

# *SPINDLY*, a Negative Regulator of Gibberellic Acid Signaling, Is Involved in the Plant Abiotic Stress Response<sup>1[W][OA]</sup>

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The *SPINDLY* (*SPY*) gene was first identified as a negative regulator of plant gibberellic acid (GA) signaling because mutation of this gene phenocopies plants treated with an overdose of bioactive GA and results in insensitivity to a GA inhibitor during seed germination. The *SPY* gene encodes an *O*-linked *N*-acetylglucosamine transferase that can modify the target protein and modulate the protein activity in cells. In this study, we describe the strong salt and drought tolerance phenotypes of *Arabidopsis* (*Arabidopsis thaliana*) *spy-1* and *spy-3* mutants in addition to their GA-related phenotypes. *SPY* gene expression was found to be drought stress inducible and slightly responsive to salt stress. Transcriptome analysis of *spy-3* revealed that many GA-responsive genes were up-regulated, which could explain the GA-overdosed phenotype of *spy-3*. Some stress-inducible genes were found to be up-regulated in *spy-3*, such as genes encoding late embryogenesis abundant proteins, Responsive to Dehydration20, and AREB1-like transcription factor, which may confer stress tolerance on *spy-3*. *CKX3*, a cytokinin (CK) catabolism gene, was up-regulated in *spy-3*; this up-regulation indicates that the mutant possesses reduced CK signaling, which is consistent with a positive role for *SPY* in CK signaling. Moreover, overexpression of *SPY* in transgenics (*SPY* overexpressing [*SPY*-OX]) impaired plant drought stress tolerance, opposite to the phenotype of *spy*. The expression levels of several genes, such as *DREB1E/DDF1* and *SNH1/WIN1*, were decreased in *SPY*-OX but increased in *spy-3*. Taken together, these data indicate that *SPY* plays a negative role in plant abiotic stress tolerance, probably by integrating environmental stress signals via GA and CK cross talk.

The *SPINDLY* (*SPY*) gene was first identified by ethyl methanesulfonate (EMS) mutagenesis screening for mutants resistant to paclobutrazol (PAC), an inhibitor of GA synthesis in plants, and isolated by map-based cloning (Jacobsen and Olszewski, 1993; Jacobsen

et al., 1996). Unlike GA-insensitive mutants, most of the identified *spy* mutants phenocopy wild-type plants treated with GA. Relative to wild-type plants, *spy* mutants exhibit lighter green-colored leaves, early flowering, increased stem elongation, and partial male sterility, suggesting that *SPY* functions as a negative regulator to repress GA signaling in plants, and mutation of the *SPY* gene gives rise to an elevated GA response.

GA is one of the most important plant hormones and has been known to play major roles in various aspects of plant biology, including seed germination, leaf expansion, stem and root elongation, flowering time, and fruit development. A major breakthrough in our understanding of GA perception and signal transduction was achieved in the last 10 years, when several components of the GA signaling pathway were identified. Initially, a GA signal is perceived by a soluble GA receptor, GA-Insensitive Dwarf1 (*GID1*; Ueguchi-Tanaka et al., 2005). A null mutation in the single *GID1* gene in rice (*Oryza sativa*) leads to an extremely short and GA-insensitive plant. In *Arabidopsis* (*Arabidopsis thaliana*), when three homologs of *GID1* (*GID1a*, *GID1b*, and *GID1c*) were disrupted, plants also displayed a similar dwarf phenotype (Griffiths et al., 2006; Iuchi et al., 2007), indicating that *GID1* has an

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essential role in GA signaling. Following *GID1* binding to bioactive GA, the signal is transduced to the DELLA proteins, leading to their degradation (Hirano et al., 2008). The DELLA proteins are conserved repressors of GA signaling that modulate all aspects of GA-induced growth and development in plants. DELLA proteins were named for an N-terminal conserved DELLA domain, which is essential for GA-dependent proteasomal degradation (Willige et al., 2007). In Arabidopsis, there are five DELLA proteins: GA Insensitive (*GAI*), Repressor of *ga1-3* (*RGA*), *RGA-like1* (*RGL1*), *RGL2*, and *RGL3*, and they form a subfamily of the GRAS family of putative transcription regulators (Pysh et al., 1999; Richards et al., 2001). The N-terminal DELLA and VHYNP domains are necessary for protein interactions with *GID1* (Griffiths et al., 2006). Loss-of-function mutations of DELLA proteins can rescue the dwarf phenotype of the GA biosynthesis mutant *ga1-3* (Dill and Sun, 2001; King et al., 2001), indicating that DELLA proteins function as negative regulators in GA signaling. Another important component of GA signal transduction is an F-box protein, *SLY1*, that functions as a subunit of the E3 ubiquitin ligase complex *SCF<sup>SLY1</sup>*. *SLY1* interacts with *GAI* and *RGA* in a yeast two-hybrid system and mediates their ubiquitination and degradation (Dill et al., 2004; Fu et al., 2004; Tyler et al., 2004). Mutations in the *GA1* locus block GA biosynthesis prior to the formation of *ent-kaurene*. The phenotypes of *gal* mutants, including dwarfism, germination failure, male sterility, and incomplete petal development, can be reversed by exogenous GA application (for review, see Richards et al., 2001).

The *SPY* gene is expressed throughout the plant and can be detected not only in all organs where the phenotypes of *spy* mutants have been observed but also in the roots, indicating a role for the gene in root development (Swain et al., 2002). The *SPY* protein is predominantly localized to the nucleus, where it modifies components of the GA signaling pathway (Swain et al., 2002). Cytosolic *SPY* activity was reported to promote cytokinin (CK) responses and to repress GA signaling (Maymon et al., 2009). The *SPY* protein consists of 915 amino acids, including multiple tetratricopeptide repeats (TPRs) at the N terminus and a Ser and Thr *O*-linked GlcNAc (*O*-GlcNAc) transferase (OGT) domain at the C terminus (Jacobsen et al., 1996). Each TPR motif consists of a highly degenerate sequence of 34 amino acids with eight loosely conserved residues. The TPR motifs and OGT domain are important for mediating protein-protein interactions and correct assembly for the enzyme activity, respectively (Kreppel and Hart, 1999; Lubas and Hanover, 2000; Tseng et al., 2001, 2004). Similar to animal OGTs, in vitro-expressed *SPY* protein possesses OGT activity (Thornton et al., 1999). OGTs transfer a GlcNAc monosaccharide to the *O*-linkage of Ser or Thr of cytosolic and nuclear proteins. In animals, more than 1,000 *O*-GlcNAc-modified proteins have been identified, and this kind of protein modification is believed to

