ABSTRACT

The study of mutants impaired in the sensitivity or synthesis of abscisic acid (ABA) has become a powerful tool to analyse the interactions occurring between the ABA and ethylene signalling pathways, with potential to change the traditional view of the role of ABA as just being involved in growth inhibition. The tss2 tomato mutant, which is hypersensitive to NaCl and osmotic stress, shows enhanced growth inhibition in the presence of exogenous ABA. The tos1 tomato mutant is also hypersensitive to osmotic stress, but in contrast to tss2, shows decreased sensitivity to ABA. Surprisingly, blocking ethylene signalling suppresses the growth defect of tss2 seedlings on ABA, NaCl, and osmotic stress, but not the osmotic hypersensitivity of tos1. The ethylene production of tss2 seedlings is increased compared with that of control seedlings under osmotic stress. In addition, the tss2 plants are hypersensitive to root growth inhibition by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). This suggests that, in addition to ABA regulation, TSS2 acts as a negative regulator of endogenous ethylene accumulation. As previously shown in Arabidopsis, it is shown here that extensive cross-talk occurs between the ABA and ethylene signalling pathways in tomato and that the TSS2 and TOS1 loci appear as regulators of this cross-talk.

Key words: Abscisic acid, ethylene production, osmotic stress, root growth, tomato, tos1, tss2.

INTRODUCTION

The hormonal regulation of plant growth and development is a complex trait. Interactions among hormones are widespread and counteracting effects of different hormones on a given developmental process are common. These interactions appear to occur at many levels, including both positive and negative reciprocal effects on synthesis (Riov et al., 1990; Ghassemian et al., 2000; Hansen and Grossman, 2000; Hussain et al., 2000; Spollen et al., 2000), and both positive and negative interactions between signalling pathways (Beaudoin et al., 2000; Ghassemian et al., 2000; Gazzarrini and McCourt, 2001; Federoff, 2002; León and Sheen, 2003).

Recently, phenotypic analyses have determined that several response mutants have altered sensitivities to more than one hormone, indicating that a single signalling component can act in two or more different hormone responses. The axr2 mutants of Arabidopsis have increased sensitivity to abscisic acid (ABA), ethylene, and auxins (Wilson et al., 1990), mutations in the brassinosteroid biosynthetic gene SAX1 confer increased ABA sensitivity to the seed (Ephritikhine et al., 1999), and mutants defective in their response to ethylene also shows altered ABA sensitivity (Beadouin et al., 2000; Ghassemian et al., 2000). Moreover, the complexity of these relationships is increased by the fact that the hormonal effects can differ in stressed and non-stressed conditions and will also depend on the developmental history of the tissue.

In Arabidopsis, the ethylene pathway regulates seed dormancy negatively by inhibiting ABA signalling, while...
these two pathways act synergistically in inhibiting root growth (Beaudoin et al., 2000; Ghassemian et al., 2000). ERA3, a gene involved in ABA sensitivity, has recently been cloned and found to be allelic to the ETHYLENE INSENSITIVE2 (EIN2) gene demonstrating a clear interaction between the ABA and ethylene signalling pathways (Ghassemian et al., 2000). This is further supported by the identification of the constitutive ethylene response mutant (ctr1) and ein2 as enhancer and suppressor mutations, respectively, of the ab1-1 mutant (Beaudoin et al., 2000).

