Arabidopsis plants deficient in plastidial glyceraldehyde-3-phosphate dehydrogenase show alterations in abscisic acid (ABA) signal transduction: interaction between ABA and primary metabolism

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Abstract

Abscisic acid (ABA) controls plant development and regulates plant responses to environmental stresses. A role for ABA in sugar regulation of plant development has also been well documented although the molecular mechanisms connecting the hormone with sugar signal transduction pathways are not well understood. In this work it is shown that Arabidopsis thaliana mutants deficient in plastidial glycolytic glyceraldehyde-3-phosphate dehydrogenase (gapcp1gapcp2) are ABA insensitive in growth, stomatal closure, and germination assays. The ABA levels of gapcp1gapcp2 were normal, suggesting that the ABA signal transduction pathway is impaired in the mutants. ABA modified gapcp1gapcp2 gene expression, but the mutant response to the hormone differed from that observed in wild-type plants. The gene expression of the transcription factor ABI4, involved in both sugar and ABA signalling, was altered in gapcp1gapcp2, suggesting that their ABA insensitivity is mediated, at least partially, through this transcriptional regulator. Serine supplementation was able partly to restore the ABA sensitivity of gapcp1gapcp2, indicating that amino acid homeostasis and/or serine metabolism may also be important determinants in the connections of ABA with primary metabolism. Overall, these studies provide new insights into the links between plant primary metabolism and ABA signalling, and demonstrate the importance of plastidial glycolytic glyceraldehyde-3-phosphate dehydrogenase in these interactions.

Key words: ABA, ABA signal transduction, Arabidopsis, GAPCp, glyceraldehyde-3-phosphate dehydrogenase, glycolysis, sugar signalling.

Introduction

The phytohormone abscisic acid (ABA) controls the development of plants and plays a crucial role in their survival when faced with adverse environmental conditions, such as salt and water stress (Schroeder et al., 2001; Himmelbach et al., 2003). The genetic dissection of ABA signalling has now identified central players of this signal transduction pathway including ABA receptors (Shen et al., 2006; Ma et al., 2009; Melcher et al., 2009; Miyazono et al., 2009; Pandey et al., 2009; Park et al., 2009; Santiago et al., 2009; Cutler et al., 2010; Nishimura et al., 2010), the group A
protein phosphatases type 2C, which negatively regulate ABA signalling (Meyer et al., 1994; Leung et al., 1997), and the SNF1-related kinase 2 (SnRK2 kinases) which are positive regulators (Yoshida et al., 2006; Vlad et al., 2009) of downstream substrates such as AREB/ABF transcription factors (Furihata et al., 2006).

A role for ABA in sugar regulation of plant development has been well documented (Arenas-Huertas et al., 2000; Leon and Sheen, 2003; Rolland et al., 2006; Rook et al., 2006). Some of the Arabidopsis sugar signalling mutants are allelic to ABA biosynthetic or signalling mutants, and many ABA biosynthetic mutants also have sugar phenotypes (Arenas-Huertas et al., 2000; Rolland et al., 2006; Ramon et al., 2008). The two main substrates for glycolysis in plants, sucrose and starch (Plaxton, 1996), are subjected to large diurnal changes in their concentrations. Thus, sugar levels and glycolysis need to be highly coordinated to provide metabolic flexibility that can respond to the differential demands of plant development and to environmental stress acclimation. In spite of considerable evidence corroborating the interactions between sugars and ABA, information about the role played by glycolysis in these interactions is lacking.

The most well-known function of glycolysis is to provide energy and precursors for anabolic processes (Plaxton, 1996). In recent years, additional non-glycolytic functions, such as the regulation of transcription or apoptosis, have also been attributed to glycolytic enzymes in mammals and yeast (Kim and Dang, 2005). These additional functions of glycolytic enzymes suggest that links between metabolic sensors and development could be established directly through the enzymes that participate in metabolism (Kim and Dang, 2005). In this sense, a role for the plant glycolytic hexokinase as a glucose sensor has been reported (Moore et al., 2003; Ramon et al., 2008). On the other hand, the participation of enolase and aldolase in the plant response to salt tolerance (Barkla et al., 2009) postulates a more direct function for glycolytic enzymes in acclimation to environmental stresses.

The glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reversibly converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate by coupling with the reduction of NAD⁺ to NADH. In mammals, GAPDHs have been implicated in transcriptional regulation, DNA repair, signal transduction cascades, and apoptosis (Hara et al., 2005; Kim and Dang, 2005; Hara and Snyder, 2006; Colell et al., 2007; Min et al., 2007; Lee et al., 2009). A family of four genes encoding putative phosphorylating glycolytic GAPDHs (GAPC1, GAPC2, GAPCp1, and GAPCp2) is present in Arabidopsis thaliana (http://www.Arabidopsis.org). GAPC1 and GAPC2 are cytosolic isoforms, while GAPCp1 and GAPCp2 are located in the plastids (Muñoz-Bertomeu et al., 2009). In spite of the low gene expression level of GAPCps in Arabidopsis, gapcp double mutants (gapcp1gapcp2) show profound alterations of plant development, mainly arrested root development and sterility (Muñoz-Bertomeu et al., 2009, 2010a, b). The strategic situation of GAPCp in the glycolytic pathway could make this enzyme an important player not only for glycolysis but also for the generation of metabolites for other anabolic pathways (Plaxton, 1996; Ho and Saito, 2001; Andre et al., 2007; Baud et al., 2007). In this same vein, it was previously demonstrated that GAPCp deficiency affected the sugar and amino acid balance in Arabidopsis (Muñoz-Bertomeu et al., 2009). Another pathway that could be affected by GAPCp deficiency is ABA biosynthesis. The substrate of GAPCp, glyceraldehyde-3-phosphate, is the first precursor of the methyl-erythritol phosphate pathway responsible for ABA biosynthesis in the plastids. Here evidence is provided that a deficiency in GAPCp is able to provide insensitivity to ABA. It is demonstrated that this insensitivity is not due to a modification of the ABA content but to an impaired ABA signal transduction in the GAPCp-deficient mutant. It is shown that the gapcp1gapcp2 ABA insensitivity is caused by the sugar/amino acid imbalance displayed by the mutant. The ABA insensitivity of gapcp1gapcp2 could be mediated, at least in part, via the transcriptional regulator ABI4. A specific role for amino acid homeostasis and/or serine metabolism in the ABA–sugar interaction is also discussed. This work provides new evidence for the links between plant primary metabolism and ABA signal transduction, and demonstrates the importance of GAPCps in these interactions.

Materials and methods

Plant material and growth conditions

Several A. thaliana (ecotype Columbia 0) gapcp1 and gapcp2 single and double T-DNA mutants were previously isolated as described in Muñoz-Bertomeu et al. (2009). For this work, mutant allele gapcp1.1 (SAIL_390_G10) of the At1g79530 gene, and gapcp2.1 (SALK_137288) and gapcp2.3 (SALK_037936) alleles of the At1g18300 gene were used. Wild-type (WT) plants expressing a GAPCp1 cDNA under the control of the 3SS promoter (3SS-GAPCp) were previously obtained as described in Muñoz-Bertomeu et al. (2009).

Unless otherwise stated, seeds were sterilized and sown on 0.8% agar plates containing 1/5 Murashige and Skoog (MS) with Gamborg vitamins, and MES (0.9 g l⁻¹) adjusted to pH 5.7 with TRIS. After a 4 d treatment at 4°C, seeds were placed in a growth chamber (SANYO, MLR-351H, Japan) at 22°C for a 16/8 h day/night photoperiod, 100 μmol m⁻² s⁻¹. Plates were supplemented with different concentrations of ABA and serine as indicated in the figure legends. Drought stress was imposed by placing the plates under the hood for 4 h before collecting the plants. For dormancy experiments, freshly harvested seeds were sown on plates containing 0.8% agar and 0.9 g l⁻¹ MES adjusted to pH 5.7 with TRIS. Because gapcp1gapcp2 is sterile, a population of seeds from heterozygous plants (GAPCp1gapcp1.1 gapcp2,1gapcp2.1) was used for the germination experiments. This population, named G1g1.1 g2.1g2.1, contained 25% of double mutants (gapcp1.1gapcp1.1 gapcp2.1gapcp2.1), 50% of heterozygous plants (GAPCp1-gapcp1.1 gapcp2.1gapcp2.1), and 25% of single mutant plants (GAPCp1GAPCp1 gapcp2.1gapcp2.1). To score seed germination, the percentage of seeds that had germinated and developed fully green expanded cotyledons was determined. For the rest of the physiological and molecular studies, double homozygous gapcp1gapcp2 plants were identified in segregating populations of vertically grown plants by the root phenotype after 7–10 d as described (Muñoz-Bertomeu et al., 2009). Then they were
subcultured on media containing different concentrations of ABA or sorine for an additional 8–13 d as indicated in the figure legends and the tables. For stomata bioassay, some plantlets were grown in a Conviron growth chamber in pots filled with a (1:1, v/v) mixture of vermiculite and fertilized peat (KEKILA 50/50; kekkilä Iberia, S.L.) irrigated with demineralized water as needed.