regulate many basic cellular and disease processes. In some cases, *O*-GlcNAc modification and phosphorylation occur at the same site on the substrate protein, leading to the hypothesis that these two modification processes compete with each other to fine-tune substrate protein activity under different circumstances (Comer and Hart, 2001; Wells et al., 2001; Slawson and Hart, 2003; Love and Hanover, 2005). In Arabidopsis, there are only two genes encoding *O*-GlcNAc transferases, *SPY* and *SECRET AGENT (SEC)* (Hartweck et al., 2002). Mutations in or knockout of the *SPY* gene leads to elevated GA responses, indicating that *SPY* functions as a negative regulator of GA signaling, whereas loss-of-function mutations of the *SEC* gene do not result in any obvious phenotypic changes in plants. It has been suggested that *SPY* plays a primary role in GA signaling. Double mutants containing loss-of-function mutations in both the *SPY* and *SEC* genes are embryonically lethal (Hartweck et al., 2002, 2006). Although the *SPY* gene has been identified as a negative regulator of GA signaling, the molecular action of *SPY* in GA signaling and the functions of plant OGTs remain largely unknown. Although the role of *SPY* in GA-related processes is indisputable, it is also known that *SPY* is involved in other cellular processes. The *spy* mutants exhibit altered phyllotaxy, CK responses, light responses, and reduced hypocotyl and rosette growth, none of which are directly related to GA responses (Swain et al., 2001; Tseng et al., 2004; Greenboim-Wainberg et al., 2005).

Recent research has begun to elucidate the molecular events involved in plant hormone responses and environmental stress adaptation. As an important plant phytohormone, GA has been reported to affect the plant abiotic stress response. The growth variability promoted by treatment with GA is able to reverse the inhibitory effects of salt, oxidative, and heat stresses on germination and seedling establishment. Additionally, GA promotes plant growth by inducing the degradation of the growth repressor DELLA in the nucleus, and a quadruple loss-of-function DELLA mutant is more sensitive to extremely high-salinity stress than the wild-type plants (Achard et al., 2006). It has been proposed that *SPY* can alter DELLA protein activity or stability via *O*-GlcNAc modification. Moreover, *SPY* can suppress GA signaling and promote CK responses in Arabidopsis (Greenboim-Wainberg et al., 2005). Interestingly, reducing CK signaling by knocking out two CK receptors, *AHK2* and *AHK3*, can enhance plant survival rates under severe salt stress conditions (Tran et al., 2007).

In this study, we describe the salt and drought stress tolerance phenotypes of *spy-1* and *spy-3* mutants in addition to their GA-related phenotypes. *SPY* gene expression was found to be drought stress inducible and slightly responsive to salt stress, indicating a role for this gene in stress response. Transcriptome analysis of the *spy-3* mutant revealed that many GA-responsive genes are up-regulated, explaining the GA-overdosed phenotype observed in the *spy-3* mutant. Some stress-

inducible genes were found to be up-regulated in *spy-3*, such as genes encoding late embryogenesis abundant (LEA) proteins, Responsive to Dehydration20 (RD20), ATP-binding cassette (ABC) transporters, and AREB1-like transcription factor under normal growing conditions. Under dehydration stress, the expression of many LEA protein genes was higher in the mutant than in the wild type, which may confer stress tolerance on the *spy-3* mutants. Increased expression of the *CKX3* gene was detected in the *spy-3* mutant under both normal and stressed conditions, which may also contribute to plant stress tolerance. Transcriptomic comparison of *spy-3* versus wild-type plants and *SPY*-overexpressing (*SPY-OX*) versus wild-type plants showed that the expression levels of *DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN1E/DWARF AND DELAYED FLOWERING1 (DREB1E/DDF1)* and *SHINE1/WAX INDUCER1 (SHN1/WIN1)* were increased by the *spy-3* mutation but decreased by *SPY* overexpression, which may result in the reversed drought stress phenotypes. The function of the *SPY* gene in plant abiotic stress response and plant hormone cross talk is also discussed.