Although it is known that ABA is an essential mediator in triggering the plant response to dehydration (Bray, 1997; Leung and Giraudat, 1998; Borsani et al., 2003; Botella et al., 2005) the hormonal relationships that determine the plant responses to water stress are not well understood. In this context, ABA has generally been regarded as an inhibitor of shoot growth (Trewavas and Jones, 1991; Davies, 1995; Munns and Cramer, 1996). This view was based on observations that (i) ABA accumulates to high concentrations in plants experiencing water deficits or other adverse conditions, often correlating with growth inhibition, and (ii) applications of ABA usually result in growth inhibition. However, the interpretation of these results is difficult due to the uncertainty as to whether the effects of applied ABA are predictive of the role of endogenous ABA (Trewavas and Jones, 1991; Sharp et al., 1994). Paradoxically, it has been observed for over 30 years that ABA-deficient mutants are often shorter and have smaller leaves than the corresponding wild types and that leaf and stem growth can be substantially restored by applying ABA (Imber and Tal, 1970; Bradford, 1983; Quarrie, 1987). The inhibited shoot growth of ABA-deficient mutants of tomato and Arabidopsis has been attributed to shoot water deficits, and the growth-promoting effect of applied ABA has been assumed to result from improvement in the plant water balance (Bradford, 1983; Neil et al., 1986; Nagel et al., 1994; Léon-Kloosterziel et al., 1996).

Although it was reported that ethylene production was greater in ABA-deficient mutants of tomato (Tal et al., 1979) and Arabidopsis (Rakitina et al., 1994), and that these tomato mutants exhibited morphological symptoms characteristic of excess ethylene, such as leaf epinasty and adventitious rooting (Tal, 1966; Nagel et al., 1994), the possibility that ethylene is a cause of shoot growth inhibition in ABA-deficient mutants was not considered until recently (Wright, 1980; Bradford and Hsiao, 1982; Sharp et al., 2000; Sharp, 2002; LeNoble et al., 2004). Recent studies have revealed that an important role of endogenous ABA is to limit ethylene production and that this reduction is required for the maintenance of root elongation at low water potentials (Spollen et al., 2000; Sharp et al., 2004). Consistent with the finding that ABA restricts ethylene production, it was reported that under well-watered conditions ethylene production was greater in shoots of the flacca (flic) tomato mutant (Tal et al., 1979) in water-stressed maize seedlings (Sharp, 2002), and in whole plants of the aba1 Arabidopsis mutant. The occurrence of the findings in maize, tomato, and Arabidopsis suggests that the restriction of ethylene production may be a widespread function of ABA, and that endogenous ABA may often function to maintain rather than inhibit plant growth (Sharp, 2002).

Identification of the tss2 and tos1 tomato (Solanum lycopersicum cv. Moneymaker) mutants indicates that both increased and decreased sensitivity to ABA may lead to a decreased tolerance to osmotic stress. Therefore, an appropriate ABA perception and/or signalling are required for osmotic tolerance (Borsani et al., 2002). In this report, it is shown that tss2 under osmotic stress or exogenous ABA shows increased ethylene production compared with control plants, while tos1 already has elevated ethylene production in control growth conditions. It is also shown that in contrast to tos1, tss2 hypersensitivity to both osmotic stress and ABA is prevented by blocking ethylene signalling. The expression of genes induced by ABA and ethylene is also altered in these mutants. These results suggest that the TSS2 and TOS1 loci are required for maintaining low ethylene production under both osmotic stress and increased ABA levels.

