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Long-distance ABA transport can mediate distal tissue responses by affecting local ABA concentrations

Running title: ABA transport and effects in grafted plants

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Abstract Environmental stresses that perturb plant water relations influence abscisic acid (ABA) concentrations, but it is unclear whether long-distance ABA transport contributes to changes in local ABA levels. To determine the physiological relevance of ABA transport, we made reciprocal- and self-grafts of ABA-deficient *flacca* mutant and wild-type (WT) tomato plants, in which low phosphorus (P) conditions decreased ABA concentrations while salinity increased ABA concentrations. Whereas foliar ABA concentrations in the WT scions were rootstock independent under normal conditions, salinity resulted in long-distance transport of ABA: *flacca* scions had approximately twice as much ABA when grafted on WT rootstocks compared to *flacca* rootstocks. Root ABA concentrations were scion dependent: both WT and *flacca* rootstocks had less ABA with the *flacca* mutant scion than with the WT scion under normal conditions. In WT scions, whereas rootstock genotype had limited effects on stomatal conductance under normal conditions, a *flacca* rootstock resulted in decreased leaf area in stressed plants, presumably due to attenuated root-to-shoot ABA transport. In *flacca* scions, a WT rootstock decreased stomatal conductance but increased leaf area of stressed plants, likely due to enhanced root-to-shoot ABA transport. Thus, long-distance ABA transport can affect responses in distal tissues by changing local ABA concentrations.

Keywords: ABA, *flacca*, grafting, phenotypic reversion, phosphorus deficiency, salt stress, *Solanum lycopersicum*

INTRODUCTION

Abscisic acid (ABA) is synthesized in response to multiple abiotic stresses that alter tissue water status (Zhang et al. 2006). Local synthesis of ABA (as indicated by enhanced expression of the *NCED* gene encoding the rate-limiting enzyme in ABA production; Iuchi et al. 2001; Speirs et al. 2013) has been observed in the leaves of well-watered plants exposed to high evaporative demand (McAdam et al. 2016b), detached leaves, and roots allowed to dry on the laboratory bench (Zdunkek-Zastocka and Sobczak 2013), as well as in the leaves and roots of plants exposed to drying soil (Speirs et al. 2013; Zdunkek-Zastocka and Sobczak 2013). In addition to acting at the site of synthesis, ABA has also been suggested to act as a long-distance signal that moves from the roots via the xylem to the shoot, where it restricts transpirational water loss by closing the stomata (Schachtman and Goodger 2008; Shabala et al. 2016). However, reciprocal grafting studies using wild-type (WT) and ABA-deficient genotypes, which should diminish the long-distance transport of ABA, suggest a relatively limited role for root-synthesized ABA in regulating gas exchange in the leaves of both *Solanum lycopersicum* (tomato) and *Arabidopsis thaliana* (Arabidopsis). For instance, soil drying induces normal levels of stomatal closure in WT tomato scions grafted to rootstocks of the ABA-deficient mutant *sitiens* (*sit*), in which any long-distance ABA signal is greatly reduced (Holbrook et al. 2002). Similarly, osmotic stress still induces stomatal closure in WT shoots grafted to rootstocks of the ABA-deficient mutant *aba2-1* in *Arabidopsis thaliana*, while *aba2-1* stomata fail to close regardless of their rootstock (Christmann et al. 2007). These results call into question the importance of long-distance ABA transport within the plant.

Other studies have found that the relative effects of ABA deficiency on stomatal responses in reciprocally grafted WT and ABA-deficient genotypes can vary according to the prevailing environmental conditions. For example, under high relative humidity, the ABA-deficient *sit* tomato rootstock did not influence stomatal conductance (g_s) of WT scions (thus g_s was independent of rootstock and root-to-shoot ABA transport) but under low relative humidity, a *sit* rootstock increased g_s of WT scions by 37% compared with the WT self-grafts (indicating a significant effect of root-to-shoot ABA transport). Conversely, under high relative humidity, a WT rootstock significantly decreased g_s of *sit* scions compared to *sit* self-grafts indicating that enhanced ABA transport from WT rootstocks could phenotypically revert an ABA-deficient scion, while the stomatal responses of ABA-deficient scions was rootstock-independent under low relative humidity conditions (Jones et al. 1987). Thus root-to-shoot ABA transport can alter stomatal responses of

both WT and ABA-deficient scions, but only under specific environmental conditions. Whereas there are few such examples where an ABA-deficient rootstock increases g_s of WT scions due to attenuated ABA transport from the ABA-deficient rootstock, about half of the previous studies show a pronounced phenotypic reversion of stomatal behavior in ABA-deficient scions grafted to a WT rootstock due to enhanced ABA transport from the WT rootstock (Albacete et al. 2015). Thus, it can be difficult to generalize the role of root-supplied ABA in regulating stomatal conductance. Similarly, grafting a WT scion onto an ABA-deficient tomato rootstock, *flacca* (*flc*), had no effect on leaf area, yet a WT rootstock substantially increased the leaf area of *flc* scions compared (Dodd et al. 2009). Again, this shows it can be difficult to generalize the role of root-supplied ABA in regulating leaf expansion, as the effects are scion-dependent. Thus, we hypothesize that the effects of root-synthesized ABA on shoot physiology (both stomatal conductance and growth) depend on the specific stresses encountered by the plant, and on the scion ABA status.

While reciprocal grafting can elucidate the relative importance of root-to-shoot and shoot-to-root ABA transport, hormone flow-modeling studies of ungrafted white lupin (*Lupinus albus*) plants identified environmental stresses in which these flows are especially important (Wolf et al. 1990). In non-salinized plants, 28% of the ABA flowing from the root to the shoot in the xylem originated in the roots (with the remainder determined to have been synthesized in the shoot, transported to the roots in the phloem, and then recycled via the xylem), while this was almost doubled in salinized plants. Since these results implied that the relative importance of root-synthesized ABA was greater under salinity, this was tested by reciprocally grafting WT and *flc* tomato plants. Although rootstock did not affect the scion biomass of the non-salinized plants or WT scions grown under salt stress, a WT rootstock doubled the biomass of *flc* scions when these plants were salinized, indicating that the importance of root-synthesized ABA varied according to scion ABA status (Chen et al. 2003). While these physiological effects were consistent with the changes in shoot ABA concentration since increased shoot ABA concentration of the *flc* scions could be explained by increased root export of ABA from WT rootstocks, scion ABA status had no effect on root ABA concentration, whether or not the plants were salinized (Chen et al. 2003). Since the local ABA concentrations did not always correlate with the tissue growth response, further work is required to reveal how different environmental stresses affect scion and rootstock contributions to the ABA concentrations of distal organs.

Historically, relatively few studies have considered the potential role of ABA as a shoot-to-root signal regulating root growth and physiology (reviewed in Kudoyarova et al. 2015), in part due to the difficulties of sampling roots. Nevertheless, there is evidence that shoot-to-root ABA transport affects rootstock phenotypes. In reciprocally grafted WT and *flc* tomatoes, root biomass and ABA concentration partially depend on

shoot-to-root ABA transport (Chen et al. 2002). In reciprocally grafted *flc* and WT tomatoes, the initial stress-induced root ABA accumulation was independent of the scion genotype, indicating limited ABA transport from shoots to roots (Manzi et al. 2015). Subsequent root ABA accumulation in WT rootstocks was attenuated by a *flc* scion, while a WT scion increased the ABA concentration in *flc* rootstocks, indicating that root ABA concentrations depend on shoot to root ABA transport. These changes in root ABA concentration are not explained by differences in root water relations (Manzi et al. 2015), although root ABA concentrations correlate with the water status of both detached roots (Cornish and Zeevaart 1985; Simonneau et al. 1998) and the roots of ungrafted plants (Zhang and Davies 1989; Puertolas et al. 2013). Resolving the relative importance of local *versus* systemic effects on root ABA concentration in grafted plants is important, especially since ABA increases root hydraulic conductance which can alter plant water relations (Thompson et al. 2007).

Most studies of root–shoot communication have imposed a single abiotic stress, but there is increasing interest in understanding plant responses to combined stresses (Mittler 2006; Atkinson and Urwin 2012; Zribi et al. 2012, 2014). A recent meta-analysis showed that low phosphorus (P) conditions increase xylem ABA concentrations more than other nutritional stresses, with additive effects caused by salinity and nutrient limitations (Peuke 2016). Although saline soils reduce P bioavailability (Xu et al. 2016), the physiological effects of combining these stresses were inconsistent (Grattan and Grieve 1999). Even in salinized tomato plants, shoot biomass was independent of substrate P concentration in one study (Mohammad et al. 1998), yet increasing P concentrations increased salt tolerance in another (Awad et al. 1990). Thus, whether limited P availability exacerbates plant responses to salinity is uncertain, as is the potential role of ABA transport in mediating such interactions. We hypothesize that the relative importance of root-to-shoot and shoot-to-root ABA transport in mediating physiological responses (growth, stomatal conductance) and tissue ABA concentrations depends on the environmental stresses imposed. Thus, self- and reciprocally-grafted WT and ABA-deficient *flacca* tomato plants were grown under a factorial combination of salinity and low P to determine root and shoot growth and water relations, leaf area and stomatal conductance, and ABA concentrations of roots, shoot and xylem sap.

