Water Deficit Induces Abscisic Acid Accumulation in Endosperm of Maize \textit{Viviparous} Mutants\textsuperscript{1}

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ABSTRACT

To determine whether abscisic acid (ABA) accumulation in endosperms of water-limited maize (\textit{Zea mays} L.) plants is from synthesis in maternal plant organs or from intraendosperm synthesis, plants heterozygous for \textit{viviparous} (\textit{vp}) genes were self-pollinated to create endosperm genotypes capable of (+/-/-; +/- +/+ or incapable (-/-/-) of carotenoid and ABA synthesis. The mutants \textit{vp2}, \textit{vp5}, and \textit{vp7}, each in \textit{W22} inbred background, were utilized. Both in wild-type endosperms capable of ABA synthesis and in mutants incapable of ABA synthesis, ABA concentrations at 15 days after pollination were substantially increased in response to plant water deficit. We conclude that ABA synthesis in maternal organs was the source of ABA that accumulated in endosperms in response to plant water deficit.

We (14) previously suggested that ABA may serve as a growth-regulating signal to alter maize reproductive development during drought. In support of this idea, we noted that, in addition to elevating the ABA concentrations in leaves (13, 16) and roots (22), water deficit also increases ABA concentrations in grain tissues of maize and other cereals (3, 13, 14, 20). The water deficit-induced increase in grain ABA concentration was tentatively thought to be of maternal origin. This interpretation was based on studies indicating a lack of change in endosperm water potentials (13, 14) and studies in which radiolabeled ABA was used, that showed that plants are capable of translocating free ABA from leaves to grains (3, 13, 19).

To test further the hypothesis that increased concentrations of ABA in endosperm of water-limited maize is maternally produced, we report here the results of a study using \textit{vp}\textsuperscript{2} mutants of maize. The mutants \textit{vp2}, \textit{vp5}, and \textit{vp7} contain blocks at various points along the carotenoid biosynthesis pathway and accumulate low amounts of ABA in embryo and endosperm (12). There is substantial support for the view that the pathway of ABA biosynthesis in plants is via carotenoid precursors (2, 18, 21). This view is consistent with observations that ABA was not detected in \textit{vp} mutant seedling (19) or root tissues (10). Studies indicated that small amounts of ABA present in mutant seeds are derived from the maternal plant (19).

The objective of the current study was to determine whether increased ABA levels in endosperms of water-limited plants is from synthesis in maternal organs or from intraendosperm synthesis. Plants heterozygous for \textit{vp} genes were self-pollinated to create endosperm genotypes capable of (+/-/-; +/- +/+ or incapable (-/-/-) of carotenoid and (hence ABA) synthesis. If ABA accumulated regardless of kernel genotype, then water deficit-induced ABA found in endosperm must be from maternal tissues. Our previous study showed that, during water limitation, endosperms from apical ear regions had higher ABA concentrations than endosperms from the middle of the ear (14). To determine whether this same positional pattern of ABA accumulation would occur in response to water deficit in the genotypes used in the current study, kernels in apical and middle regions of ears were compared.

MATERIALS AND METHODS

Each seed stock of maize, \textit{Zea mays} L., contained a mutant allele of one of the \textit{vp} loci (\textit{vp2}, \textit{vp5}, \textit{vp7}), backcrossed into a \textit{W22} inbred background. Seed was sown in 12-L pots, and plants were cultured in a greenhouse as previously described (13). Beginning at 1 DAP, plants were subjected to either well-watered or water deficit treatments as previously described (13). Plants were self-pollinated (13). At about 15 DAP, endosperms of the (-/-/-) genotype could be identified on ears by the lack of yellow carotenoid pigmentation; the other three endosperm genotypes (+/-/+; +/- +/+, -/-/-) exhibited a yellow kernel phenotype. The "mutant" kernels referred to in this study were kernels of the (-/-/-) genotype, and the other genotypes with normal pigmentation were termed "wild-type" kernels. Kernels were harvested from ears at 15 DAP and immediately frozen in liquid N\textsubscript{2}. Endosperms and embryos were excised from frozen kernels for subsequent analysis. ABA and starch measurements were conducted as described previously (14).

RESULTS AND DISCUSSION

We used self-pollinated heterozygous plants, each carrying a mutant allele of one of the \textit{vp} loci (\textit{vp2}, \textit{vp5}, \textit{vp7}), to provide segregating mutant and wild-type kernels on each ear. Thus, any plant-to-plant differences due to growth conditions or genotype (residual heterozygosity following backcross introduction of mutant genes into \textit{W22} inbred background) would exert equivalent effects on both mutant and wild-type kernels and not contribute to experimental error. Examination of wild-type kernels also provided a comparison with results of previous studies in which a commercial hybrid was used (13, 14).