**Materials and methods**

**Plant materials and growth conditions**

Wild-type, tss2 and tos1 (Solanum lycopersicum cv. Moneymaker) seeds used in this study were obtained as described previously (Borsani et al., 2001, 2002). Seed were sterilized with 40% (v/v) commercial bleach for 30 min and washed several times with sterile water. The seeds were first germinated until radicle emergence in sterile water in order to improve germination uniformity. The basal agar medium contained Murashige and Skoog (MS) salts, (Murashige and Skoog, 1962) with 3% (w/v) sucrose, and 0.7% (w/v) agar. The MS medium consists of the following: 1690 mg 1−1 NH4NO3, 1900 mg 1−1 KNO3, 370 mg 1−1 MgSO4·7H2O, 170 mg 1−1 KH2PO4, 378 mg 1−1 CaCl2·2H2O, 27.8 mg 1−1 FeSO4·7H2O, 37.2 mg 1−1 disodium EDTA, 0.7495 mg 1−1 NaCl, 6.3 mg 1−1 H3BO3, 16.9 mg 1−1 MnSO4·H2O, 8.6 mg 1−1 ZnSO4·7H2O, 0.25 mg 1−1 Na2MoO4·2H2O, 0.025 mg 1−1 CuSO4·5H2O, and 0.025 mg 1−1 CoSO4·6H2O. The various agar plates used in this work were made by adding the appropriate amount of mannitol, ABA, Ag+, and AVG to the molten basal medium. Light provided by cool-white fluorescent bulbs was at 50 μE m−2 s−1 with 16 h of light at 22 °C, 8 h of dark at 18 °C, and 70% relative humidity. Varying levels of Ag+ and AVG in the media were achieved by adding appropriate amounts of AgNO3 and AVG. The KMnO4 treatment was performed by including 15 g of solid KMnO4 into a sterile filter paper bag taped to the lid of the Petri dish. The appropriate amount of 1-MCP gas (Ethylbloc™, Floralife, Walterboro, SC) was generated according to the manufacturer instructions and injected into a Petri dish with a sealed rubber septum in the lid.

**Growth measurements**

For growth measurements, 10 seedlings were used per treatment, and three replicates were made for each treatment. Three-day-old seedlings with 2 cm long roots were transferred from vertical agar plates...
containing MS medium onto a second agar medium that was supplemented with different treatments. Increases in root length were measured after 2 d of treatment.

**Ethylene measurements**

Seeds were germinated and six homogenous seedlings were grown in 150 ml glass tubes filled with 50 ml of liquid MS medium and capped with cotton to permit gas exchange. The seedlings were grown for 7 d and were thereafter subjected to different treatments by changing the growth medium for MS (control treatment), MS+200 mM mannitol and MS+20 μM ABA. After the medium change the tubes were capped with a rubber cap used to take gas samples and the seedlings remained in the same conditions for 3 d. Ethylene accumulated in this time was measured in a gas chromatograph provided with a flame ionization detector.

**Real-time quantitative RT-PCR (QRT-PCR)**

The protocols used for RT-PCR were essentially as described by Benitez et al. (2005). The primers used for LapA amplifications were LapAF 5'-ACAGCTTGATTCCGAATTGAAT-3' and LapAR 5'-TGGCAGGAGGCAGATTTCTT-3'. The primers used for GluB amplifications were GluBF 5'-ACGTGATTTCGAAATCTT-3' and GluBR 5'-TTCCTATATGACGCGATCCCAT-3'.

**Statistical analysis**

Analyses of variance was performed with data from three independent experiments and means from ethylene blocker experiments were compared using Duncan’s test at the *P*=0.05 level. Percentages analysis was performed using previous angular transformation.

**Results**

**Blocking ethylene signalling abolishes the ABA hypersensitivity of tss2**

The *tss2* and *tos1* tomato mutants were identified as hypersensitive to NaCl and mannitol, respectively (Borsani et al., 2001, 2002). The *tss2* mutant is hypersensitive to Na⁺, Li⁺, as well as to general osmotic stresses created by mannitol, sorbitol, and choline chloride (Borsani et al., 2001). The *tss2* mutant is hypersensitive to ABA (Borsani et al., 2001), while the *tos1* mutant exhibits reduced sensitivity to ABA (Borsani et al., 2002). Extensive interactions between ABA and ethylene signalling pathways in *Arabidopsis* and tomato have been shown (Beaudoin et al., 2000; Ghassemian et al., 2000; Le Noble et al., 2004). As shown in Fig. 1A, root growth of *tss2* and *tos1* is hypersensitive and hyposensitive, respectively, to all ABA concentrations analysed. It was determined whether blocking ethylene perception could affect the ABA root growth sensitivity exhibited by *tss2* and *tos1*. For this purpose, the ABA responsiveness in wild-type, *tos1*, and *tss2* seedlings was measured in the presence of Ag⁺, which blocks the perception of ethylene (Tanimoto et al., 1995; Morgan and Drew, 1997). As shown in Fig. 1B, the hypersensitivity of *tss2* to ABA was abolished by adding Ag⁺ to the growth medium. In fact, no significant differences in ABA response were found between wild type, *tos1*, and *tss2* when the medium was supplemented with Ag⁺.