## RESULTS

### *spy* Mutants Are More Tolerant to High-Salinity Stress

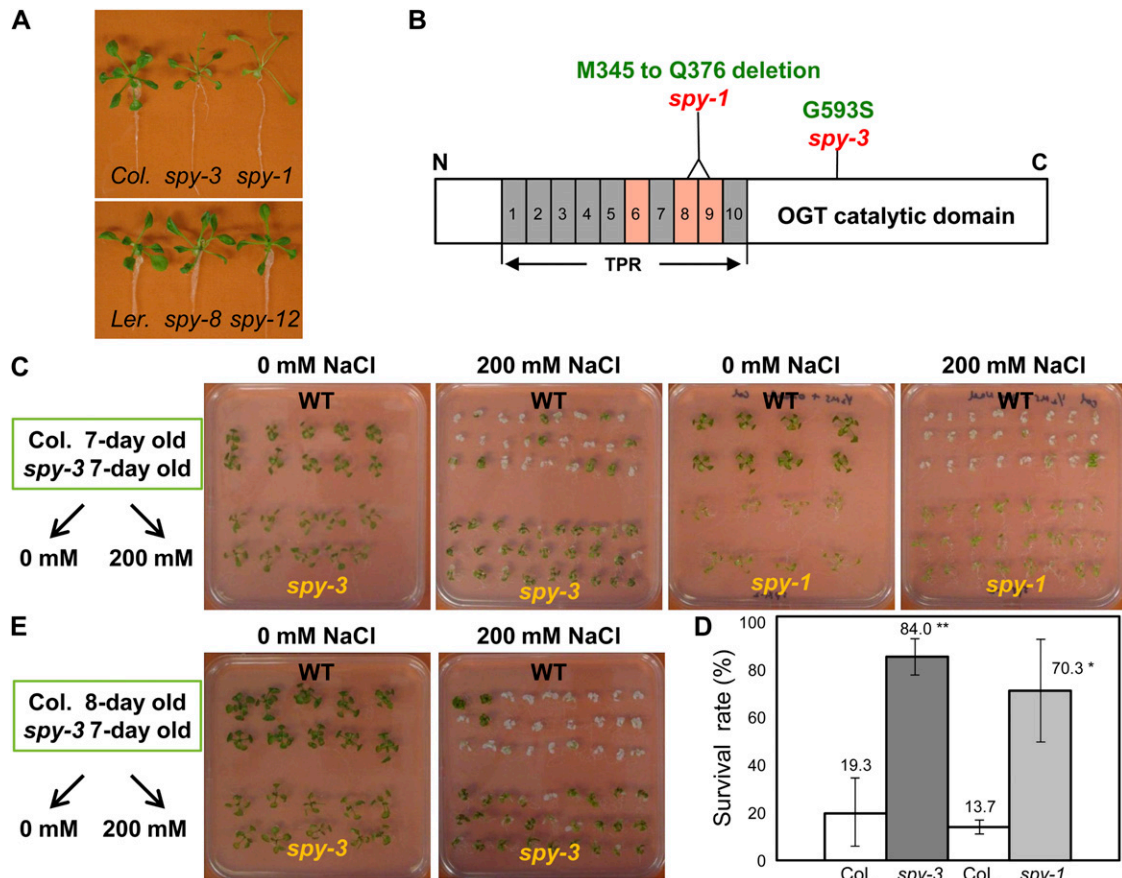
To examine whether the *SPY* gene also plays a role in the plant abiotic stress response, we obtained point mutation mutants of *spy-1* and *spy-3* (Jacobsen and Olszewski, 1993; Jacobsen et al., 1996). Both the *spy-1* and *spy-3* mutants were generated by EMS mutagenesis screening of Arabidopsis ecotype Columbia (Col) for plants that were more tolerant to PAC during seed germination compared with wild-type plants. The seedlings of both mutants exhibited a GA-overdosed phenotype. The *spy-1* mutant exhibited a stronger phenotype than the *spy-3* mutant in terms of characteristics affected by GA responses, such as elongated stem, pale leaves, slender seedlings, and male sterility, whereas the *spy-3* mutant exhibited only a mild phenotype, and this was probably due to a more severe mutation in the *spy-1* mutant (Fig. 1A). The *spy-3* mutation caused an amino acid substitution in the OGT domain, while the *spy-1* mutation caused an in-frame deletion of 23 amino acids of the protein, located in the eighth and ninth TPRs (Jacobsen et al., 1996; Fig. 1B). We compared the ability of the *spy* mutants and wild-type Col plants to survive severe high-salinity stress conditions. If 7-d-old plants were transferred from normal growth conditions to 200 mM NaCl-containing plates after 7 to 10 d, less than 20% of the wild-type plants survived. In contrast, more than 80% of *spy-3* and 70% of *spy-1* plants continued to grow and retained a greenish color, which suggested that these two mutants were strongly salt tolerant (Fig. 1C). Statistical analysis of the data obtained in these experiments is presented in Figure 1D. Because the *spy* mutants grew a bit more quickly after germination

than the wild-type plants, we compared the salt tolerance of 8-d-old Col and 7-d-old *spy-3* to eliminate any differences due to plant age and size. Similarly, we found that the survival rate of *spy-3* plants was much higher than that of the wild-type plants (Fig. 1E). In order to verify that *spy* mutations really altered plant stress tolerance, we obtained two additional *spy* alleles, *spy-8* and *spy-12*, which were generated from an EMS mutagenesis screen for suppressors of the *gal-3* mutant (Silverstone et al., 2007). An increased resistance to the inhibitory effects of salt on germination was clearly observed in these mutants (Supplemental Fig. S1). Taken together, these data strongly suggested that mutations in the *SPY* locus could enhance plant resistance to salinity stress.

### *spy* Mutants Consume Less Water and Survive Longer under Water Deficit Stress

Because salt and drought stresses usually cause similar water stress in plant cells, we extended our plant survival tests to water deficit conditions. First, we compared the plant survival rates in individual soil pots. When the water supply for 4-week-old plants was terminated for 2 weeks, most of the Col plants died, whereas almost all of the *spy-3* and *spy-1* mutants survived (Fig. 2A). Since the rosette leaves of the 4-week-old mutants were smaller than those of wild-type plants, which could bring about the differences in transpiration and water content in the soil in different pots after withholding water, we exerted out efforts to dehydrate the plants similarly by ensuring that the soil water content dropped to the same level in the soil pots. The water supply to 4-week-old plants growing in different pots was terminated. The soil water content was recorded daily from day 12 after watering was cut off. Lower water content was observed in the soil in which wild-type plants were growing compared with soil containing *spy-3* plants, indicating a higher water usage or transpiration of the wild-type plants. On day 16, when the total water amount had fallen to approximately 10% of the original in the pots containing wild-type plants, watering was resumed. Approximately 28% of the plants recovered from severe wilt. Watering was reinitiated for the *spy-3* plants after 19 d of dehydration, when the soil water content in these pots had dropped to a similar level to that in the pots of the wild type (Fig. 2B). Although the plants were dehydrated for 3 d longer than the wild-type plants, 88.0% of the *spy-3* plants survived (Fig. 2C).