Stomata bioassays

Stomatal responses were analysed in 3- to 4-week-old plants. Leaves were floated for 2.5 h in stomatal opening solution containing 50 mM KCl, 7 mM iminodiacetic acid, and 10 mM MES (pH 6.2). After incubation in 1 μM and 10 μM ABA for 2 h, leaf epidermal peels were obtained and the stomatal aperture was measured as described (Pei et al., 1997). Control experiments were performed in parallel with no ABA added. t-Test (one-tailed, homoscedastic) P-values were calculated.

Microarrays

Fifteen day-old seedlings (gapcp1gapcp2 and WT) vertically grown in 1/5 MS plates were used for the microarray experiments. On day 8 seedlings were subcultured on media containing 0.75 μM ABA as described above. Total RNAs from three pools of 15-day-old seedlings (gapcp1gapcp2 and WT) vertically grown in 1/5 MS plates at the same time were extracted using the RNeasy plant mini kit (Qiagen). RNA integrity was determined using RNA 6000 Nano Labchips in an Agilent 2100 Bioanalyzer following the manufacturer’s protocol. The purified RNA (8 μg) was used to generate first-strand cDNA in a reverse transcription reaction (One-Cycle Target Labeling and Control Reagents; Affymetrix). After second-strand synthesis, the double-stranded cDNAs were used to generate cRNA via an in vitro transcription reaction. The cRNA was labelled with biotin, and 20 μg of the labelled cRNA was fragmented. The size distribution of the cRNAs and fragmented cRNAs was assessed using an Eppendorf Biophotometer and electrophoresis. Fragmented cRNA (15 μg) was added to 300 μl of hybridization solution, and 200 μl of this mixture was used for hybridization on Arabidopsis ATH1 Genome Arrays for 16 h at 45 °C. The standard wash and double-stain protocols (EukGE-WS2xv5-450) were applied using an Affymetrix GeneChip Fluidics Station 450. The arrays (three replicates of each line) were scanned on an Affymetrix GeneChip scanner 3000. Fluorescence images were normalized with the software GCOS from Affymetrix. Expression data of ABA-treated seedlings were compared with images were normalized with the software GCOS from Affymetrix.

Metabolite determination

Starch and total soluble glucose were determined with the ENZYTEC starch kit (ATOM). ABA content was determined by liquid chromatography-electrospray tandem mass spectrometry (Durgbanshi et al., 2005). Total glutathione was extracted and subsequently quantified by reverse-phase HPLC after derivatization with monobromobimane (Molecular Probes) following previously described methods (Dominguez-Solis et al., 2001)

Results
gapcp1gapcp2 are ABA insensitivity

At the seedling stage, gapcp1gapcp2 are smaller than WT plants when grown in a medium without ABA (Fig. 1A).

![Fig. 1.](image-url) The gapcp1gapcp2 mutant shows ABA insensitivity to growth. (A) Fresh weight of the aerial part of 3-week-old gapcp1gapcp2 seedlings (g1.1g1.1 g2.1g2.1) as compared with the wild type (WT). (B) Fresh weight (%) of the aerial part of 3-week-old gapcp1gapcp2 seedlings (g1.1g1.1 g2.1g2.1) as compared with the WT under different ABA concentrations. Seedlings were treated for 13 d with the ABA concentrations indicated in the figure. Data are the mean ± SD, n ≥ 5 plates, each plate containing four (WT) and six (g1.1g1.1 g2.1g2.1) plants. *Significant at a P-value < 0.05 as compared with the WT. For simplicity, g stands for gapcp.
The fresh weight of 3-week-old gapcp1gapcp2 plants grown in MS medium was almost three times lower than that of the WT (Fig. 1A). However, gapcp1gapcp2 showed less sensitivity to growth inhibition by ABA. While WT growth was inhibited by ~50% by 0.5 μM ABA, the growth of gapcp1gapcp2 was not significantly affected by this concentration (Fig. 1B). A 2-fold higher ABA concentration (1 μM) was necessary to achieve the same level of growth inhibition in gapcp1gapcp2 when compared with the WT (Fig. 1B).

It may be argued that gapcp1gapcp2 ABA growth insensitivity could be associated with the slower growth rate of the mutant under non-ABA conditions as compared with the WT. To rule out this possibility other typical ABA responses were examined. Stomata bioassay experiments revealed that gapcp1gapcp2 responds to ABA by closing stomata, but the response needs a higher ABA concentration if compared with the response of the WT (Fig. 2).