To confirm that the effect of Ag⁺ was specifically due to ethylene perception, the ABA sensitivity of *tss2* and *tos1* was analysed after the application of 1-methylcyclopropene (1-MCP), l-α-(2-aminoethoxyvinyl)-glycine (AVG), and solid KMnO₄. 1-MCP is an ethylene antagonist used for blocking ethylene perception (Sisler and Serek, 1999).
AVG is an inhibitor of ACC synthase and has been used for blocking ethylene biosynthesis (Ghassemian et al., 2000). KMnO₄ is a potent oxidant that has been used to remove ethylene produced by seedlings from the air (Tieman et al., 2000). As shown in Fig. 2, Ag⁺, 1-MCP, and AVG treatments suppressed the growth defect of tss2 in ABA. Only when the seedlings were grown in the presence of KMnO₄ did the tss2 mutant remain slightly hypersensitive to ABA. However, in this treatment wild-type and tos1 growth was also reduced compared with that of the other treatments, perhaps because KMnO₄ did not adequately oxidize all of the endogenous ethylene produced or the ethylene effect was faster than the capacity of KMnO₄ to remove this compound.

**Blocking ethylene signalling abolishes the osmotic and NaCl hypersensitivity of tss2 but has no effect on tos1**

The hypersensitivity of tss2 to ABA could be overcome by blocking ethylene perception. It was therefore determined whether tss2 and tos1 mutants were affected in their normal responses to NaCl and mannitol in the presence of Ag⁺. As previously reported, tss2 but not tos1 is hypersensitive to NaCl (Fig. 3; Borsani et al., 2002). The tss2 hypersensitivity to NaCl was rescued by blocking ethylene by the use of Ag⁺ in the growth medium. In the presence of mannitol, both tos1 and tss2 were hypersensitive (Fig. 3). Blocking ethylene perception by including Ag⁺ in the medium abolished the osmotic hypersensitivity of tss2. By contrast, tos1 remained hypersensitive to osmotic stress.

**tss2 but not tos1 is hypersensitive to ACC**

Because both the insensitivity of tos1 and the hypersensitivity of tss2 to ABA could be overcome by blocking ethylene perception, it was determined whether these mutants were affected in their response to ethylene. As shown in Fig. 4, root growth of the wild type, tos1, and tss2 was measured in the presence of different concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) that is rapidly converted to ethylene (Beaudoin et al., 2000). It was found that the tss2 root growth did not differ from that of the wild type at 0.5 μM ACC. However, it was hypersensitive when grown on media containing 1, 5 or 10 μM ACC (Fig. 4). This result suggests that TSS2 is a negative regulator of both ethylene and ABA signalling pathways. While tos1 growth was diminished relative to wild type on control medium, its growth was similar to that of the wild type when the medium was supplemented with up to 10 μM ACC, resulting in some insensitivity to ACC in terms of relative root growth (Fig. 4).

Studies of several species indicate an important role of endogenous ABA in limiting ethylene production and maintaining primary root growth during periods of low water potential (Sharp, 2002). It was therefore determined whether ethylene content was affected in tss2, tos1, and the

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**Fig. 2.** Effect of blocking ethylene signalling on ABA response for root growth. Effect of Ag⁺, 1-MCP, KMnO₄, and AVG on ABA inhibition of tos1 and tss2 root growth. Seed of wild type, tos1, and tss2 were germinated and grown for 3 d on MS medium. The resulting seedlings were incubated vertically on MS medium containing 20 μM ABA (ABA) or MS medium containing 20 μM ABA and 15 μM Ag⁺ (ABA Ag⁺), ~1 ppm 1-MCP (ABA 1-MCP), 15 g of solid KMnO₄ (ABA KMnO₄) and 0.015 μM AVG (ABA AVG). The root length was scored after 2 d. Root growth was expressed as a percentage relative to controls incubated on MS medium or MS medium supplemented with Ag⁺, 1-MCP, KMnO₄, and AVG. Results are the means of three independent experiments and values followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan’s multiple range test.