RESULTS

Scion ABA status has dominant effects on shoot and root biomass

Compared with plants grown under the control conditions (high phosphorus, non-salinized), which had the highest shoot biomass, combined low P and salinity stress had a greater effect than either stress alone, with the low P, salt, and combined stresses decreasing shoot biomass by 30%, 56%, and 79%, respectively (averaged across all graft combinations; Figure 1A). In all treatments, WT self-grafts had the highest shoot biomass. Shoot biomass was decreased in the (scion/rootstock) *flc*/WT and *flc*/*flc* plants by 39% and 60%, respectively (averaged across all treatments), while the *flc* rootstock had no significant effect on shoot biomass of WT scions under any of the treatments. WT rootstocks increased the shoot biomass of the *flc* scions (except under the combined stress) by 70% (averaged across the remaining treatments). Taken together, these results indicate that the scion genotype had a dominant effect on shoot biomass, although WT rootstocks stimulated the growth of the *flc* scions, except under combined low P and salinity stress.

Root biomass was decreased under low P stress, salt stress, and the combined stress by 33%, 49%, and 66% (averaged across all graft combinations; Figure 1B), respectively, in comparison with the control condition. Root biomass varied similarly to shoot biomass, as there was no difference in the root/shoot ratios between graft combinations. The root biomass of the WT self-grafts was 2.4-fold higher than the *flc* self-grafts (averaged across treatments). The *flc* scion decreased the growth of the WT rootstock by 47% (averaged across all treatments), while a WT scion increased the growth of *flc* rootstocks by 60%. Thus, the scion genotype had a dominant effect on root biomass.

Rootstock genotype determines stomatal conductance (g_s) and leaf area of flc scions

Compared with the control conditions, combined low P and salinity stress had a greater effect than either stress alone, since the low P, salt, and the combined stresses decreased g_s by 30%, 73%, and 81% respectively (averaged across all graft combinations; Figure 2A). Generally, the *flc* scions had a higher g_s than the WT scions. A *flc* rootstock had no significant effect on the g_s of WT scions, except under the control conditions (50% increase compared with the WT self-grafts). By contrast, the WT rootstock significantly affected the g_s of *flc* scions; minimal (< 10%) differences were observed in the non-salinized plants, whereas in salinized plants, a WT rootstock decreased the g_s of *flc* scions by approximately 70% compared with the *flc* self-grafts. Overall, the effects of rootstock on g_s were scion-dependent, with limited effects in WT scions but pronounced stomatal closure of salinized *flc*/WT plants compared to *flc* self-grafts.

Leaf area decreased under low P stress, salt stress, and combined stress by 21%, 58%, and 74%, respectively, in comparison with the control condition (averaged across all graft combinations; Figure 2B). While low P stress decreased leaf area similarly across all graft combinations, in salinized plants the severity

of the growth inhibition increased as ABA levels declined. The WT self-grafts had the largest leaf area under all treatments, with WT/*flc*, *flc*/WT, and *flc*/*flc* plants having 21%, 54%, and 67% less leaf area, respectively (averaged across treatments). Although the WT self-grafts and the WT/*flc* plants had the same leaf area under control conditions, the *flc* rootstock resulted in a 27% smaller leaf area across all other treatments. The *flc* self-grafts and *flc*/WT plants had the same leaf area when grown under combined stress; however, the WT rootstock increased the leaf area of *flc* scions by an average of 47% in the other treatments. These experiments indicate that scion ABA status had a dominant effect on leaf area, although significant rootstock effects occurred in both the WT and *flc* scions.

Scion ABA status generally determines plant water relations

In isolation, low P stress did not significantly change leaf water potential (Ψ_{leaf}), whereas salt stress decreased Ψ_{leaf} by 0.45 MPa (averaged across all graft combinations; Figure 3A); however, low P exacerbated the decrease in Ψ_{leaf} induced by salinity by 0.33 MPa (averaged across all graft combinations). WT self-grafts always had a higher Ψ_{leaf} than *flc* self-grafts (0.43 MPa and 0.86 MPa higher in non-salinized and salinized plants, respectively). In WT scions, Ψ_{leaf} was rootstock-independent except under combined stress, where a *flc* rootstock decreased Ψ_{leaf} by 0.12 MPa compared with the WT self-grafts. In contrast, Ψ_{leaf} was rootstock-dependent in the *flc* scions, with a WT rootstock increasing Ψ_{leaf} by 0.30 MPa (averaged across all treatments). Taken together, we conclude that the scion genotype had a dominant effect on Ψ_{leaf}, but a WT rootstock universally increased Ψ_{leaf} of *flc* scions.

In non-salinized plants, all graft combinations had a similar root water potential (Ψ_{root}; Figure 3B). Salt stress universally decreased Ψ_{root}, with combined stress further decreasing Ψ_{root} by 0.21 MPa (averaged across graft combinations). In salinized plants, the Ψ_{root} of WT self-grafts was 0.46 MPa higher than in the *flc* self-grafts. In WT rootstocks exposed to salinity, Ψ_{root} was scion-dependent, with a *flc* scion decreasing Ψ_{root} by 0.18 MPa in comparison with the WT self-grafts. In *flc* rootstocks exposed to salinity, Ψ_{root} was scion-dependent; a WT scion increased the Ψ_{root} by 0.48 MPa compared with the *flc* self-grafts. Overall, the scion genotype had a dominant effect on Ψ_{root}.

Long-distance ABA transport affects local ABA concentrations in both roots and shoots

Low P stress decreased foliar ABA concentrations ($[ABA]_{leaf}$) by 25% compared with plants grown under the control condition, while salt stress increased $[ABA]_{leaf}$ by 65% (averaged across all graft combinations; Figure 4A). The $[ABA]_{leaf}$ of plants grown under combined stress was generally similar to that

of plants grown in the control conditions (except for *flc*/WT plants, which showed a 65% increase under the combined stress). WT self-grafts had a 4-fold higher $[ABA]_{leaf}$ than the *flc* self-grafts (averaged across treatments), and the *flc* rootstock did not significantly influence the $[ABA]_{leaf}$ of the WT scions. By contrast, a WT rootstock significantly increased the $[ABA]_{leaf}$ of *flc* scions, by at least 1.4-fold and 2.8-fold in non-salinized and salinized plants, respectively. Although the scion genotype had a dominant effect on $[ABA]_{leaf}$, WT rootstocks significantly increased the ABA concentration of *flc* scions, except under low P stress.

Compared with the control condition, low P stress decreased the ABA concentration of root xylem sap ($[ABA]_{xylem}$) by 43% (averaged across all graft combinations; Figure 4B). Salt stress resulted in an 18-fold increase in the $[ABA]_{xylem}$ of WT self-grafts and a 2.9-fold average increase in the other graft combinations compared with plants grown in the control conditions. The combined stress attenuated this response however, generating a 28% lower $[ABA]_{xylem}$ (averaged across all graft combinations) than under salt stress alone. WT self-grafts had a 2.9-fold and a 24.7-fold higher $[ABA]_{xylem}$ than *flc* self-grafts in non-salinized and salinized plants, respectively. The *flc* scion did not affect the $[ABA]_{xylem}$ of WT rootstocks under control conditions; however, across the three stress treatments, the *flc* scion decreased the $[ABA]_{xylem}$ of the WT rootstocks by an average of 71%. WT scions universally increased $[ABA]_{xylem}$ of *flc* rootstocks (by 2.4-fold averaged across all treatments) compared with the *flc* self-grafts. Taken together, we found the rootstock genotype had a dominant effect on $[ABA]_{xylem}$, but this was influenced by the scion genotype, especially in the salinized plants.

Low P stress decreased the root ABA concentrations ($[ABA]_{root}$) by 30% compared with the control condition, while salt stress resulted in a 1.9-fold increase in the $[ABA]_{root}$ (averaged across all graft combinations; Figure 4C). The combined stress attenuated the salt-induced ABA accumulation, with a 25% lower $[ABA]_{root}$ (averaged across all graft combinations) than under salt stress alone. WT self-grafts had approximately double the $[ABA]_{root}$ of the *flc* self-grafts. Universally, *flc* scions decreased the $[ABA]_{root}$ of WT rootstocks by an average of 25% (across all treatments) compared with the WT self-grafts, while a WT scion resulted in a 1.7-fold increase in the $[ABA]_{root}$ of *flc* rootstocks (averaged across all treatments) compared with the *flc* self-grafts. Thus, although the rootstock genotype had a dominant effect on $[ABA]_{root}$, the scion genotype also had significant effects.