We also compared plant survival in same tray. In this experiment, the wild-type plants and *spy-3* mutants were planted side by side in one soil plot in which the soil water content was more comparable between plants than that in different pots. The earlier bolting and flowering phenotypes of the *spy-3* mutant as compared with the wild-type plants were typical GA-elevated phenotypes (Fig. 2D). In our drought experiments, water was usually withheld when the *spy-3* mutant was flowering. Water consumption at



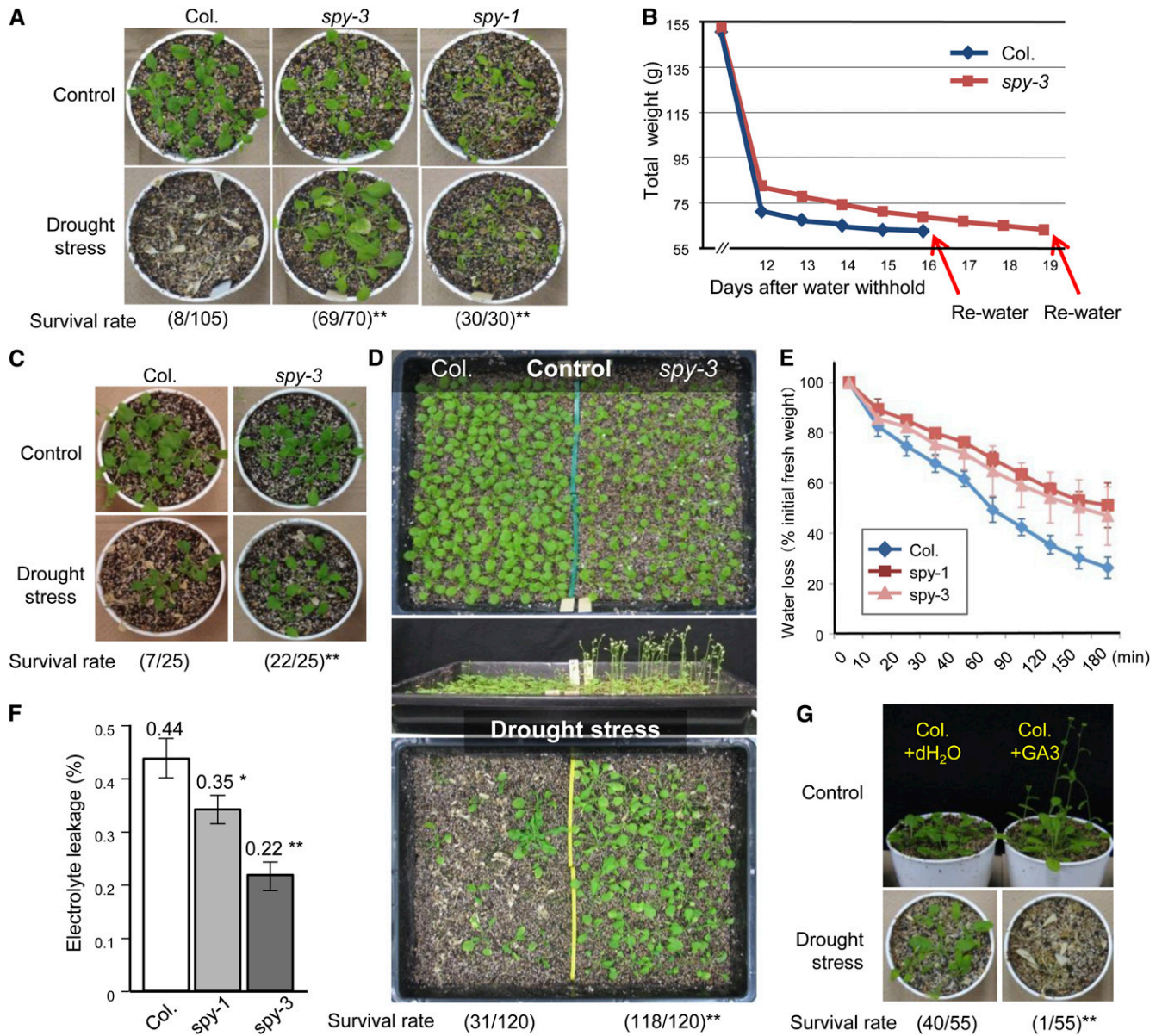
**Figure 1.** *spy* mutants were more tolerant of high-salinity stress compared with wild-type plants. A, Morphological phenotypes of *spy* mutants. Three-week-old Col, *spy-1*, and *spy-3* plants growing on GM agar plates were photographed. Ler, Ecotype Landsberg *erecta*. B, Schematic structure of the SPY protein and the locations of the mutations in the mutants. C, *spy-1*, *spy-3*, and wild-type (WT) plants grown on GM plates for 7 d and subsequently transferred onto 0.5× MS medium plates with or without 200 mM NaCl. D, The survival rate was calculated from independent experiments ( $n \geq 3$ ; \*\*  $P < 0.01$ , \*  $P < 0.05$ , Student's *t* test). E, Salt stress tolerance was compared between 8-d-old Col and 7-d-old *spy-3* plants, as described in A.

this stage is considered to be most crucial for the plant. However, we again observed a much higher survival rate of the *spy-3* mutants compared with the wild-type plants (Fig. 2D). Additionally, the water loss from detached leaves of *spy-3* and wild-type plants was evaluated using an assay that is considered to be less affected by the size of the plants. The aerial parts of 15-d-old plants were placed in empty petri dishes and periodically weighed. As expected, both the *spy-1* and *spy-3* plants were better able to retain water than the wild-type plants (Fig. 2E). The leaf electrolyte leakage of the mutants and the wild type was compared after dehydrating samples for 2.5 h in petri dishes. Significantly lower ion leakage was observed in the *spy* mutants as compared with the wild-type plants (Fig. 2F). From all these observations, we concluded that loss-of-function mutation of the *SPY* gene led to increased water stress tolerance in comparison with the wild type, which was probably due to less leaf water loss and better membrane integrity under drought stress. Because *spy* mutants exhibit elevated GA sig-

naling and a GA-overdosed phenotype, we wondered whether the increased GA response caused the drought tolerance. We treated wild-type plants with either water or a bioactive GA<sub>3</sub> solution. This treatment led, as expected, to the typical GA phenotype, characterized by earlier flowering and light green coloring, which is similar to *spy-3* mutants. The plants were then subjected to drought stress. Interestingly, we found that, unlike the *spy-3* mutants, the GA-treated plants were much more sensitive to water stress, with a much lower survival rate compared with the water-treated plants (Fig. 2G). This result indicated that although *SPY* is involved in the GA response, the salt and drought stress tolerance observed in the *SPY* mutation was probably not due to the increased GA signaling.