Seed germination assays were also performed. Since gapcp1gapcp2 seeds are sterile, single gapcp mutants (gapcp1 and gapcp2) and a mixed population of GAPCp-deficient seeds (single mutant, double mutant, and heterozygous), named Glg1.1 g2.1g2.1, originating from heterozygous mother plants (GAPCp1gapcp1gapcp2gapcp2), were used in these experiments. Seed dormancy was not altered by GAPCp deficiency as compared with the WT (Fig. 3A). However, germination of GAPCp-deficient seeds was insensitive to ABA. The ABA insensitivity phenotype was already observed in gapcp single mutants (Fig. 3B) and this phenotype was also observed in G1g1.1 g2.1g2.1 (Fig. 3C).

Plants overexpressing GAPCp1 (Oex-GAPCp1) did not show hypersensitivity to ABA (Fig. 3C), which suggests that either only loss of GAPCp1 function affects the ABA response or that other compensatory and/or regulatory mechanisms are responsible for this lack of phenotype.

ABA and glutathione levels are not modified in gapcp1gapcp2

The ABA-insensitive phenotype of gapcp1gapcp2 may indicate that this mutant is ABA deficient. The methylerythritol phosphate pathway responsible for ABA biosynthesis is located in the plastids. The first precursor in this
pathway is glyceraldehyde-3-phosphate, the substrate of GAPCp. The ABA content in the aerial part of gapcp1-gapcp2 did not differ significantly from that of the WT under non-drought stress conditions. In addition, the mutant was able to respond to imposed drought stress by increasing the ABA content similarly to the WT (Table 1).

gapcp1gapcp2 roots display serine deficiency (Muñoz-Bertomeu et al., 2009). Since serine is the precursor of glycine and cysteine, both of which form part of the glutathione molecule, the levels of this metabolite were also measured. The glutathione contents in both the aerial part and the roots of gapcp1gapcp2 did not differ significantly from those of the WT (Table 1).

gapcp1gapcp2 plants display an altered gene expression in response to ABA

To assess the effect of ABA on the gene expression of gapcp1gapcp2, a microarray analysis comparing gapcp1-gapcp2 and the WT after ABA treatment was performed. These data were compared with those obtained under identical conditions with non-ABA-treated plants (Muñoz-Bertomeu et al., 2009). Full details of the microarray analysis are provided in Supplementary Table S2 at JXB online.

ABA treatment deregulated 321 genes (a >2-fold difference relative to the WT; P <0.05) in gapcp1gapcp2 as compared with the non-ABA-treated mutants and 193 genes in the WT as compared with the non-ABA-treated WT (Fig. 4A, and Supplementary Table S2 at JXB online). ABA-treated WT and gapcp1gapcp2 only shared 24% of the deregulated genes. Thus, the gene expression response of gapcp1gapcp2 to ABA differed from that of the WT, and could be related to the insensitivity phenotypes observed in the mutant.

### Table 1. ABA (ng g⁻¹ DW) and glutathione (nmol g⁻¹ DW) content in the wild-type (WT) and gapcp1gapcp2 (g1.1 g1.1 g2.1 g2.1) with or without drought stress

Three-week-old plants were grown in plates. For drought stress, plates were left open under the hood for 4 h before harvesting. Results are the mean ± SD, n ≥3.

| Lines                | ABA Aerial part | Glutathione Aerial part | Roots |
|----------------------|-----------------|-------------------------|-------|
| WT (control)         | 26.6±1.4        | 2454±169                | 1586±57 |
| g1.1 g1.1 g2.1 g2.1 (control) | 24.1±1.7       | 2513±128                | 1627±81 |
| WT (drought stress)  | 179.8±5.3       |                         | –     |
| g1.1 g1.1 g2.1 g2.1 (drought stress) | 173.8±4.6     |                         | –     |