Tissue ABA status had limited effects on tissue element concentrations

In comparison with plants grown in the control condition, low P stress decreased leaf and root P

concentrations ($[P]_{leaf}$ and $[P]_{root}$) by 57% and 76%, respectively (averaged across all graft combinations; Table S1). Salt stress resulted in a 1.7-fold increase in the $[P]_{leaf}$ and $[P]_{root}$ of plants with WT scions (averaged across both tissues), but had no effect on $[P]_{leaf}$ and $[P]_{root}$ of plants with *flc* scions. Under the combined stress, $[P]_{leaf}$ and $[P]_{root}$ values were similar to those of plants grown under low P stress. The ABA status of the scion had no consistent effect on the $[P]_{leaf}$; however, the $[P]_{root}$ of plants with *flc* scions was generally higher, with an average 1.4-fold increase relative to the WT scions (averaged across all treatments and graft combinations). Overall, the tissue P concentration was consistent with the P treatment applied, while salt stress increased P accumulation. Leaf and root ABA status had no consistent effect on $[P]_{leaf}$ and $[P]_{root}$.

In non-salinized plants, leaf and root Na concentrations ($[Na]_{leaf}$ and $[Na]_{root}$) were similar between graft combinations and generally did not exceed 5 mg kg^{-1} dry weight (DW), although the $[Na]_{root}$ of WT/*flc* plants under the control condition was 13 mg kg^{-1} DW (Table S2). Salinity increased $[Na]_{leaf}$ and $[Na]_{root}$ by 6.5-fold and 4.3-fold respectively (averaged across graft combinations and P levels), with consistent effects across P levels. Although the $[Na]_{leaf}$ of salinized WT self-grafts and the $[Na]_{root}$ of salinized *flc* self-grafts were each approximately half that of the other graft combinations, generally tissue ABA status did not affect sodium accumulation.

Correlation analysis reveals unifying behavior across multiple graft combinations

Across all treatments and graft combinations, leaf area decreased linearly with Ψ_{leaf} (Figure 5a). Leaf area was not correlated with $[P]_{leaf}$ (Figure 5B), but increased linearly with $[ABA]_{leaf}$, with unique relationships observed in the salinized and non-salinized plants (Figure 5C).

Within each salt treatment, g_s increased linearly as Ψ_{leaf} declined (Figure 6A), although these variables were not significantly correlated across all treatments and graft combinations. Stomatal conductance was not correlated with $[P]_{leaf}$ (Figure 6B), but decreased as $[ABA]_{leaf}$ increased, although this relationship was more sensitive in non-salinized plants (Figure 6C). Although g_s was correlated with $[ABA]_{root}$, $[ABA]_{xylem}$, and $[ABA]_{leaf}$, the latter variable explained more of the variance in g_s than the other two (Supp. Table 3).

In plants grown under low P conditions, there was no relationship between root biomass and $[P]_{root}$ (Figure 7a), or shoot biomass and $[P]_{leaf}$ (Figure 7C). In plants grown with an optimal P supply, root biomass increased as $[P]_{root}$ decreased (Figure 7A) while shoot biomass increased as P_{leaf} decreased (Figure 7C). Within each salt treatment, root biomass increased with $[ABA]_{root}$ (Figure 7B) and shoot biomass increased with $[ABA]_{leaf}$ (Figure 7D).

The above findings highlighted the importance of understanding the regulation of tissue ABA concentrations. Higher $[ABA]_{leaf}$ was correlated with higher Ψ_{leaf} in both salinized and non-salinized plants, with salinity decreasing the Ψ_{leaf} by 1.02 MPa at the same $[ABA]_{leaf}$ (Figure 8A). Although $[ABA]_{root}$ was independent of Ψ_{root} in non-salinized plants, a higher $[ABA]_{root}$ was correlated with a higher Ψ_{root} (Figure 8C) in salinized plants. Leaf and root P concentrations were not correlated with their respective ABA concentrations (Figure 8B, D). Taken together, these correlations suggest that endogenous ABA concentrations regulate the water status of local tissues.

DISCUSSION

Since there has been considerable controversy on the physiological importance of root-sourced ABA in plants responses to environmental stresses that are expected to stimulate root ABA biosynthesis (eg. Holbrook et al. 2002; Manzi et al. 2015), WT and ABA-deficient tomato plants were self- and reciprocally-grafted and grown under factorial combinations of salt and low P stress. Even though the scion genotype moderated rootstock ABA status in reciprocally grafted plants, many physiological variables (shoot biomass, leaf area, leaf water potential and $[ABA]_{leaf}$) were rootstock-dependent even in WT (ABA-replete) scions, implying pronounced effects of root-to-shoot ABA transport. This conclusion was confirmed by comprehensive profiling of leaf, root and xylem sap ABA concentrations (Figure 4), which established that long-distance ABA transport (in both directions) can mediate distal tissue responses by affecting local ABA concentrations.

Combining our data with previous reports of self- and reciprocally grafted WT and ABA-deficient tomatoes (Figure 9D-F; Table S5) is timely, given the paradigm shifts in the perceived physiological importance of long-distance ABA transport. Currently, rootstock ABA status is believed to have negligible effects on g_s and $[ABA]_{leaf}$ (Holbrook et al. 2002; Christmann et al. 2007), and scion ABA status is thought to regulate $[ABA]_{root}$ (Manzi et al. 2015; McAdam et al. 2016a). We performed a meta-analysis of the effects of a distal organ on local ABA concentrations (Figure 9A, B) and the concomitant physiological effects (Figure 9C) in tomato, which highlighted three main conclusions. First, ABA-deficient scions decrease the ABA concentrations of WT rootstocks, while WT scions increase the ABA concentrations of ABA-deficient rootstocks. Second, ABA-deficient rootstocks have no significant influence on the ABA concentrations of WT scions, while WT rootstocks significantly increase the ABA concentrations of ABA-deficient scions.

Third, ABA-deficient rootstocks rarely affect the g_s of WT scions, while WT rootstocks typically decrease the g_s of ABA-deficient scions. Thus, while the effects of root-to-shoot (and shoot-to-root) ABA transport may depend on environmental stress(es) (Jones et al. 1987; Albacete et al. 2015), we found that the responses were surprisingly consistent (Figure 9A–C), despite different environmental stresses having very different effects on tissue water relations (Figure 3).

Physiological responses to P deprivation are independent of ABA

Contrary to previous studies where limited P increased $[ABA]_{leaf}$ (Jeschke et al. 1997; Rothwell et al. 2015), we found that low P decreased $[ABA]_{leaf}$ and $[ABA]_{xylem}$ in all graft combinations (Figure 4), which may result from changes in ABA biosynthesis, degradation, or long-distance transport (Jeschke et al. 1997; Peuke 2016). The decreased root hydraulic conductance of P-deprived plants (Radin and Matthews 1989) should decrease leaf turgor, thereby stimulating leaf ABA accumulation, but no change in Ψ_{leaf} was detected in our study (Figure 3A). Nevertheless, limited ABA accumulation (1.7-fold increase) in P-deficient castor bean leaves was attributed to a high net degradation of ABA (Jeschke et al. 1997). Since P deprivation in isolation did not change Ψ_{leaf} and Ψ_{root} (Figure 3), a possible increase in ABA degradation without a corresponding turgor-mediated increase of ABA biosynthesis supports a model in which limited P availability does not directly stimulate ABA accumulation, but rather indirectly causes it as a side effect of decreasing turgor.

P deprivation decreased the shoot and root biomass of all graft combinations (Figure 1). Linear and positive relationships between the root biomass and $[ABA]_{root}$ (Figure 7B), and shoot biomass and $[ABA]_{leaf}$ (Figure 7D), indicate that local ABA accumulation maintains growth. This growth may be at least partially maintained through the limitation of the production of the growth inhibitor ethylene (Sharp et al. 2000; Dodd et al. 2009), which could be stimulated by P deprivation (Garcia et al. 2015). Similarly, the positive relationship between $[ABA]_{leaf}$ and leaf area (Figure 5C) may be accounted for by stress-enhanced ethylene evolution regulating tomato leaf expansion (Sobeih et al. 2004).

Direct ABA-mediated growth maintenance appears to be more important than any possible stomatal limitation of photosynthesis induced by P deprivation (Fujita et al. 2003; Rothwell et al. 2015). Although decreased g_s correlated with suboptimal plant P concentrations under well-watered conditions (Biddinger et al. 1998; Rothwell et al. 2015), we found that g_s was independent of P_{leaf} (Figure 6B). Under both P treatments, the higher g_s (and lower Ψ_{leaf}) of the *flc* self-grafts suggests that g_s regulates Ψ_{leaf} (Figure 6A), as previously reported for these graft combinations (Dodd et al. 2009) and for reciprocally grafted WT and

strigolactone-deficient tomato plants (Visentin et al. 2016). Foliar ABA accumulation was previously reported to correlate with low-P-induced stomatal closure (Radin 1984; Rothwell et al. 2015). Although $[ABA]_{leaf}$ determined the maximal g_s (Figure 6C), we found that low P decreased g_s and $[ABA]_{leaf}$ in all graft combinations involving *flc* as either the rootstock or the scion, suggesting that other phytohormones (Martínez-Andújar et al. 2017) mediate stomatal responses to P status.