#### *SPY* Gene Expression Is Stress Inducible

Because the analysis of salt and drought tolerance indicated a role for the *SPY* gene in the plant stress



**Figure 2.** *spy-1* and *spy-3* mutants were more tolerant of drought stress. A, The plant survival rate under drought stress was compared in individual soil pots. B, Four-week-old Col and *spy-3* plants were subjected to dehydration in pots containing the same amounts of soil. After 12 d of water withholding, the weight of each pot was recorded daily. Watering of the plants was reinitiated once they were equally dehydrated, which occurred on days 16 and 19 for wild-type and *spy-3* plants, respectively. C, Representative photographs for the data in B are shown. D, Plant drought tolerance was compared in a large tray containing soil in which the plants were cultivated side by side. Water was withheld from the plants for approximately 14 d, after which the significant difference was observed between *spy-3* and wild-type plants. E, Water loss rates of detached *spy-1*, *spy-3*, and wild-type shoots. Wild-type Col is shown as the control. The mean and sd were obtained from four plants in each assay. F, Leaf electrolyte leakage was compared among *spy-1*, *spy-3*, and Col plants after 2.5 h of dehydration stress. G, Three-week-old Col plants treated with 50  $\mu$ M GA<sub>3</sub> or water after germination were subjected to drought stress, and their survival rates were compared. For all drought tolerance experiments, photographs were taken after 1 week of reinitiated watering. Survival rates were calculated from at least three independent experiments. In all panels, \* indicates  $P < 0.05$  and \*\* indicates  $P < 0.01$  (by Student's  $t$  test).

response, we extended our study to determine whether *SPY* expression is stress inducible. We found that *SPY* expression was responsive to dehydration stress. After a 10-min dehydration treatment, there was a clear

increase in *SPY* gene expression, and this remained high for up to 24 h. Gene expression of *SPY* was slightly responsive to NaCl treatment but not to treatment with abscisic acid (ABA). The responsiveness of the gene to

cold was not clear (Fig. 3A). From the eFP Browser database, which is a digital resource that indicates gene expression information based on collective microarray analyses, we found that *SPY* is expressed at higher levels in guard cells relative to epidermal cells, but this expression does not respond to ABA treatment (Fig. 3B).

### Drought-Inducible Expression of Some Genes Is Enhanced in the *spy-3* Mutant

Because the *spy* mutants exhibited increased tolerance to both salinity and water deficit stresses, we compared genome-wide gene expression changes between the *spy* mutants and wild-type plants (Col ecotype). For this analysis, gene expression was compared between *spy-3* and wild-type plants, because the *spy-3* mutant displayed a strongly tolerant phenotype with the fewest morphological changes. The expression of 506 genes was found to be enhanced in the *spy-3* mutant compared with the wild type, and 287 genes were down-regulated in the mutant. The criteria for identifying up- or down-regulated genes were a fold change absolute (FCA)  $\geq 2$  and  $P < 0.05$  (Supplemental Table S1). To obtain an overview of the genes with altered expression, the top 200 up- or down-regulated genes were submitted to Genevestigator to determine their expression in response to various treatments, including chemical, biotic, abiotic, and hormone treatments. The 200 most strongly up-regulated genes did not show any predominant response to the various treatments, except that many genes were found to be drought stress inducible (Supplemental Fig. S2A). However, among the 200 most strongly down-regulated genes, many were found to be ABA or drought repressible (Supplemental Fig. S2B). Furthermore, microarray analysis of Col plants growing in soil that were dehydrated for 3 d, which identified drought-inducible genes genome

wide (Maruyama et al., 2009), showed that of the 506 genes up-regulated in the *spy-3* mutant, 59 were drought stress inducible, suggesting that these genes may contribute to the enhanced drought tolerance of the *spy* mutant (Fig. 4A; Supplemental Table S2).

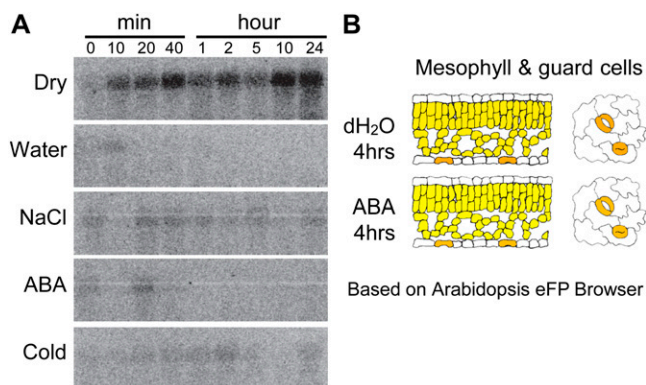
### Expression of GA-Responsive Genes Is Increased in the *spy-3* Mutant

Because the *spy-3* mutant displays many of the same phenotypes as GA-treated plants, we were interested to determine whether the expression of GA-responsive genes was altered in the *spy-3* mutant. Microarray analysis of *ga1-3* mutants versus wild-type plants using flower buds identified 826 GA-responsive genes (Cao et al., 2006). When we compared our data with these results, we found that of the 506 genes up-regulated in the *spy-3* mutant, 114 were GA responsive, including *RGL1* (Fig. 4B; Supplemental Table S3). Such a large overlap in gene expression may provide a good explanation for the GA-overdosed phenotype of the *spy-3* mutant. In addition to these 114 genes, there were at least another three GA-responsive genes found to be up-regulated in the *spy-3* mutant: *RGL2*, *GA 2-oxidase*, and *GA-responsive gene (At5g59845)*. Although DELLA proteins are degraded by treatment with GA, the expression of these genes was found to be slightly up-regulated in the *spy-3* mutant.

We also examined the correlation between gene expression in the *spy-3* mutant and ABA-treated Col plants. According to the microarray analysis of the Col plants treated with ABA for 6 h, there were 2,119 and 2,427 genes that were up- and down-regulated, respectively (Fujita et al., 2009). Of the 2,119 ABA-up-regulated genes, the expression of 79 was found to be increased, and of the 2,427 ABA-down-regulated genes, the expression of 46 was decreased in the *spy-3* mutant (Fig. 4C). Compared with the overlap observed between the genes up-regulated in both GA-treated and *spy-3* mutant plants, those up-regulated in ABA-treated plants overlapped with those up-regulated in the *spy* mutants much less, indicating that the *spy-3* mutation has strong effects on GA-dependent gene expression, whereas it does not considerably affect ABA-responsive gene expression.