Fig. 4. ABA gene expression is deregulated in gapcp1gapcp2. (A) Comparison of the wild type (WT) versus WT+ABA and gapcp1gapcp2 (g1.1 g1.1 g2.1 g2.1) versus g1.1 g1.1 g2.1 g2.1+ABA. (B) Comparison of the WT versus g1.1 g1.1 g2.1 g2.1 and WT+ABA versus g1.1 g1.1 g2.1 g2.1+ABA. (C) Quantification of transcript level changes in g1.1 g1.1 g2.1 g2.1 upon ABA treatment. RNA was extracted from 18-day-old gapcp1gapcp2 and WT seedlings grown in the presence or absence of 0.75 μM ABA for 10 d. The expression of a selection of up- and down-regulated genes in gapcp1gapcp2 was quantified using real-time PCR. Data are the mean ± SD; n=3. For simplicity, g stands for gapcp.
More than 270 genes were deregulated in gapep1gapcp2 as compared with the WT (Fig. 4B, and Supplementary Table S2). Surprisingly, after long-term ABA treatment there were no significant differences in gene expression between gapep1gapcp2 and the WT (Fig. 4B). Some of the genes deregulated by ABA in the WT, especially the down-regulated genes (46%), were already deregulated in gapep1gapcp2 prior to ABA treatment (Fig. 4A). Furthermore, in 41% of genes deregulated in gapep1gapcp2, differences in gene expression between gapep1gapcp2 and the WT were attenuated by the hormone treatment (Fig. 4A). The effect of ABA on several genes deregulated in gapep1gapcp2 was reconfirmed by real-time Q-RT-PCR. As shown in Fig. 4C, the gene expression differences between gapep1gapcp2 and the WT were attenuated upon ABA treatment.

ABA affects carbohydrate metabolism and deregulates ABI4 expression in gapep1gapcp2

gapep1gapcp2 present high levels of carbohydrates in both the aerial part and roots as compared with the WT (Munoz-Bertomeu et al., 2009). ABA treatment reduced both starch and total soluble glucose levels in the aerial part of gapep1gapcp2 (Fig. 5). The effect of ABA on the root carbohydrate level of gapep1gapcp2 was not as clear. Although there was a trend to a reduction in the starch and an increase in the glucose content, these changes were not significant (Fig. 5). As the ABA-INSENSITIVE transcription factor ABI4 has been described as a central transcriptional regulator connecting ABA with sugar signalling, the gene expression of ABI4 was investigated in 48 h imbibed seeds (±0.25 μM ABA) from the WT and from a mixed population of GAPCp-deficient seeds (G1g1.1 g2.1g2.1). ABI4 induction by ABA was reduced by GAPCp inactivation as compared with the WT (Fig. 6).

**ABA insensitivity of gapep1gapcp2 is partially dependent on serine supply**

It was previously demonstrated that serine supplementation to the growing medium restored normal carbohydrate levels in gapep1gapcp2 (Munoz-Bertomeu et al., 2009). In order to investigate whether the gapep1gapcp2 ABA insensitivity phenotype may be related to only its impaired carbohydrate metabolism, the ABA phenotypes of gapep1gapcp2 were investigated in those mutants supplemented with serine. The presence of serine in the growing medium abolished gapep1gapcp2 insensitivity to growth inhibition by ABA at all the concentrations used (Fig. 7). Serine also significantly reduced the ABA insensitivity of gapep1gapcp2 during germination (Fig. 8). In the presence of serine, the
inhibition by ABA of gapcp1gapcp2 germination did not differ from that of the WT not supplied with serine (Fig. 8). Interestingly, serine supplementation produced hypersensitivity to ABA in both the WT and gapcp1gapcp2 (Fig. 8).

**Discussion**

**gapcp1gapcp2 has an impaired ABA signal transduction pathway**

*gapcp1gapcp2* was insensitive to ABA, but the mutant’s dormancy response was not altered as compared with the WT, indicating that the ABA phenotype observed in the mutants is hormone specific. The impaired *gapcp1gapcp2* ABA response could indicate an alteration either of ABA biosynthesis or of the hormone signalling. The ABA levels in *gapcp1gapcp2* were normal, suggesting that GAPCp affects ABA signal transduction. In this sense, the gene expression response of *gapcp1gapcp2* to ABA differs from that of the WT. Thus, GAPCp inactivation does not eliminate gene responses to ABA but modifies them. The altered gene expression upon ABA treatment can be related to the ABA insensitivity of *gapcp1gapcp2* and suggests that ABA signal transduction in the mutant is impaired.

**The ABA response of gapcp1gapcp2 interferes with carbohydrate metabolism**

The *gapcp1gapcp2* ABA insensitivity phenotype may relate to its impaired carbohydrate metabolism. Starch levels have been seen to decrease upon stress, such as cold (Nagao et al., 2005), salt (Kempa et al., 2007), or ABA treatment (Akihiro et al., 2005). It could be argued that *gapcp1gapcp2* display more ABA insensitivity than the WT because they have a higher starch content than the WT under non-stress conditions. ABA would induce the mobilization of starch to obtain compatible solutes under stress conditions. In fact, the ABA treatment of *gapcp1gapcp2* was associated with a reduction in the starch and soluble glucose levels in the aerial part. This result indicates that *gapcp1gapcp2* respond to ABA. Furthermore, the results are a direct proof that ABA interferes with starch metabolism.

