P > 95 %) from -4 DFP onwards while cell wall invertase activity was reduced (ns.) on 3 and 7 DFP. Previous work (e.g. 5; 10) has emphasised the role of cell wall invertases in controlling sucrose hydrolysis in the ovary and young kernel. However, in our study the hexose to sucrose ratio was better correlated to activity of vacuolar invertase (R^2 = 0.69***) than to activity of cell wall invertase or to the sum of activities.

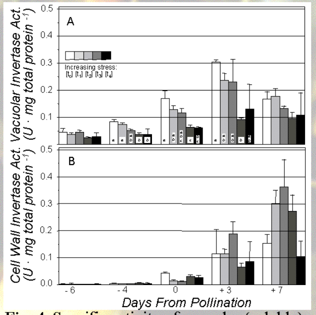


Fig. 4. Specific activity of vacuolar (soluble) and cell wall bound (insoluble) acid invertase (µmol sucrose converted per minute per mg total protein) at 30 °C and pH=5.0.

Abundance of mRNA transcribed from *Ivr2* (Fig. 5A-B upper panels) was decreased (P > 99 %) by drought at +3 DFP and over all dates. Vacuolar (R^2 = 0.83***) and cell wall bound invertase (R^2 = 0.76***) activity correlated well with *Ivr2* and *Incw2* expression, respectively, indicating prominent transcriptional regulation.

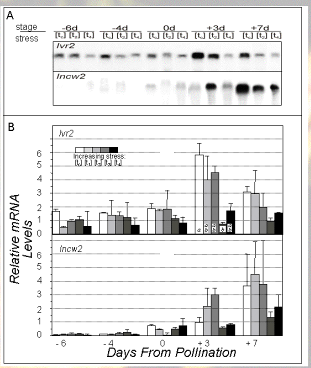


Fig. 5. Northern blot of vacuolar (*Ivr2*) and cell wall bound (*Incw2*) invertase mRNA.

In situ localization indicate a role in sucrose unloading

Ivr2 vacuolar invertase mRNA (Fig. 6) as well as invertase activity (Fig. 7) was predominantly localized to terminal regions of vascular strands.

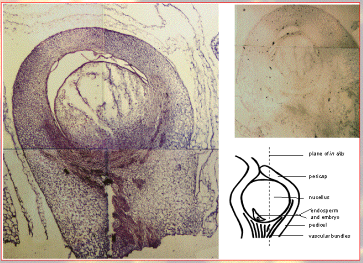
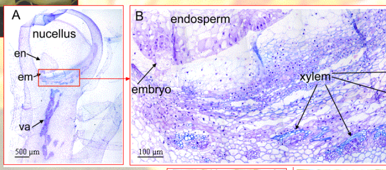


Fig. 6. In situ localization of *Ivr2* vacuolar invertase mRNA in young maize kernel at +6 DFP.



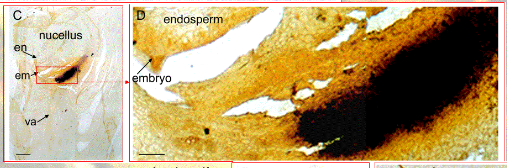
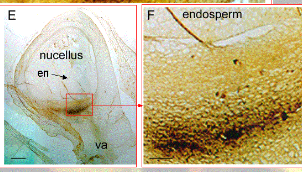
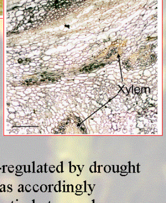


Fig. 7. In situ localization of invertase activity in young maize kernels at +7 DFP. 5 µm sections of methacrylat embedded tissue was stained with toluidine blue (A, B) for overview, or DAB in a coupled enzymatic reaction with glucose oxidase and peroxidase producing a yellow-brown precipitate at the sites of invertase activity. (C, D): kernel from well watered plant. (E, F): kernel from severely drought stressed plant showing less activity. (G): Negative control without substrate added to reaction.





Conclusion

Expression of the *Ivr2* gene encoding a vacuolar invertase was down-regulated by drought with preceding changes in ABA levels. Vacuolar invertase activity was accordingly decreased by drought, and seemed to be the main determinant of the ratio between hexoses and sucrose in young ovaries. The localization was clearly distinct from cell wall invertase (*Incw2*) in basal endosperm tissue later in kernel development (8, 10). The drought-induced decrease in hexose to sucrose ratio may affect phloem unloading, turgor and ovary differentiation and thus implicates a key role for *Ivr2* vacuolar invertase during the early, abortion-sensitive phase of ovary development.

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