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\textsuperscript{2} Abbreviations: \textit{vp}, \textit{viviparous}; DAP, days after pollination.
ABA in Wild-Type Kernels

In water-limited plants heterozygous for vp2 and vp5, leaves were visibly wilted and rolled and had lost their glossy sheen by 15 DAP. Wild-type endosperms on heterozygous vp2 and vp5 plants accumulated substantially higher ABA concentrations in response to water-limited compared with well-watered treatment (Fig. 1, A–D). These results are similar to those previously observed in maize hybrid Pioneer 3925 (13, 14). In plants heterozygous for vp7, water deficit symptoms (leaf wilting and rolling) were considerably less severe than in vp2 and vp5 heterozygotes. Although wild-type endosperms in the apical region of vp7 heterozygous plants also appeared to accumulate higher ABA concentration in response to water limitation (Fig. 1E), this difference and that in middle region kernels (Fig. 1F) was not statistically significant (P ≤ 0.05). In well-watered heterozygous vp7 plants, the ABA concentrations of wild-type endosperms were about the same as those in wild-type endosperms in well-watered heterozygous vp2 and vp5 plants (about 10–20 ng/g fresh weight). The heterozygous vp7 plants were much more robust than the other vp genotypes, with tall, thick stems, perhaps indicating residual heterozygosity following the backcross introduction of the mutant allele into the W22 background. It is plausible that the imposed water deficit was less deleterious in these plants due to higher stomatal resistance or greater water storage capacity in stems (6). Endosperm from apical ear regions accumulated higher concentrations of ABA (Fig. 1) and lower amounts of starch (Table 1) than endosperm from middle ear regions. These data confirm earlier findings using a commercial maize hybrid, in which the gradient in ABA accumulation within the ear was negatively associated with endosperm cell number and starch accumulation (14).

ABA in Mutant Kernels

In homozygous mutant kernels, ABA synthesis in endosperm and embryo tissues is almost completely blocked (12).

Hence, any accumulation of ABA in response to water limitation may be attributed to import from maternal sources. Consistent with this latter possibility, mutant endosperms in plants heterozygous for vp2 and vp5 had substantially higher ABA concentrations in water-limited than well-watered treatments (Fig. 1, A–D). In middle kernels of vp2 (Fig. 1B) and in apical kernels of vp5 (Fig. 1C), the extent of ABA increase in response to water limitation was about the same in mutant compared with wild-type endosperms. In apical kernels of vp2 (Fig. 1A) and in middle kernels of vp5 (Fig. 1D), the ABA increase in response to water limitation was less in wild-type than in mutant endosperms. Nevertheless, the mutant endosperms on water-limited plants had substantially greater ABA concentration than that in mutant endosperms on well-watered plants. Mutant endosperms on heterozygous vp7 plants appeared to have slightly higher ABA concentrations in water-limited than in well-watered treatments (Fig. 1, E and F); however, such differences were not significant (P ≤ 0.05). This result is consistent with the less extreme water deficit symptoms and effects on ABA concentration in wild-type endosperm of vp7 compared with other vp genotypes, as noted above.

We expressed ABA data per gram of fresh weight (Fig. 1)

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**Table 1. Starch Content of vp5 Mutant or Wild-Type Endosperms in Apical and Middle Ear Regions of Control and Water-Limited Plants at 15 DAP**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ear Region</th>
<th>Control</th>
<th>Water Limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant</td>
<td>Apical</td>
<td>6.38 ± 0.66</td>
<td>0.57 ± 0.23</td>
</tr>
<tr>
<td>Wild type</td>
<td>Apical</td>
<td>5.58 ± 0.36</td>
<td>0.89 ± 0.13</td>
</tr>
<tr>
<td>Mutant</td>
<td>Middle</td>
<td>9.16 ± 0.63</td>
<td>1.24 ± 0.26</td>
</tr>
<tr>
<td>Wild type</td>
<td>Middle</td>
<td>8.54 ± 0.41</td>
<td>1.05 ± 0.20</td>
</tr>
</tbody>
</table>

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**Figure 1.** Concentration of ABA (fresh weight [FW] basis) in vp2 (A and B), vp5 (C and D), and vp7 (E and F) mutant (−/−/−) (left side of panels) and wild-type (+/+−/−, +/+−/−, +/+−/+−) (right side of panels) endosperms on plants subjected to well-watered (open bars) or water-limited (hatched bars) treatments. Plants heterozygous (+/−) at each genetic locus were self-pollinated 1 d before water was withheld. Endosperms were harvested at 15 DAP from apical (A, C, and E) and middle (B, D, and F) ear regions. Error bars, SEM (n = 4).
because we expected that tissue would respond to concentration rather than content per se. Moreover, increased ABA accumulation in response to water deficit was also apparent in both mutant and wild-type endosperms on \( vp_2 \) and \( vp_5 \) heterozygous plants when expressed as content per endosperm (data not shown).