Physiological responses to salinity are independent of ABA

As expected, when plants were grown under optimal P supply, salinity increased the $[ABA]_{leaf}$ and $[ABA]_{xylem}$ of all graft combinations (except *flc* self-grafts), while increasing the $[ABA]_{root}$ of all graft combinations (Figure 4). The salinity-induced ABA accumulation in the roots of *flc* self-grafts contradicts the lack of response in observed in other studies using self-grafted *flc* tomato plants (Chen et al. 2003; 200 mM NaCl), as well as those observed in ungrafted plants of the ABA-deficient *notabilis* tomato mutant (Mulholland et al. 2003; <120 mM NaCl). This result is particularly surprising because *flc* is deficient in the root aldehyde oxidase activity that catalyzes the penultimate stage of ABA biosynthesis (Sagi et al. 1999). Nevertheless, *flc* accumulates trans-ABA alcohol (Linforth et al. 1987) which can be converted via a shunt pathway to ABA (Rock et al. 1991) which seems to have especially active in the salinized *flc* plants. While this root ABA accumulation indicates local root ABA synthesis in response to decreased Ψ_{root} (Figure 3B) or the accumulation of ionic factors (Na^+ and Cl^-) (Table S2), pronounced shoot-to-root ABA transport also augmented the $[ABA]_{root}$ of WT/*flc* plants. Similarly, a *flc* scion diminished root ABA accumulation of WT rootstocks, indicating that the $[ABA]_{root}$ partially depends on the import of ABA from the shoot.

Salt-induced foliar ABA accumulation in tomato (Albacete et al. 2008; Figure 4A here) may result from local ABA biosynthesis in response to decreased Ψ_{leaf} (Figure 3A) or root ABA export (Figure 4B). A previous study found that WT self-grafts accumulate twice as much ABA in their leaves as WT/*sit* plants during soil drying, indicating the importance of root ABA export, since $[ABA]_{xylem}$ was 70% lower in the WT/*sit* plants (Holbrook et al. 2002). Conversely, root ABA export did not affect the $[ABA]_{leaf}$ of WT scions but substantially altered the $[ABA]_{leaf}$ of *flc* scions (cf. Figure 4A, B). The relative importance of foliar ABA biosynthesis *versus* root ABA export in determining $[ABA]_{leaf}$ may therefore vary according to the graft combination.

The positive relationships between root and shoot biomass and their respective ABA concentrations (Figure 7B, D) suggest that ABA accumulation is necessary to maintain growth in salinized plants. Although salinity induced root and leaf ABA accumulation and decreased g_s (Figure 2A, Figure 6C), Ψ_{leaf} and Ψ_{root} still

decreased in these plants (Figure 3). Nevertheless, absolute Ψ_{leaf} values are unlikely to directly affect growth, since all graft combinations showed similar salt-induced growth inhibition despite large differences (0.29–0.63 MPa) in Ψ_{leaf} (Figure 3A). Moreover, since Ψ_{leaf} increased with $[ABA]_{leaf}$ in unique linear relationships according to salt treatment (Figure 8A), and since Ψ_{root} increased linearly with $[ABA]_{root}$ in salinized plants (Figure 8C), leaf and root ABA concentrations could instead regulate tissue water status by controlling stomatal behavior (Figure 6A, C). There was limited variation in the plant Na^+ concentrations between graft combinations (Table S2), as was reported previously (Chen et al. 2003), which was consistent with the similar salt-induced growth inhibition in all graft combinations. Our results (Figure 1) corroborate reports (Chen et al. 2003; Mäkelä et al. 2003; Mulholland et al. 2003) that the shoot biomass response to salinity was generally ABA-independent, although the concurrent salt stress and high evaporative demand of ABA-deficient plants desiccated their leaves (Mäkelä et al. 2003) and thus exacerbated their salt-induced growth inhibition compared to WT plants.

Low P has variable effects on the physiological effects of salt stress

For almost all variables (except $[ABA]_{root}$ and leaf area), the salt × P interaction (Table S4) was significant, since the effects of combined salt and P deficiency were different from those predicted from the addition of the effects of the component stresses. For example, averaged across all graft combinations, shoot biomass was reduced 30% by low P and 56% by salt stress, yet the combination of these stresses only decreased shoot biomass by 79% (Figure 1). Similar non-additive effects on whole plant biomass (Zribi et al. 2012) and the relative growth rate of wild and cultivated barley (Zribi et al. 2014) were found under combined salt (100 mM NaCl) stress and P deprivation (5 µM P). Moreover, since individual low P and salt stresses had opposing effects on foliar ABA concentrations, the $[ABA]_{leaf}$ of the plants grown under control conditions and those under combined stress were similar (except in the *flc*/WT plants), although the combined stress substantially increased the $[ABA]_{root}$ and $[ABA]_{xylem}$ compared with the control plants (Figure 4). By contrast, combined salt and P stress exacerbated the salt-induced decrease in Ψ_{leaf} and Ψ_{root} (Figure 3), suggesting that the diminished ABA accumulation under low P affects plant water relations when combined with salinity stress.

Root ABA accumulation is partially regulated by shoot ABA export

Of the two abiotic stresses applied, only salinity increased the $[ABA]_{root}$ (irrespective of P status), suggesting that decreased Ψ_{root} (Figure 3B) stimulates ABA accumulation. Although $[ABA]_{root}$ and Ψ_{root} were

linearly related in salinized plants across all graft combinations (Figure 8C), this relationship was the opposite of that seen when WT plants were exposed to drying soil (Puertolas et al. 2015). These contrasting relationships between $[ABA]_{root}$ and Ψ_{root} suggest the need for flow modelling experiments (Wolf et al. 1990; Peuke 2016) with reciprocally grafted plants, to distinguish local and long-distance effects on root ABA status.

In agreement with previous reports, WT scions increased the $[ABA]_{root}$ of *flc* rootstocks (Figure 4C; Manzi et al. 2015; McAdam et al. 2016a), while *flc* scions decreased the $[ABA]_{root}$ of WT rootstocks (Chen et al. 2002; Manzi et al. 2015). $[ABA]_{root}$ is highly dependent on the import of precursors from the shoot during short-term stresses (such as transplanting to dry soil; Manzi et al. 2015) or in response to defoliation or shading (Ren et al. 2007), whereas long-term experiments under steady-state conditions (as applied here) may minimize scion effects on $[ABA]_{root}$. Although published experiments reveal considerable variation in the relative magnitude of grafting effects on $[ABA]_{root}$ (Figure 9d), summarizing these data demonstrated that reciprocally grafted plants had root ABA concentrations that were intermediate between the respective self-grafts (Figure 9a), indicating scion modulation of $[ABA]_{root}$.

Scion-dependent physiological effects on root ABA export

In WT scions, $[ABA]_{leaf}$ was independent of root ABA export (cf. Figure 4A, B), yet the physiological effects of root ABA export depended on the stress(es) encountered and the physiological processes considered. The g_s of WT/*flc* plants was almost double that of WT self-grafts under control conditions (Figure 2A), contrary to the rootstock-independent g_s of WT scions under individual and combined low P and salt stresses, which was consistent with an analysis of stomatal responses in reciprocally grafted ABA-deficient and WT tomato plants (Figure 9C). While leaf area was independent of the rootstock genotype under control conditions (Figure 2B), as previously reported (Dodd et al. 2009), various stresses decreased the leaf area of WT/*flc* plants compared with the WT self-grafts (Figure 2B). Different physiological processes (stomatal conductance *versus* leaf expansion) are therefore differentially sensitive to root ABA export, making it difficult to generalize on the role of root-supplied ABA.

Likewise, the effects of WT rootstocks on *flc* scions depended on the stress(es) applied. While there were limited rootstock effects on the g_s of *flc* scions in non-salinized plants, stomatal closure (near-equivalent to that of WT scions) occurred in salinized plants (Figure 2A). Differences in the magnitude of this stomatal phenotypic reversion are likely due to both (stress-induced) variation in root ABA export (Figure 4B) and catabolism of xylem-supplied ABA in the leaves (Gowing et al. 1993). Stomatal phenotypic reversion of

ABA-deficient scions by WT rootstocks varies considerably (Albacete et al. 2015), yet the consensus response in tomato is a decreased g_s when compared with the ABA-deficient self-grafts (Figure 9C). Moreover, WT rootstocks partially phenotypically reverted the leaf area of *flc* under all treatments except the combined salt and low P stresses (Figure 2B). In well-watered plants, this phenotypic reversion was independent of Ψ_{leaf} and associated with the direct effects of increased root ABA export and the normalization of shoot ethylene relations (Dodd et al. 2009). Taken together, a rootstock's ability to influence scion physiology depends on whether it affects $[ABA]_{leaf}$ (cf. Figure 9B, C), which is moderated by the rootstock genotype and the environmental stress(es) encountered.