The transcription factor ABI4 has been implicated in both ABA and sugar signal transduction cascades during early seedling development and could be one of the links between these two pathways (Acevedo-Hernandez et al., 2005; Rook et al., 2006; Bossi et al., 2009). ABI4 has also been implicated in the retrograde signalling between plastids and the nucleus (Koussevitzky, 2007). The induction of the ABI4 gene by ABA in GAPCp-deficient seeds was attenuated as compared with that observed in the WT. These results indicate that the ABA insensitivity of *gapcp1gapcp2* may be mediated, at least at the initial developmental stages, through ABI4. Since these expression experiments were performed in a mixed population of single mutants, double mutants, and heterozygous seeds, much higher differences in ABI4 expression should be expected between a homogeneous population of *gapcp1gapcp2* and WT seeds. Finally, the deregulation of ABI4 expression in *gapcp1gapcp2* supports the microarray conclusions indicating that GAPCp inactivation modifies the ABA-induced gene response.

**Involvement of serine metabolism in ABA signal transduction**

As ABI4 expression is narrowed down to the initial stages of seedling development, it is possible that other signalling molecules participate in the connection between sugar and ABA signal transduction. Serine supply restored normal
ABA growth sensitivity in gapcp1gapcp2, which could be due to its effect on carbohydrate metabolism, as already discussed. However, it could also be related, at least in part, to changes in the amino acid pools. A recent metabolomic study has shown a correlation among drought stress, ABA production, and amino acid accumulation in Arabidopsis (Urano et al., 2009). The total free amino acids in gapcp1gapcp2 increased as compared with the control plants.

**Fig. 7.** gapcp1gapcp2 ABA insensitivity to growth is abolished by serine supplementation. Fresh weight (%) of the aerial part of 18-day-old gapcp1gapcp2 seedlings (g1.1g1.1 g2.1g2.1) as compared with the wild type (WT) under different concentrations of ABA and serine. Seedlings were treated for 9 d with the concentrations of ABA and serine indicated in the figure. Data are the mean ±SD, n ≥5 plates; each plate contained 4 (WT) and six (g1.1g1.1 g2.1g2.1) plants. *Significant at a P-value <0.05 as compared with the WT. For simplicity, g stands for gapcp.

**Fig. 8.** Serine supplementation confers hypersensitivity to ABA during germination. Germination of 10-day-old seeds from wild-type (WT) and heterozygous gapcp (G1g1.1 g2.1g2.1) plants under different concentrations of ABA and serine. Data are the mean ±SD; each experiment consisted of at least four plates with 60 seeds each. The experiment was repeated several times with different pools of seeds. ●Significant at a P-value <0.05 as compared with the non-serine-treated WT seeds. *Significant at a P-value <0.05 as compared with the non-serine-treated seeds within each genotype. For simplicity, g stands for gapcp and G for GAPCp.
factor. Finally, the results suggest that serine (directly or indirectly) may play a role in connecting primary metabolism and ABA signal transduction.

Accession numbers

*Arabidopsis* Genome Initiative locus identifiers of *Arabidopsis* genes used in this article are as follows: GAPCp1 (At1g79530), GAPCp2 (At1g16300).

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Quantification of GAPCp1 transcript level in the roots and aerial part of GAPCp1-overexpressing (Oex-GAPCp1) lines as compared with the wild type (WT). Data from a representative overexpressing line are presented.

Table S1. Primers used in this work

Table S2. Microarray data. The data were compared using the dChip software.

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References

Acevedo-Hernandez GJ, Leon P, Herrera-Estrella LR. 2005. Sugar and ABA responsiveness of a minimal RBCS light-responsive unit is mediated by direct binding of ABI4. *The Plant Journal* 43, 506–519.

Akihiro T, Mizuno K, Fujimura T. 2005. Gene expression of ADP-glucose pyrophosphorylase and starch contents in rice cultured cells are cooperatively regulated by sucrose and ABA. *Plant and Cell Physiology* 46, 937–946.

Andre C, Froehlich JE, Moll MR, Benning C. 2007. A heteromeric plastidic pyruvate kinase complex involved in seed oil biosynthesis in Arabidopsis. *The Plant Cell* 19, 2006–2022.

Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, Leon P. 2000. Analysis of Arabidopsis glucose insensitive mutants, gin5 and gin6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. *Genes and Development* 14, 2085–2096.
Barkla BJ, Vera-Estrella R, Hernandez-Coronado M, Pantoja O. 2009. Quantitative proteomics of the tonoplast reveals a role for glycolytic enzymes in salt tolerance. The Plant Cell 21, 4044–4058.

Baud S, Wuilleme S, Dubreucq B, de Almeida A, Vuagnat C, Lepiniec L, Miquel M, Rochat C. 2007. Function of plastidial pyruvate kinases in seeds of Arabidopsis thaliana. The Plant Journal 52, 405–419.

Bossi F, Cordoba E, Dupre P, Mendoza MS, Roman CS, Leon P. 2009. The Arabidopsis ABA-INSENSITIVE (ABI) 4 factor acts as a central transcription activator of the expression of its own gene, and for the induction of ABI5 and SBE2.2 genes during sugar signaling. The Plant Journal 59, 359–374.

Colell A, Ricci JE, Tait S, et al. 2007. GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. Cell 129, 983–997.

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. Annual Review of Plant Biology 139, 5–17.

Czechowski T, Stitt M, Altman T, Udvardi MK, Scheible WR. 2005. Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiology 139, 5–17.

Dominguez-Solis JR, Gutierrez-Alcala G, Vega JM, Romero LC, Gotor C. 2001. The cytosolic O-acetylserine(thiol)lyase gene is regulated by heavy metals and can function in cadmium tolerance. Journal of Biological Chemistry 276, 9297–9302.

Durgbanshi A, Arbona V, Pozo O, Miersch O, Sancho JV, Gomez-Cadenas A. 2005. Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. Journal of Agricultural and Food Chemistry 53, 8437–8442.

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshiha R, Shinozaki K, Yamaguchi-Shinozaki K. 2006. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. Proceedings of the National Academy of Sciences, USA 103, 1988–1993.

Guan LM, Zhao J, Scandalios JG. 2000. Cis-elements and trans-factors that regulate expression of the maize Cat1 antioxidant gene in response to ABA and osmotic stress: H$_2$O$_2$ is the likely intermediary signaling molecule for the response. The Plant Journal 22, 87–96.

Hara MR, Agrawal N, Kim SF, et al. 2005. S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nature Cell Biology 7, 665–674.

Hara MR, Snyder SH. 2006. Nitric oxide–GAPDH–Siah: a novel cell death cascade. Cellular and Molecular Neurobiology 26, 527–538.

Himmelbach A, Yang Y, Grill E. 2003. Relay and control of abscisic acid signaling. Current Opinion in Plant Biology 6, 470–479.

Ho CL, Saito K. 2001. Molecular biology of the plastidial phosphorylated serine biosynthetic pathway in Arabidopsis thaliana. Amino Acids 20, 243–259.

Kempa S, Rozhon W, Samaj J, et al. 2007. A plastid-localized glycogen synthase kinase 3 modulates stress tolerance and carbohydrate metabolism. The Plant Journal 49, 1076–1090.

Kim JW, Dang CV. 2005. Multifaceted roles of glycolytic enzymes. Trends in Biochemical Sciences 30, 142–150.

Kousaveitzky S. 2007. Signals from chloroplasts converge to regulate nuclear gene expression. Science 316, 1698–1698.

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI. 2003. NADPH oxidase AtRbohd and AtRbohf genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO Journal 22, 2623–2633.

Lee MN, Ha SH, Kim J, Koh A, Lee CS, Kim JH, Jeon H, Kim DH, Suh PG, Ryu SH. 2009. Glycolytic flux signals to mTOR through glyceraldehyde-3-phosphate dehydrogenase-mediated regulation of Rheb. Molecular and Cellular Biology 29, 3991–4001.

Leon P, Sheen J. 2003. Sugar and hormone connections. Trends in Plant Science 8, 110–116.

Leung J, Merlot S, Giraudat J. 1997. The Arabidopsis ABSCISIC ACID-INSENSITIVE2 (ABI2) and ABI1 genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. The Plant Cell 9, 759–771.

Li C. 2008. Automating dChip: toward reproducible sharing of microarray data analysis. BMC Bioinformatics 9, 231.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324, 1064–1068.

Melcher K, Ng LM, Zhou XE, et al. 2009. A gate–latch–lock mechanism for hormone signalling by abscisic acid receptors. Nature 462, 602–608.

Meyer K, Leube MP, Grill E. 1994. A protein phosphatase 2C involved in ABA signal transduction in Arabidopsis thaliana. Science 264, 1452–1455.