Wild-type kernels accumulate ABA during grain filling and maturation, reaching a peak concentration at approximately 48 days after midsilking (4). The endosperm in the present study was sampled before the initiation of rapid ABA biosynthesis by kernel tissues, and thus import of ABA from maternal sources may have constituted a relatively higher proportion of endosperm ABA at the observed growth stage than at later growth stages.

In summary, these \( vp \) data demonstrate that the water deficit-induced ABA accumulation by endosperm tissue was not due to intraendosperm ABA synthesis. Two likely sources of the ABA that accumulated in kernels of water-limited plants are leaves and roots. This interpretation is supported by previous observations that, in plants under the same treatment regimen reported here, ABA concentration increased in both mutant and wild-type leaf and root tissues (13, 14). In addition, translocation of radiolabeled ABA from leaves to kernels was demonstrated previously (3, 13, 19), providing evidence that movement of ABA from vegetative tissues to reproductive sinks can operate in vivo.

**Starch Accumulation**

Starch accumulation was not affected by carotenoid deficiency in the \( vp \) mutants, although it decreased substantially in both genotypes in response to water deficit (Table I). We previously observed similar decreases in endosperm starch content in response to water deficit in a commercial hybrid (14). Carotenoids are essential for chloroplast development and may play a role in plastid differentiation (17). Moore and McClean (9) reported fewer and smaller plastids in columnella cells of \( vp_9 \) seedling roots. On this basis, it was expected that carotenoid-deficient \( vp_5 \) endosperms would have defective amyloplast development, resulting in decreased starch accumulation. It is possible that at 15 DAP starch accumulation was just beginning and differences in starch contents were difficult to detect. However, by 15 DAP, amyloplast formation and starch granule initiation are near completion (1, 11) and enzymes of the starch synthesis pathway approach their maximal activity (15); therefore, the capacity for starch synthesis and accumulation was present at high levels by this stage. Hence, if carotenoid deficiency had affected amyloplast formation, an effect on starch synthesis should have been detected at 15 DAP. There are several possible explanations for the lack of a \( vp_5 \) effect on starch accumulation. First, endosperm amyloplasts may not require carotenoids for the assembly of membrane systems as is the case in chloroplasts (8). Second, the carotenoid precursor phytoene, which accumulates because of the block caused by the \( vp_5 \) mutation (12), may be sufficient for normal amyloplast formation. Third, changes in amyloplast development might have occurred, but this may not have interfered with their ability to accumulate starch.

**Roles of ABA in Seed and Caryopsis Development**

Seed developmental processes differ in sensitivity to ABA. Based on studies of ABA mutants of *Arabidopsis thaliana* [Heynh.], Koornneef *et al.* (7) concluded that seed development processes such as storage protein synthesis respond to much lower concentrations of ABA than does the induction of dormancy. The basis of this conclusion was the observation that storage protein synthesis and other embryo development processes occurred normally in mutant seeds having decreased ABA responsiveness and in mutants with low capability for ABA synthesis; however, induction of dormancy was blocked in such mutants and could not be restored by maternally produced or exogenously applied ABA.

A two-phasic pattern of ABA accumulation has been observed in *Arabidopsis* seeds: a peak early in seed development is primarily of maternal origin and a later peak in ABA is seed produced (5). A similar dual pattern of ABA accumulation was observed in maize kernels (4). Thus, maternally produced ABA, such as that observed at 15 DAP in the present study, may play an important role in early maize endosperm development. Developmental processes that occur early in seed development, such as cell division and plastid formation, may be sensitive to ABA translocated from the mother plant. If concentrations of ABA increase in maternal tissues during a water deficit, seed levels of ABA may also increase; this case is supported by the data presented here. Higher than normal concentrations of ABA during early endosperm development were correlated with decreased cell division (14) and starch accumulation (14; Table I). These data add strength to the hypothesis that ABA may act as a signal between vegetative tissues experiencing water deficit and reproductive structures. Such an ABA signal may be responsible, in part, for altering growth and development of the endosperm.

**LITERATURE CITED**