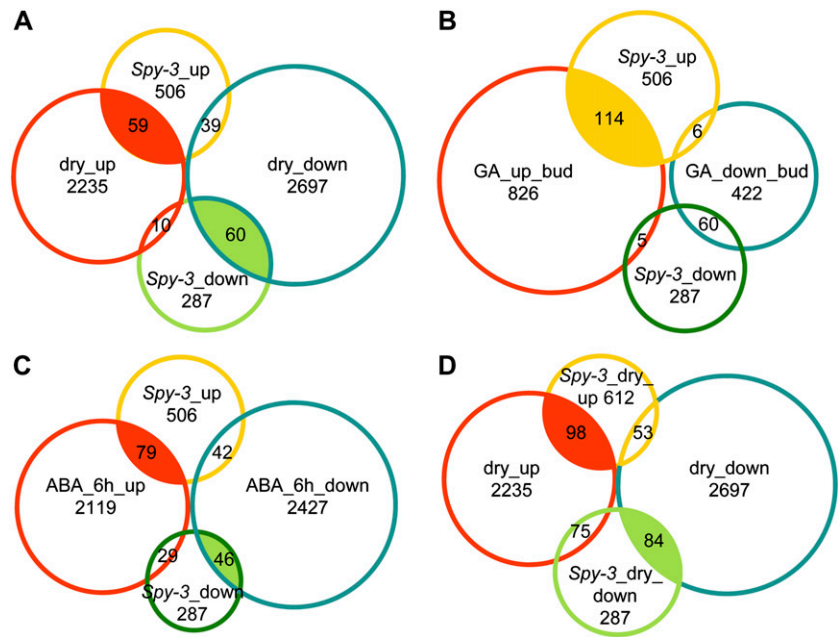
### Transcriptome Alterations in Dehydrated *spy-3* Mutants Compared with Wild-Type Plants

We also compared the gene expression changes in *spy-3* and wild-type plants that were dehydrated for 2 h. Overall, the expression of 612 genes was found to be enhanced in the *spy-3* mutant compared with Col, and 720 genes were down-regulated in the mutant, as determined by selecting genes with FCA  $\geq 2$  and  $P < 0.05$  (Supplemental Table S4). The top 200 up- or down-regulated genes were analyzed using Genevestigator. Similarly, among the top 200 up-regulated genes, many were found to be drought stress inducible (Supplemental Fig. S3A). However, among the top 200



**Figure 3.** A, Expression of the *SPY* gene under various stress treatments. Twenty micrograms of total RNA from 3-week-old plants that had been treated as indicated was used for RNA-blot hybridization with a gene-specific probe. B, *SPY* was preferentially expressed in guard cells in leaf tissue based on data from the Arabidopsis eFP Browser.

**Figure 4.** Venn diagrams of gene expression alterations in *spy-3* plants under normal or dehydration stress conditions based on microarray analysis. A, Overlap between Col plants dehydrated for 3 d and nondehydrated *spy-3* plants. B, Overlap between genes up- or down-regulated by GA with those of untreated *spy-3* plants. C, Overlap between Col plants treated for 6 h with ABA and untreated *spy-3* plants. D, Overlap between Col plants dehydrated for 3 d and *spy-3* plants dehydrated for 2 h.



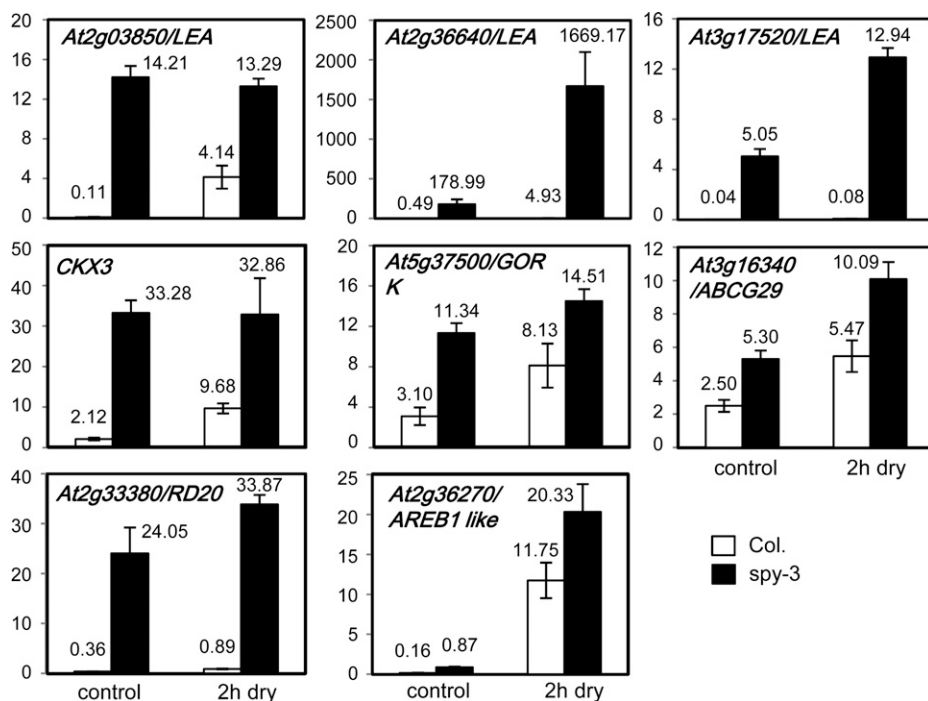
down-regulated genes, there were many genes that were ABA or drought stress repressible, which was similar to the results of the microarray analysis of the untreated *spy-3* mutants. Unexpectedly, many biotic stress-inducible genes were revealed to be down-regulated in the *spy-3* mutant (Supplemental Fig. S3B). Compared with the results of the microarray analysis of Col plants growing in soil that were dehydrated for 3 d (Maruyama et al., 2009), we found that of the 612 up-regulated genes, 98 were drought stress inducible (Fig. 4D; Supplemental Table S5). Importantly, 16 LEA protein genes were found to be up-regulated in the *spy-3* plants that were dehydrated for 2 h, and all of them were drought stress inducible, which might contribute to the drought stress tolerance of plants. Similarly, the expression of five lipid transfer proteins and seven transporter genes was found to be enhanced in the *spy-3* mutants dehydrated for 2 h. Some transcription factor- and protein enzyme-encoding genes were also revealed to be transcriptionally enhanced.