Min J, Kim YK, Cipriani PG, et al. 2007. Forward chemical genetic approach identifies new role for GAPDH in insulin signaling. Nature Chemical Biology 3, 55–59.

Miyazono KI, Miyakawa T, Sawano Y, et al. 2009. Structural basis of abscisic acid signalling. Nature 462, 609–614.

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J. 2003. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signalling. Science 300, 332–336.

Muñoz-Bertomeu J, Cascales-Minana B, Alaiz M, Segura J, Ros R. 2010a. A critical role of plastidial glycolytic glyceraldehyde-3-phosphate dehydrogenase in the control of plant metabolism and development. Plant Signaling and Behavior 5, 67–69.

Muñoz-Bertomeu J, Cascales-Minana B, Ires-Segura A, Mateu I, Nunes-Nesi A, Fernie AR, Segura J, Ros R. 2010b. The plastidial glyceraldehyde-3-phosphate dehydrogenase is critical for viable pollen development in Arabidopsis. Plant Physiology 152, 1830–1841.

Muñoz-Bertomeu J, Cascales-Miñana B, Mulet JM, Baroja-Fernandez E, Pozueta-Romero J, Kuhn JM, Segura J, Ros R. 2009. Plastidial glyceraldehyde-3-phosphate dehydrogenase deficiency leads to altered root development and affects the sugar and amino acid balance in Arabidopsis. Plant Physiology 151, 541–558.

Nagao M, Minami A, Arakawa K, Fujikawa S, Takezawa D. 2005. Rapid degradation of starch in chloroplasts and concomitant
accumulation of soluble sugars associated with ABA-induced freezing tolerance in the moss Physcomitrella patens. *Journal of Plant Physiology* **162**, 169–180.

Nishimura N, Sarkeshik A, Nito K, et al. 2010. PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. *The Plant Journal* **61**, 290–299.

Pandey S, Nelson DC, Assmann SM. 2009. Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. *Cell* **136**, 136–148.

Park SY, Fung P, Nishimura N, et al. 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071.

Pei ZM, Kuchitsu K, Ward JM, Schwarz M, Schroeder JI. 1997. Differential abscisic acid regulation of guard cell slow anion channels in Arabidopsis wild-type and abi1 and abi2 mutants. *The Plant Cell* **9**, 409–423.

Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **406**, 731–734.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, e45.

Plaxton WC. 1996. The organization and regulation of plant glycolysis. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 185–214.

Ramon M, Rolland F, Sheen J. 2008. Sugar sensing and signaling. In: Somerville C, Meyerowitz M, eds. The Arabidopsis book. Rockville, MD: American Society of Plant Biologistsdoi: 10.1199/tab.0117, http://www.aspb.org/publications/arabidopsis/.

Rolland F, Baena-Gonzalez E, Sheen J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**, 675–709.

Rook F, Hadingham SA, Li Y, Bevan MW. 2006. Sugar and ABA response pathways and the control of gene expression. *Plant, Cell and Environment* **29**, 426–434.

Santiago J, Dupeux F, Round A, Antoni R, Park SY, Jamin M, Cutler SR, Rodriguez PL, Marquez JA. 2009. The abscisic acid receptor PYR1 in complex with abscisic acid. *Nature* **462**, 665–668.

Schroeder JI, Kwak JM, Allen GJ. 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* **410**, 327–330.

Shen YY, Wang XF, Wu FQ, et al. 2006. The Mg-chelatase H subunit is an abscisic acid receptor. *Nature* **443**, 823–826.

Suhita D, Raghavendra AS, Kwak JM, Vavasseur A. 2004. Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. *Plant Physiology* **134**, 1536–1545.

Urano K, Maruyama K, Ogata Y, et al. 2009. Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. *The Plant Journal* **57**, 1065–1078.

Vlad F, Rubio S, Rodrigues A, Sirichandra C, Belin C, Robert N, Leung J, Rodriguez PL, Lauriere C, Merlot S. 2009. Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in Arabidopsis. *The Plant Cell* **21**, 3170–3184.

Waditee R, Bhuiyan NH, Hirata E, Hibino T, Tanaka Y, Shikata M, Takabe T. 2007. Metabolic engineering for betaine accumulation in microbes and plants. *Journal of Biological Chemistry* **282**, 34185–34193.

Yoshida R, Umezawa T, Mizoguchi T, Takahashi S, Takahashi F, Shinozaki K. 2006. The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. *Journal of Biological Chemistry* **281**, 5310–5318.