**Fig. 3.** Effect of blocking ethylene signalling on NaCl and mannitol response. Effect of Ag⁺ on NaCl and mannitol inhibition of tos1 and tss2 root growth. Root elongation of wild type (WT), tos1, and tss2 seedlings was measured to quantify their sensitivities to 100 mM and 210 mM mannitol in either the absence or 15 mM Ag⁺. Root growth was expressed as a percentage relative to controls incubated on MS medium or MS medium supplemented with Ag⁺. The experiment was performed similarly as described in the legend of Fig. 1. Results are the means of three independent experiments and values followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan’s multiple range test.
corresponding wild-type genotype Moneymaker in control conditions and after exogenous ABA application as well as after osmotic stress. The ethylene production in the ABA-deficient mutant flaca and the corresponding control genotype Ailsa Craig was also analysed.

As shown in Table 1, wild type and tss2 showed similar levels of ethylene production, while the tos1 and the flaca mutant both showed increased levels of ethylene production when growing in control MS medium. This result may explain why tos1 always exhibits reduced growth in control medium compared with the wild type (Borsani et al., 2002). When ABA was added to the growth medium, wild-type seedlings reduced the production of ethylene while tos1 did not show any difference in the rate of ethylene after ABA treatment, probably due to the insensitivity showed by this mutant to this hormone. Interestingly, tss2 showed ~2 fold increase in the production of ethylene in contrast to the wild type, which reduced its production of ethylene. This increased ethylene production after exogenous ABA application may explain the hypersensitivity of tss2 root growth in medium containing ABA. Similar responses were found in Ailsa Craig and the flaca mutant, where exogenous ABA reduced the production of ethylene. It has previously been shown that mannitol treatment increases the endogenous ABA content in wild type, tss2 and tos1 (Borsani et al., 2002). Despite this ABA increase, osmotic stress generated by mannitol produced a small increase in ethylene production in the wild type. This increase in ethylene production was much higher in tss2 and tos1 indicating the incapacity of these mutants to regulate ethylene production under osmotic stress.

**Genes regulated by ABA and ethylene in tomato show altered expression in tss2 and tos1 mutants**

Because TSS2 and TOS1 are genes involved in both ABA sensitivity and the regulation of ethylene production after exogenous ABA application and mannitol stress, there was interest in determining the role of these loci in regulating gene expression under ABA and after mannitol treatment. For this purpose, the LapA and GluB genes were selected and their expression patterns were studied in wild-type, tss2, and tos1 seedlings after ABA and mannitol treatment by real-time quantitative RT-PCR (Fig. 5). No difference in the expression was detected under control conditions in either of the genes in all the genotypes analysed (data not shown). The LapA gene encodes a leucine aminopeptidase, whose transcripts are up-regulated by ABA, salinity and water deficit (Chao et al., 1999). GluB encodes a basic β-1,3-glucanase whose transcripts are induced by ethylene (Chao et al., 1999; Wu and Bradford, 2003).

Expression of LapA was induced by ABA and mannitol stress in the wild-type, tss2, and tos1 seedlings (Fig. 5). However, the induction of LapA expression was higher in the tss2 than in the tos1 and the wild type, which showed similar induction after mannitol treatment. The expression of GluB followed a similar pattern to LapA with the exception that the gene was not induced after ABA treatment (Fig. 5). The data suggest that the TSS2 and the TOS1 loci play a negative role in the expression of LapA and GluB after osmotic stress.