Pairwise comparisons of self- and reciprocal grafts of different ABA-deficient mutants and WT plants grown under different environmental stresses revealed divergent physiological responses to root- or shoot-supplied ABA, ranging from no measurable effect to substantial phenotypic reversion (Fig 9D–F). Despite these differences, summarizing the available data revealed a consensus view (Figure 9A–C) that long-distance ABA transport has limited physiological effects unless it modifies local ABA status, which occurs under some specific stresses.

MATERIALS AND METHODS

Plant material and culture

Seed of isogenic wild-type (WT; cv. Ailsa Craig) and *flc* genotypes of tomato (*Solanum lycopersicum* Mill.) were obtained from the Tomato Genetic Resources Centre (UC Davies, USA). The *flc* genotype is impaired in the oxidation of ABA-aldehyde to ABA (Taylor et al. 1988), resulting in leaf ABA concentrations of only 30% of the WT level (Netting et al. 2012). The experiments were carried out in a naturally lit glasshouse compartment (5 m x 3 m) at the Lancaster Environment Centre, with supplementary lighting provided (for a photoperiod of 06:00–20:00 h) using high pressure sodium lamps (Osram Plantastar 600W; Munich, Germany) when the ambient photosynthetic photon flux density was less than 500 µmol m^{-2} s^{-1}. The daily maximum temperature in the greenhouse was 30°C, with a minimum night temperature of 17°C. Environmental conditions in the center of the glasshouse were recorded using an Ektron II (HortiMaX Growing Solutions, Pijnacker, The Netherlands). Plants were randomly arranged on the glasshouse bench, and the position of each plant was changed daily.

Seeds were sown in seedling trays filled with a 1:1 mixture of sand (Grade 16/30; Sibelco UK Ltd., Sandbach, UK) and vermiculite (fine grade 1.0–3.0 mm, density approximately 100 kg m^{-3}, neutral pH 6.0–7.0; William Sinclair Horticulture Ltd., Lincoln, UK). A single seed was sown into each separate

compartment (3 cm deep × 2 cm × 2 cm). After 12 d, when the cotyledons and one true leaf had emerged, plants were transferred to individual 0.9 L cylindrical pots (6.9 cm diameter x 24 cm height) containing the same substrate. The pots were plastic tubes with a mesh base, designed to fit in a pressure chamber (Puértolas et al. 2013). Following transplantation, the plants were immediately watered with tap water. The pot surface was covered with black tape to minimize evaporation from the substrate, with a 1-cm^2 hole in the center to allow for the plant stems.

To ensure that the stem diameters were of a similar size for grafting, 2.0-week-old WT seedlings and 3.5-week-old *flc* seedlings were used for self- and reciprocal grafts. High phosphorus (HP) Hoagland solution (2 mM P, pH 6.0) was supplied every four days to maintain growth. Extra irrigation (tap water) was supplied daily according to transpirational demands, to avoid wilting. The HP solution comprised (in mM) 5 KNO_3, 5 $Ca(NO_3)_2$•$4H_2O$, 2 $MgSO_4$•$7H_2O$, 3 NaFe-EDTA, 1 NH_4NO_3, 2 KH_2PO_4, and (in µM) 2 B, 2 Mn, 2 Zn, 2 Cu, and 2 Mo. The LP (low phosphorus) solution (0.2 mM P, pH 6.0) was prepared by substituting in 0.2 mM KH_2PO_4, as previously described (Wang et al. 2016). To ensure the HP and LP solutions had similar K^+ and nitrogen concentrations, the LP solution also contained 6.8 mM KNO_3 and 0.1 mM NH_4NO_3 to compensate for the lower KH_2PO_4 concentration.

Graft unions were established just below the cotyledonary node, as previously described (Dodd et al. 2009). These grafted plants were irrigated with HP or LP solutions daily at 17:00–18:00 h. After irrigation for three weeks with HP or LP solutions, half the plants in each P treatment were irrigated with the same solutions supplemented with 75 mM NaCl. Non-salinized plants with WT scions received extra distilled water daily at 09:00 h, while non-salinized plants with *flc* scions were also irrigated at 12:00 h and 14:30 h (according to the transpirational needs of the plant) to avoid excessive drying of the substrate. Salinized plants only received the daily salt solutions. There were four treatments: control (HP, no salt), low P (LP, no salt), salt (HP, salt), and combined stress (LP, salt).

Physiological measurements, xylem sap collection, and analyses

After nine days of salt treatment, whole plant transpiration was estimated gravimetrically by weighing the pots between 09:00 h and 12:00 h. During this time, gas exchange of the six fully expanded leaf (from the top of the plant) was determined using infra-red gas analysis (Li-6400; Li-COR Inc., NE, USA). Leaves of six plants per graft combination per treatment were allowed to equilibrate to the ambient (glasshouse) temperature, in 400 ppm CO_2 and with a photosynthetic photon flux density (500 µmol m^{-2} s^{-1}) for 4 min before data were recorded. The water potentials of the leaves were determined using a pressure chamber

(Model: 3005; Soilmoisture Equipment Corp, CA, USA). About 3 cm^2 of fresh tissue from the third completely expanded leaf from the shoot apex was frozen in liquid nitrogen and stored at -20°C. The shoot was then severed at the stem base (below the graft union), the cut surface rinsed 2–3 times with distilled water to remove any contaminating cell debris, and then blotted dry with filter paper. Root water potential was investigated using a pressure chamber, and was determined to be the pressure at which there was no spontaneous sap exudation from the cut surface. Root xylem sap was collected into pre-weighed Eppendorf tubes. Sufficient overpressure was applied to ensure the xylem sap flow rate closely matched the whole plant transpiration rate. The collected root xylem sap was immediately stored at -20°C for ABA analysis. Roots were quickly (<1 minute) and carefully removed from the pot and washed, and 1 g fresh root tissue was frozen using liquid nitrogen and stored at -20°C.

Total leaf area was recorded using a Li-3100 Area Meter (Li-COR Inc.). Shoots and roots were oven-dried and weighed, then the total phosphorus (P) and sodium (Na^+) concentrations were measured in these tissues. Approximately 0.1 g of each sample was ground into powder and digested in H_2SO_4 (98%)-H_2O_2 (30%) with 4:1 (V/V). The P content in the digested solution was measured as previously described (Wasaki et al. 2003). The Na^+ concentration in the digested solution was determined using a Flame photometer (FP640; Shanghai Jingke Scientific Instrument Co., Ltd., Shangai, China).

The ABA concentrations of the leaf, root, and xylem sap were measured using the radioimmunoassay method as previously described (Quarrie et al. 1988), with minor modifications. Frozen leaf and root tissues were freeze-dried then ground into powder. Approximately 20 mg dry leaf tissue or 30 mg dry root tissue were mixed with distilled water at a ratio of 1:70 (WT leaves), 1:50 (*flc* leaves), or 1:25 (root samples), respectively, and then shaken at 4°C overnight to extract ABA. The homogenates were centrifuged at 15000 rpm for 5 min, and the supernatant was directly used for the ABA assay.

Statistical analysis

Data were subjected to four-way ANOVA (analysis of variance) to investigate the effects of the scion, rootstock, salinity, and phosphorus concentration (Table S4). Each experiment was repeated four times, with six biological replicates in each group. Across all treatments and graft combinations, the means were compared using Duncan's multiple range tests at the 5% level of probability. Linear regressions established significant ($P < 0.05$) relationships between variables (Table S3).

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AUTHOR CONTRIBUTIONS

Concept and experimental design: W.L. and I.C.D.. Performed experiment: W.L. with technical guidance from C.d.O.. Analyzed data: W.L.. Manuscript preparation: W.L. with editorial contributions from C.d.O. and I.C.D.. All authors have read and approved the submitted manuscript.

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SUPPORTING INFORMATION

Figure legends

Figure 1. Shoot (A) and root (B) biomass in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock) grown under low (LP; 0.2 mM) or sufficient (HP 2.0 mM) phosphorus supply with (S) or without 75 mM NaCl

(A) The shoot biomass of grafted combinations under different phosphate/salt conditions. **(B)** The root biomass of grafted combinations under different phosphate/salt conditions. Data are means ± SE of six replicates. Bars labeled with different letters are significantly different at $P < 0.05$.