To verify the microarray results, the expression levels of several genes that are potentially related to water stress response in the *spy-3* mutant were monitored by quantitative reverse transcription (qRT)-PCR analyses. Three LEA protein-encoding genes, *At2g03850*, *At2g36640*, and *At3g17520*, were determined to be significantly up-regulated under both normal growing conditions and stress conditions in the *spy-3* mutant as compared with wild-type plants (Fig. 5). These LEA proteins may confer the stress-tolerant phenotype of the *spy-3* mutant. Two genes encoding transporters, an ABC transporter gene (*ABCG29*) and an outward  $K^+$  channel gene (*GORK*) that may be involved in leaf water transpiration or ABA response, were revealed to be up-regulated in the

*spy-3* mutant (Fig. 5). Moreover, the *CKX3* gene encoding a CK oxidase/dehydrogenase was found to be 15.6- and 3.4-fold up-regulated in the *spy-3* mutant under normal and dehydration conditions, respectively, according to the qRT-PCR analyses (Fig. 5). Recently, it was reported that overexpression of the *CKX3* gene, which leads to a reduction in CK content, enhanced the drought and salt tolerance of Arabidopsis and tobacco (*Nicotiana tabacum*) transgenic plants (Werner et al., 2010; Nishiyama et al., 2011). Increased expression levels of two other stress-related genes, *RD20* and an *AREB1-like* gene (*At2g36270*), were also found in the mutant under both normal and stress conditions (Fig. 5). Taken together, we concluded that the microarray data are reliable and reproducible. The enhanced expression of LEA genes, genes encoding transporters, stress-related genes, and *CKX3* might contribute to the salt- and drought-tolerant phenotypes of the *spy-3* mutant. It is worth mentioning that among the 720 genes down-regulated in the dehydrated *spy-3* mutants, the expression of 84 was found to be drought repressible. Many genes encoding disease resistance proteins, Leu-rich repeat protein kinases, and receptor-like protein kinases were transcriptionally down-regulated, which was consistent with the results of Genevestigator analysis of the gene expression responsiveness of the top 200 down-regulated genes (Supplemental Table S4).

#### Overexpression of the *SPY* Gene Reduces Plant Drought Stress Tolerance

To further elucidate the function of the *SPY* gene in the plant water deficit stress response, we generated transgenic Arabidopsis plants overexpressing the *SPY*



**Figure 5.** qRT-PCR analysis of the expression of genes that were identified as being up-regulated in the *spy-3* mutant under normal growing and 2-h-dehydrated conditions. Primer sequences are listed in Supplemental Table S11.

gene using the 35S constitutive promoter (35S:SPY-OX). Several lines of transgenic plants were obtained, and transgene expression was checked by RNA gel-blot analysis (Fig. 6A). In contrast with *spy* mutants, the *SPY* overexpressors did not exhibit dramatic morphological changes (Fig. 6B). Three lines of 35S:SPY-OX plants, a, b, and c, with comparably high levels of transgene expression, were tested for plant drought stress tolerance. Water was withheld from 4-week-old plants for approximately 2 weeks, and the plants were then watered again. The survival rate was recorded and compared between the vector-transformed control and 35S:SPY-OX lines. While 78.9% of the control plants survived, 19.4%, 15.6%, and 13.9% of the 35S:SPY-OXa, -b, and -c lines, respectively, survived after the drought stress treatment. Statistical analysis of the plant survival rates from three replicate experiments is shown in Figure 6C and Supplemental Table S6. It suggested that drought stress tolerance was decreased in these three types of 35S:SPY-OX transgenic plants.

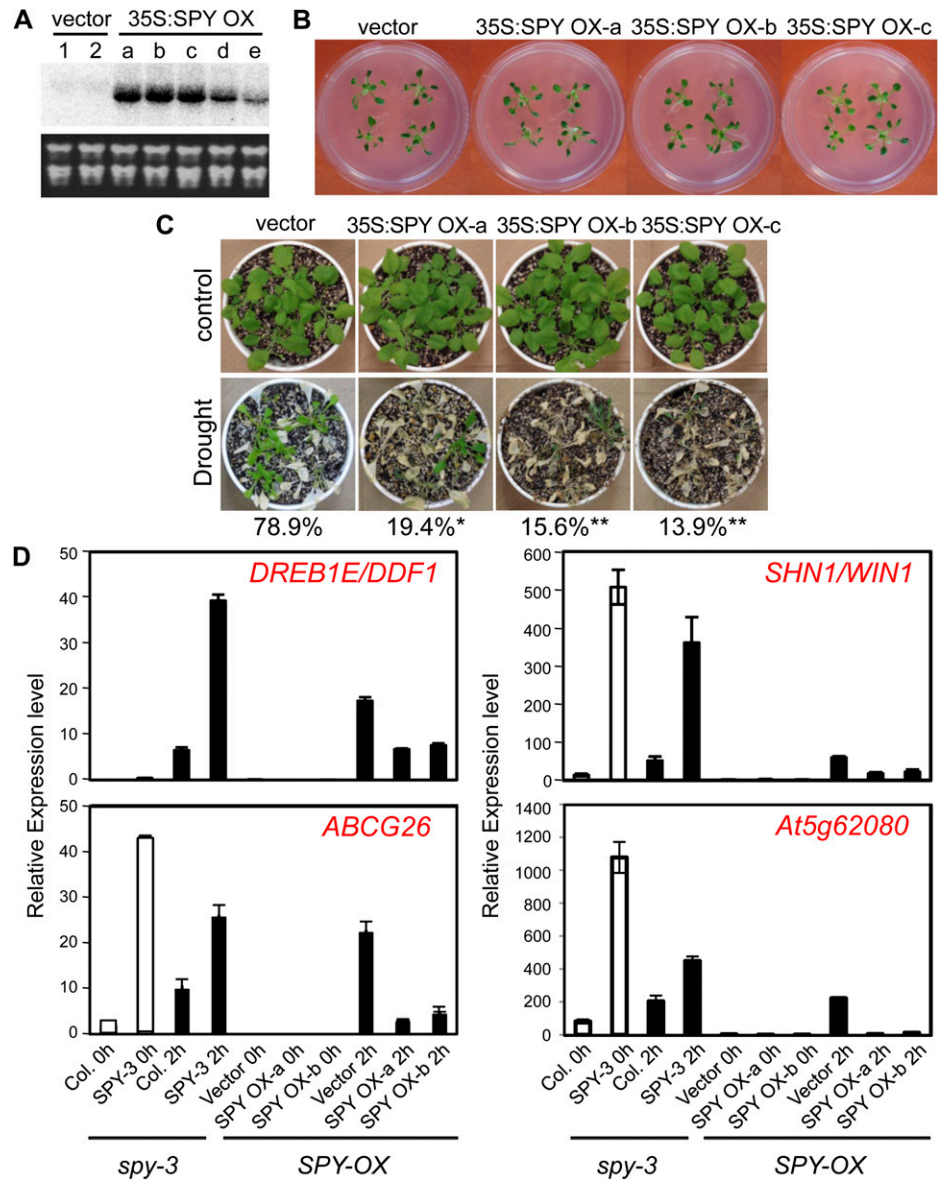
We were further interested in exploring alterations of gene expression in SPY-OX plants because the expression levels of hundreds of genes were changed in the *spy-3* mutants. Mutations in *spy-3* affected GA and CK signaling and led to a variety of morphological changes, whereas *SPY* overexpression only negatively altered plant stress tolerance without any obvious morphological effects. Analyzing SPY-OX plants may facilitate the identification of a direct relationship between *SPY* function and plant stress tolerance. Two additional microarray experiments were constructed comparing two independent 35S:SPY-OX-a and -c lines with vector-transformed transgenic plants. Transcriptomic changes were compared under both