**Discussion**

**Cross-talk between ABA and ethylene**

There are many reports in the literature of interactions between ethylene and ABA. Antagonistic interactions between ABA and ethylene mediate the rate of grain filling in rice, whereby a high ratio of ABA to ethylene enhances grain-filling rate (Yang et al., 2004). Cross-talk studies between ethylene and ABA signal transduction in *Arabidopsis*...
using an ethylene-overproducing mutant and ethylene-insensitive mutants have shown inhibitory effects of ethylene on ABA-induced stomatal closure (Tanaka et al., 2005). There are additional genetic analyses showing the existence of substantial interactions between ABA and ethylene signalling cascades in Arabidopsis (Beaudoin et al., 2000; Ghassemian et al., 2000). However, the cross-talk between ABA and ethylene signalling pathways appears to be rather complex and dependent upon the tissue analysed.

Various types of stresses have been reported to promote ethylene production in different tissues in a number of plant species (Narayana et al. 1991; Morgan and Drew, 1997). An overproduction of ethylene induced by drought has frequently been related to fruit abortion in cotton (Guinn, 1976) and reduction in grain weight in wheat (Xu et al., 1995; Beltrano et al., 1999). By contrast, applications of ethylene inhibitors increase grain weight in wheat (Beltrano et al., 1994, 1999) and maize (Cheng and Lur, 1996). In addition, it improves dry matter partitioning and grain-filling of basal rice kernels (Mohapatra et al., 2000; Naik and Mohapatra, 2000), whereas the application of ethophon, an ethylene-generating growth regulator, produces an opposite effect.

Maintenance of root growth at low water potential is an adaptive trait of osmotic stress tolerance. ABA is likely to play a role in this process by regulating the activity of the putative wall-loosening enzymes such as xylol glucan endotransglycosylase (Wu et al., 1994) or by inducing the accumulation of proline (Ober and Sharp, 1994). Other studies demonstrate an important role for endogenous ABA in restricting ethylene production at low water potentials in tomato (Sharp et al., 2000; Spollen et al., 2000; LeNoble et al., 2004). Consistent with this, it was found that ethylene production is enhanced in the ABA-deficient tomato mutant flacca (Tal et al., 1979; Table 1) and the ABA-hyposensitive tomato mutant tosl (Borsani et al., 2002; Table 1). This enhanced ethylene production could not be fully restored to normal levels with the application of exogenous ABA and higher ethylene production remained in the flacca mutant compared with that of the wild type (Tal et al., 1979; this study). However, ABA treatment did not have any effect on ethylene production in the tosl mutant, which maintained a high ethylene production constitutively. This role of ABA in controlling ethylene production is supported by further genetic and biochemical data, i.e. a reduction of root elongation at low water potential in the maize ABA-deficient vp5 mutant and after application of fluridone, a chemical inhibitor of ABA biosynthesis (Spollen et al., 2000).

**TSS2 and TOS1 loci regulate ethylene production under osmotic stress**

The tosl mutation increases root growth sensitivity to both ABA and to the ethylene precursor ACC. Blocking ethylene perception abolished ABA hypersensitivity of tosl, suggesting that TSS2 could be a negative regulator of both signalling pathways. It was proposed that the ethylene signal transduction pathway might have ethylene-independent functions with other hormones (Gamble et al., 1998). Therefore, an alternative possibility is that ABA can stimulate the ethylene signal transduction pathway independent of ethylene. A study in Arabidopsis has shown that different genes of ACC synthase are up-regulated by ABA treatment (Wang et al., 2005). Similar effects of ABA in tomato could explain the increase of ethylene production under ABA and osmotic treatment.