Figure 2. Single leaf stomatal conductance (A) and whole plant leaf area (B) in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock) grown under low (LP; 0.2 mM) or sufficient (HP; 2.0 mM) phosphorus supply with (S) or without 75 mM NaCl

(A) The stomatal conductance of grafted combinations under different phosphate/salt conditions. **(B)** The leaf area of grafted combinations under different phosphate/salt conditions. Data are means ± SE of six replicates. Bars labeled with different letters are significantly different at $P < 0.05$.

Figure 3. Leaf (A) and root (B) water potential in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock) grown under low (LP; 0.2 mM) or sufficient (HP; 2.0 mM) phosphorus supply with (S) or without 75 mM NaCl

(A) The leaf water potential of grafted combinations under different phosphate/salt conditions. **(B)** The root water potential of grafted combinations under different phosphate/salt conditions. Data are means ± SE of six replicates. Bars labeled with different letters are significantly different at $P < 0.05$.

Figure 4. Leaf tissue (A), root xylem sap (B), and root tissue (C) ABA concentrations in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock) grown under low (LP; 0.2 mM) or sufficient (HP; 2.0 mM) phosphorus supply with (S) or without 75 mM NaCl

(A) The leaf ABA concentration of grafted combinations under different phosphate/salt conditions. **(B)** The xylem ABA concentration of grafted combinations under different phosphate/salt conditions. **(C)** The root ABA concentration of grafted combinations under different phosphate/salt conditions. Data are means ± SE of six replicates. Bars labeled with different letters are significantly different at $P < 0.05$.

Figure 5. Relationships between leaf area and leaf water potential (A), leaf phosphorus concentration (B), and leaf ABA concentration (C) in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock)

(A) Relationships between leaf area and leaf water potential. **(B)** Relationships between leaf area and leaf phosphorus concentration.**(C)** Relationships between leaf area and leaf ABA concentration. Each point represents a treatment × graft combination, and linear regressions were fitted across all points (**A)** and by salt concentration (**C**) when $P < 0.05$.

Figure 6. Relationships between stomatal conductance and leaf water potential (A), leaf phosphorus concentration (B), and leaf ABA concentration (C) in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock)

(A) Relationships between stomatal conductance and leaf water potential. **(B)** Relationships between stomatal conductance and leaf phosphorus concentration. **(C)** Relationships between stomatal conductance and leaf ABA concentration. Each point represents a treatment × graft

combination, and linear regressions were fitted by salt concentration (**A, C**) when $P < 0.05$.

Figure 7. Relationships between root biomass and root phosphorus concentration (A) and root ABA concentration (B), and between shoot biomass and leaf phosphorus concentration (C) and leaf ABA concentration (D) in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock)

(A) Relationships between root biomass and root phosphorus concentration.

(B) Relationships between root biomass and root ABA concentration.

(C) Relationships between shoot biomass and leaf phosphorus concentration.

(D) Relationships between shoot biomass and leaf ABA concentration. Each point represents a treatment × graft combination, and linear regressions were fitted by P level (**A, C**) and by salt concentration (**B, D**) when $P < 0.05$.

Figure 8. Relationships between leaf ABA concentration and leaf water potential (A) and leaf phosphorus concentration (B), and between root ABA concentration and root water potential (C) and root phosphorus concentration (D) in reciprocal and self-grafted wild-type (WT) and *flacca* (*flc*) tomato plants (indicated as scion/rootstock)

(A) Relationships between leaf ABA concentration and leaf water potential. **(B)** Relationships between leaf ABA concentration and leaf phosphorus concentration. **(C)** Relationships between root ABA concentration and root water potential. **(D)** Relationships between root ABA concentration and root phosphorus concentration. Each point represents a treatment × graft combination, and linear regressions were fitted by salt concentration (**A, C**) when $P < 0.05$.

Figure 9. Relative influence of ABA deficiency in self- and reciprocally grafted ABA-deficient (aba) and wild-type (WT) tomato plants on root ABA concentration (A, D), leaf ABA concentration (B, E), and stomatal conductance (C, F), normalized against WT self-grafts (A, B, D, E) or ABA-deficient self-grafts (C, F)

(A) Relative influence on root ABA concentration (average). **(B)** Relative influence on leaf ABA concentration (average).**(C)** Relative influence on stomatal conductance (average). **(D)** Relative influence on root ABA concentration. **(E)** Relative influence on leaf ABA concentration. **(F)** Relative influence on stomatal conductance. Each point in A–C indicates the means

± SE of the data summarized in panels (**D–F**), with different letters (a, b, c) indicating significant ($P < 0.05$) differences from the normalized response. Data from the original studies and their level of replication are summarized in Table S5. Values from plants grown under low (LP; 0.2 mM) or sufficient (HP; 2.0 mM) phosphorus supply with (S) or without 75 mM NaCl were compiled from this study (HP, magenta circles; LP, dark magenta circles; HPS, light cyan circles; and LPS, dark cyan circles) and included in D–F. In (**D**), data from Manzi et al. (2015) reflect grafts with the ABA-deficient *flacca* mutant measured in moistened (filled triangles) and dry (filled inverted triangles) perlite; data from McAdam et al. (2016a) reflect grafts with the ABA-deficient *sitiens* mutant measured under well-watered conditions (filled squares); and data from Chen et al. (2002) reflect grafts with *flacca* measured under hydroponic conditions (filled circles), The data in (E) are as above, but with additional data from Holbrook et al. (2002) reflecting grafts with *sitiens* measured under well-watered (hollow triangles) and drying soil (hollow inverted triangles) conditions; and from Chen et al. (2003), reflecting grafts with *flacca* measured under optimal (hollow circles) and saline (hollow squares) conditions. The data in (F) also include measurements from Jones et al. (1987) reflecting grafts with *sitiens* at high (hollow triangles) and low (hollow inverted triangles) relative humidity, and from grafts with *flacca* measured in the first (hollow circles) and last (hollow squares) six hours of the photoperiod; data from Holbrook et al. (2002) reflecting grafts with *sitiens* measured under well-watered conditions (filled inverted triangles); data from Chen et al. (2002) reflecting grafts with *flacca* measured under hydroponic conditions (filled circles); data from Dodd et al. (2009) reflecting grafts with *flacca* measured under well-watered conditions (filled triangles); and data from Ntatsi et al. (2014) reflecting grafts with the ABA-deficient *notabilis* mutant measured under hydroponic conditions (filled squares).

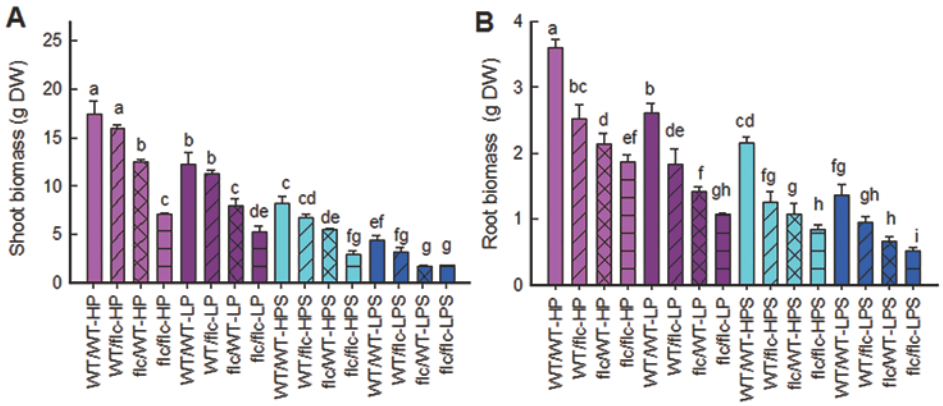

Figure 1

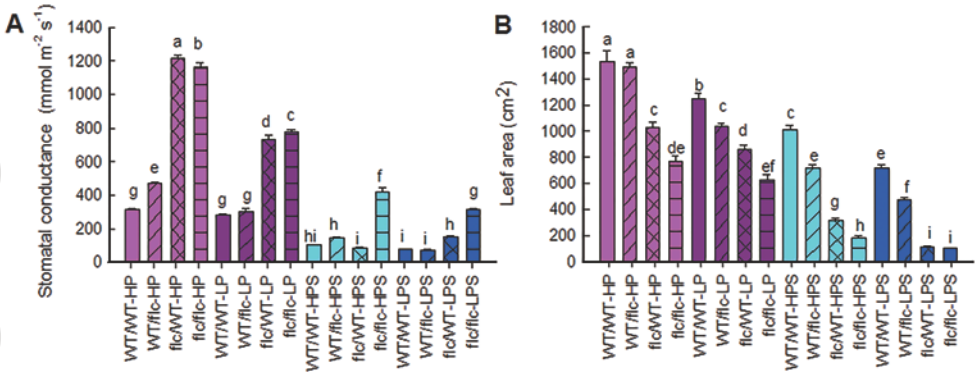

Figure 2

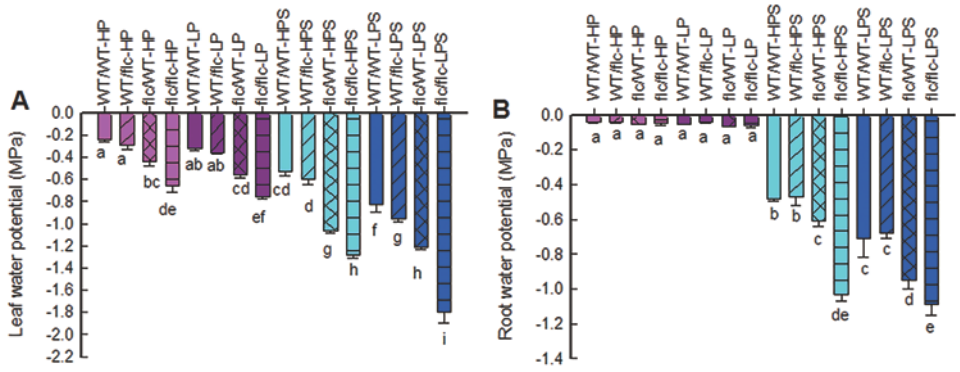

Figure 3

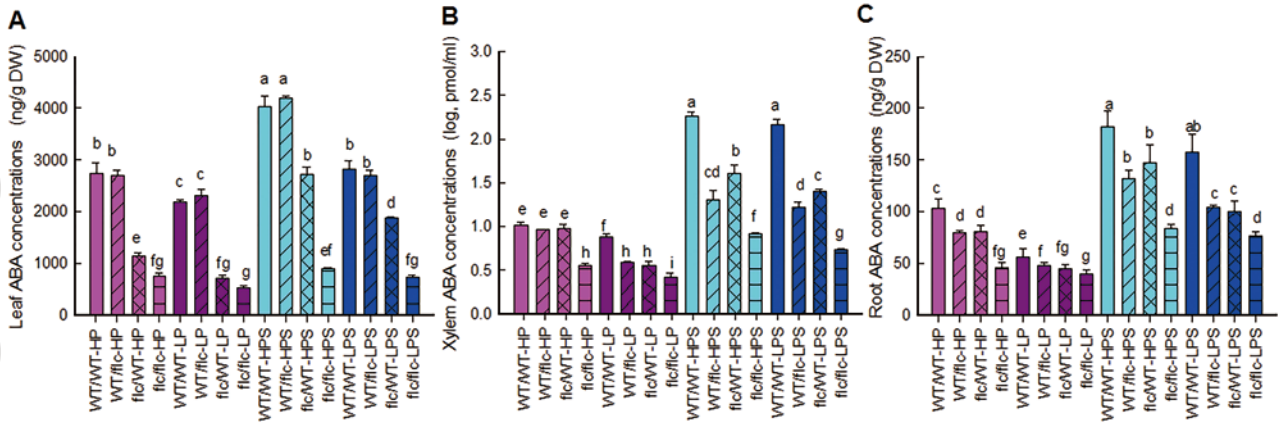

Figure 4

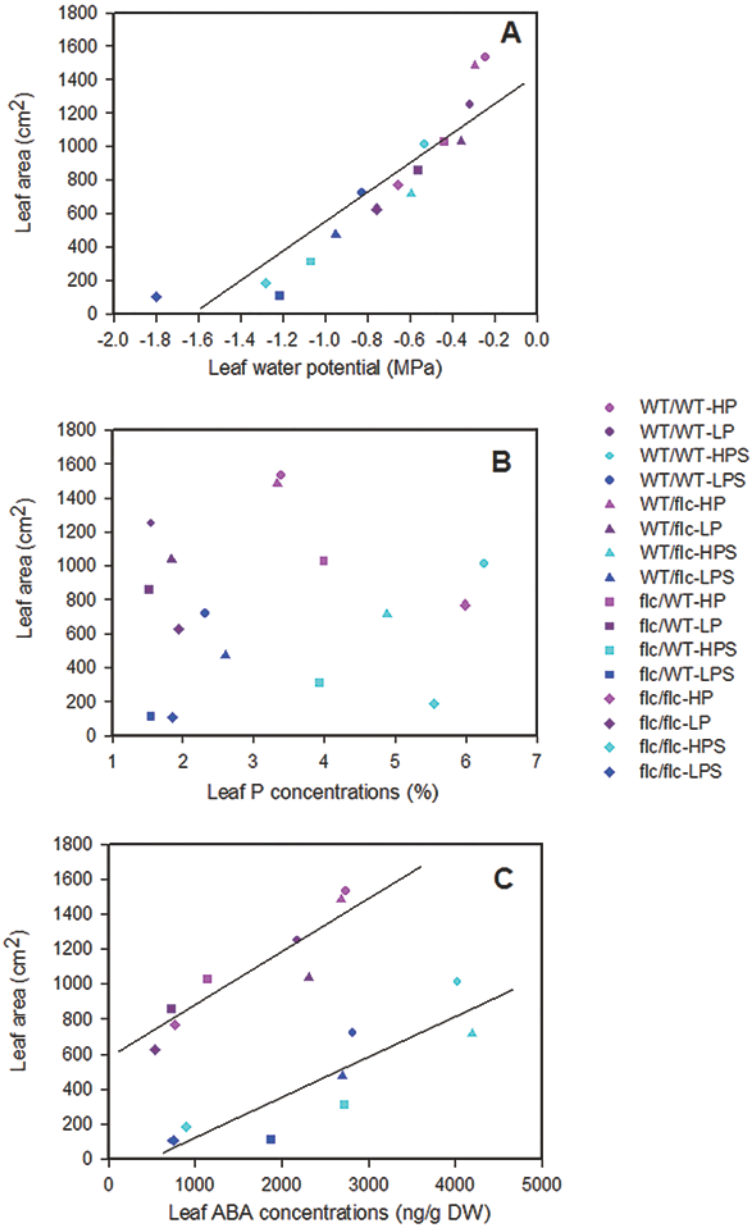