The results suggest that interactions between ABA and ethylene signalling cascades in tomato are synergistic for inhibiting root growth. This conclusion is supported by the fact that tosl, an ABA hyposensitive mutant, exhibits some insensitivity to ACC. Therefore, root growth was improved and tosl ABA hypersensitivity was abolished whether the ethylene synthesis was reduced by AVG or the ethylene sensitivity was reduced by Ag⁺. This is in apparent
contradiction to previous results obtained in Arabidopsis, where AVG increased the sensitivity of the roots to ABA (Ghassemian et al., 2000). However, it is important to note that AVG could produce different effects in tomato and Arabidopsis, because tomato roots are far more sensitive to AVG than Arabidopsis roots. Arabidopsis roots can grow on medium containing 2 μM AVG without apparent inhibition (Ghassemian et al., 2000). In these experimental conditions the 2 μM AVG in the medium completely inhibited tomato root growth. Furthermore, a 20-fold reduction in the concentration of AVG in the medium (0.1 μM) was enough to reduce tomato root growth by ~50% (data not shown).

As shown in Fig. 2, blocking ethylene perception or action encourages tos1 root growth to similar levels as in a medium supplemented with ABA, but only reaches ~60% growth of plants in the control medium, with the remaining 40% of the root growth independent of the ethylene–ABA interaction pathway. This result confirms that ABA inhibition of root growth does not appear to be exclusively mediated by this common ABA–ethylene signal transduction pathway.

The increase of endogenous ABA in tss2 and wild-type seedlings was similar when grown in MS medium and after mannitol stress (Borsani et al., 2002). Therefore, the increase in ethylene production in tss2 after osmotic stress cannot be explained by a reduced ABA concentration, as occurred in mutants defective in ABA biosynthesis, such as flacca (Table 1). In fact, when wild-type plants, such as Moneymaker or Ailsa Craig, or the ABA-deficient flacca were treated with exogenous ABA the amount of ethylene production was reduced compared with tss2 and tos1. The increased ethylene production of tss2 when either ABA or mannitol was applied suggests that TSS2 is required to restrict ethylene production under osmotic stress through ABA action. The ethylene production in the tos1 mutant is higher than in the wild type in the control medium, probably due to the insensitivity that this mutant shows to ABA (Borsani et al., 2002). This insensitivity explains why exogenous ABA produced similar amounts of ethylene in tos1 as observed when grown in the control medium. Mannitol treatment, however, increased the ethylene production in tos1, indicating that as for TSS2, TOS1 is required for the regulation of ethylene production under osmotic stress.

**Altered gene regulation in tss2 and tos1**

The difference in ABA sensitivity as well as ethylene production of tss2 compared with the wild type could explain the differences in gene expression for LapA and GluB. The LapA gene is induced by ABA in tomato, but shows little response to ethylene (Chao et al., 1999), which explains the absence of difference in induction between tss2 and the wild type. However, the induction is higher in tos1 indicating a de-regulation LapA expression in this mutant. After mannitol treatment, expression of LapA in tss2 was significantly higher than in the wild type, suggesting that tss2 is a negative regulator of LapA expression under osmotic stress conditions. The expression of GluB in tss2 and tos1 also showed differences compared with the wild type, which partially reflects the differences in ethylene production of the genotypes.

Use of mutant analysis shows that the network involving ABA, salt, and osmotic signalling is rather complex and includes a multiplicity of intersecting signalling pathways (Ishitani et al., 1997; Foster and Chua, 1999; Borsani et al., 2005). Mutational analysis is especially suited for making inroads into complex systems such ABA and ethylene because mutations in individual components can reveal their effects on the entire system. It is reported here that both TSS2 and TOS1 seem to be involved in ABA responses. However, while all the tss2 phenotypes can be explained by an uncontrolled ethylene production after osmotic stress, the tos1 phenotypes are more complex and suggest additional roles for TOS1 in ABA responses, in addition to ethylene regulation. Initial mapping work in tos1 showed that the gene mutated is localized in chromosome III (data not shown). Future identification and cloning of TOS1 and TSS2 will provide more answers in order to complete the complex puzzle of ethylene–ABA interaction.

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