Figure 5

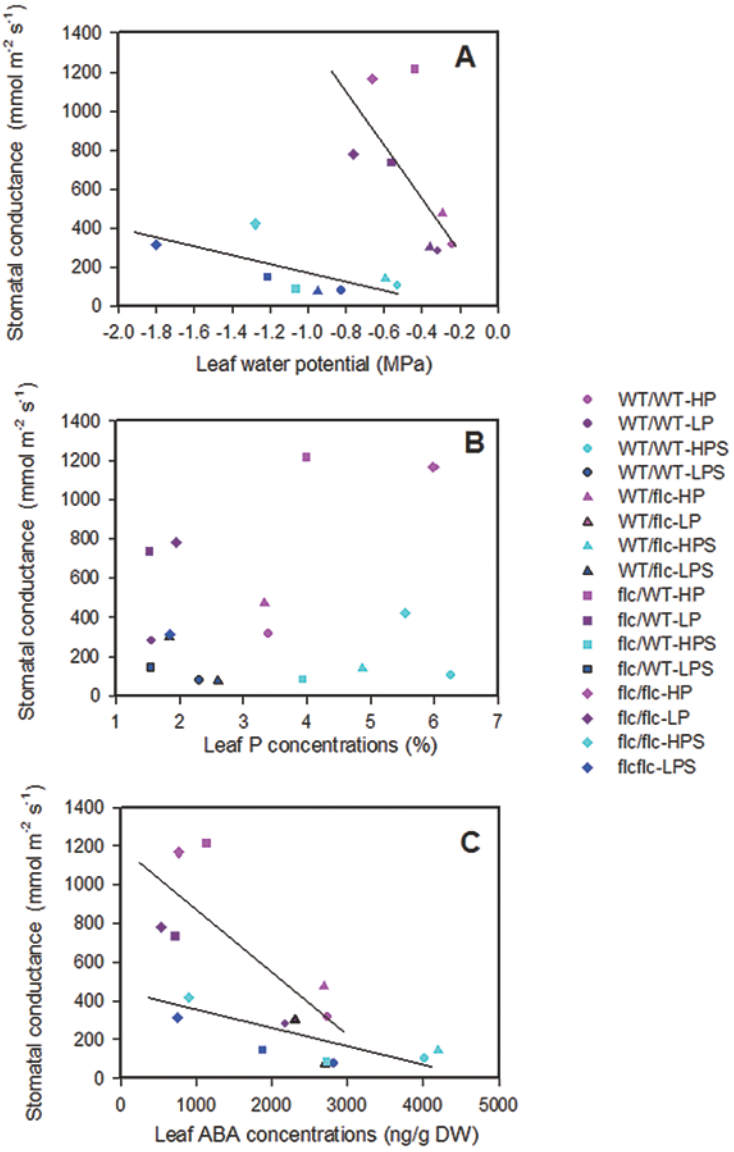

Figure 6

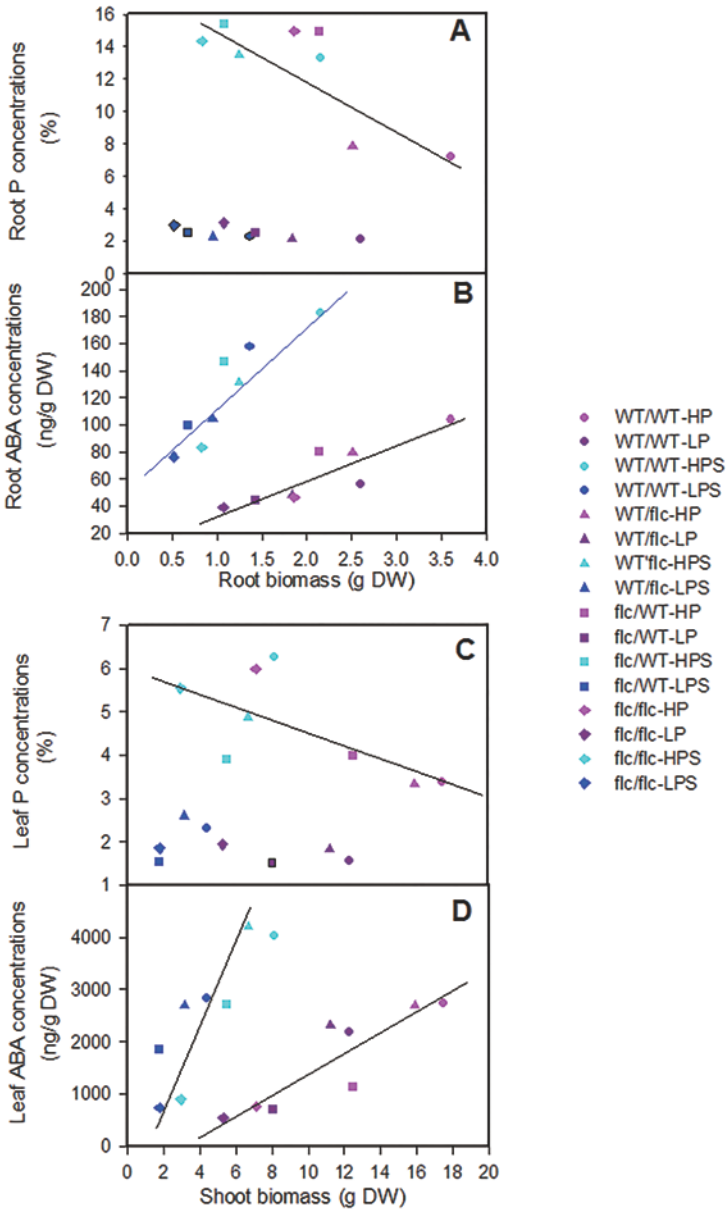

Figure 7

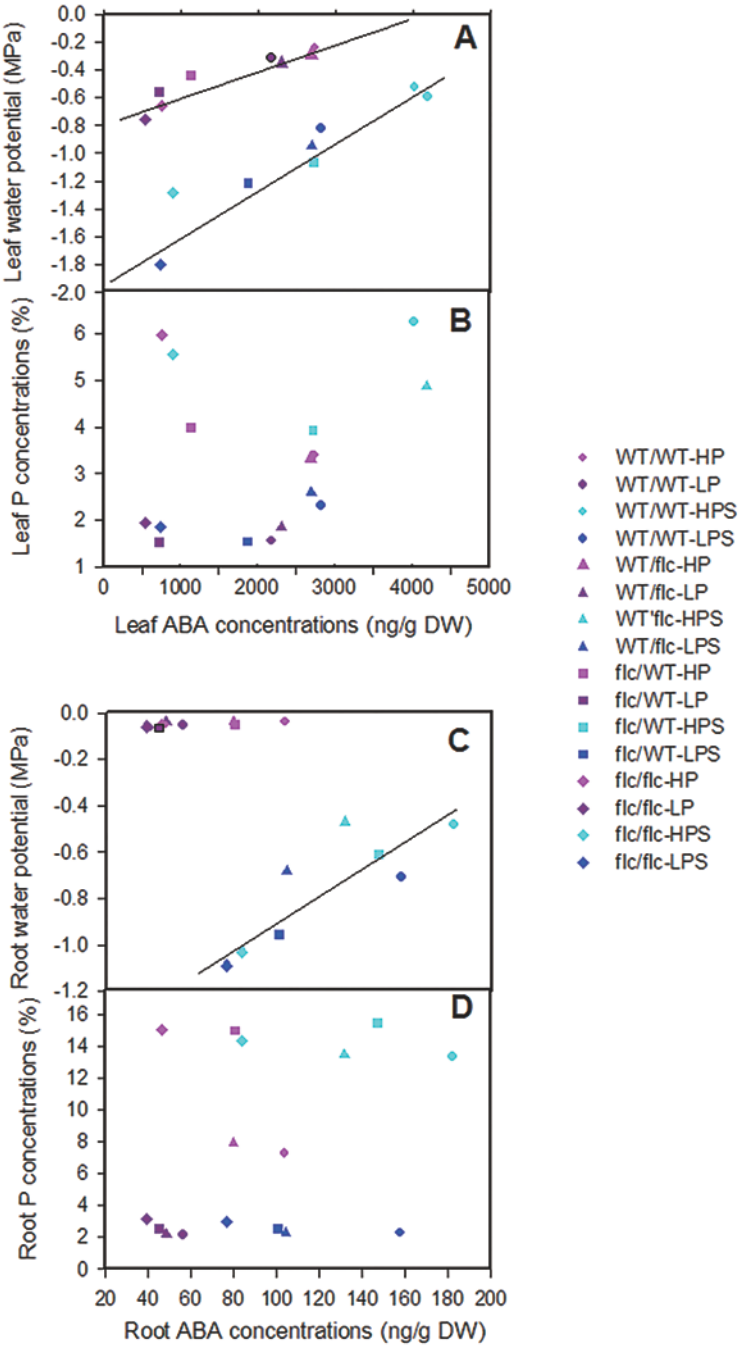

Figure 8

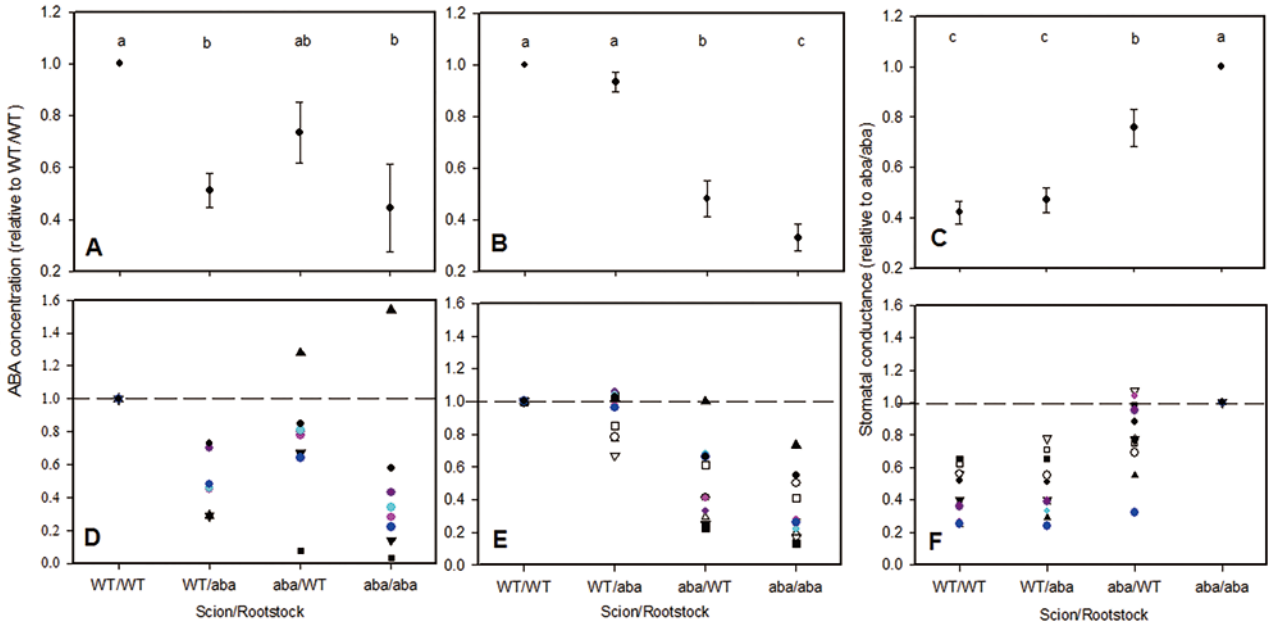

Figure 9