normal and 2-h-dehydrated conditions. In total, 48 genes were up-regulated and 156 genes were down-regulated ( $FCA \geq 2$ ,  $P < 0.05$ ) in SPY-OX plants during normal growth. Among them, the expression of the *SPY* gene was 12.1-fold higher in the transgenic plants as compared with the wild-type plants (Supplemental Table S7). According to the same criteria, 66 and 184 genes were found to be up- and down-regulated in 2-h-dehydrated SPY-OX plants (Supplemental Table S8). From both microarray experiments, more genes were found to be down-regulated than up-regulated in the SPY-OX plants, indicating a negative, genome-wide effect of *SPY* on gene expression regulation. Based on the drought-tolerant phenotype of the *spy-3* mutants and sensitive of SPY-OX plants, we were particularly interested in the genes that were up-regulated in *spy-3* and down-regulated in the SPY-OX plants, which might be the genes most affected by the *SPY* protein. Out of 156 down-regulated genes in the SPY-OX plants, 19 genes were found to be up-regulated in the *spy-3* mutant under normal growing conditions ( $FCA \geq 2$ ,  $P < 0.05$ ). Six of these genes are GA-up-regulated genes in Arabidopsis buds, but none of these genes is GA repressive; this result is consistent with a negative role for *SPY* in GA signaling (Supplemental Table S9). Indeed, there is no overlap of genes up-regulated in the SPY-OX plants that are also down-regulated in the *spy-3* plants.

Results from the 2-h-dehydrated samples showed that among the 184 genes down-regulated in the SPY-OX plants, three were up-regulated in the *spy-3* mutant ( $FCA \geq 2$ ,  $P < 0.05$ ; Supplemental Table S10). These genes are *SHN1/WIN1*, *ABCG26*, and *APETALA3 (AP3)*. Two additional genes, *DREB1E/DDF1* and



**Figure 6.** Overexpression of *SPY* reduced plant drought stress tolerance. A, RNA gel-blot analysis of transgene expression in vector and *35S:SPY*-transformed plants; 10  $\mu$ g of total RNA was loaded. B, Morphological phenotype of 3-week-old *SPY*-OX plants as compared with the wild type. C, Survival rate of vector-transformed and *SPY*-OX lines. The averaged data were obtained from three independent experiments (\*\*  $P < 0.01$ , \*  $P < 0.05$ , Student's  $t$  test). D, qRT-PCR analysis of the expression of genes that were up-regulated in *spy-3* mutants but down-regulated in *SPY*-OX plants. White bars indicate the relative expression level in the untreated plants, and black bars indicate the relative expression level in the 2-h-dehydrated plants. Primer sequences are listed in Supplemental Table S11.



*At5g62080*, were verified to be inversely regulated by *SPY* overexpression and *spy-3* mutation based on further qRT-PCR analyses. The expression of all these genes was found to be drought stress inducible (Fig. 6D). *SHN1/WIN1* encodes an AP2/ERF transcription factor belonging to the A-6 subgroup. Overexpression of *SHN1/WIN1* increased the leaf cuticular wax content, leading to reduced water loss and enhanced tolerance to drought stress (Aharoni et al., 2004; Broun et al., 2004; Kannangara et al., 2007). *DREB1E/DDF1* is also an AP2/ERF transcription factor, classified as a member of the A-1 subgroup, that can bind the DRE sequence in the promoter of dehydration-responsive genes (Sakuma et al., 2002). Transgenic plants overexpressing the *DREB1E/DDF1* gene exhibited improved tolerance to drought, cold, and heat stresses (Kang et al., 2011). The up-regulated expression of *SHN1/WIN1*

and *DREB1E/DDF1* may contribute to the maintenance of higher water content, resulting in stronger drought stress tolerance of the *spy-3* mutant (Fig. 2). Although expression of the *ABCG26* and *ABCG29* genes was increased in the *spy-3* mutant, only *ABCG26* transcription was repressed by overexpression of *SPY* under both normal and stress conditions (Figs. 5 and 6D). The function of *ABCG26* was reported to be associated with male fertility and pollen maturation (Quilichini et al., 2010). Together with the *AP3* gene, *ABCG26* might cause the flowering-related phenotypes of the *spy* mutant.

## DISCUSSION

To properly respond to environmental signals, plants need to integrate external and internal signals

and a complex network of signal transduction pathways. In recent years, different components involved in these signal cascades have been identified, including genes involved in stress-induced transcriptional regulation and complex cross talk among different hormones at either the biosynthesis or action level (Fujita et al., 2006; Shinozaki and Yamaguchi-Shinozaki, 2007). GA is an important plant phytohormone involved in several processes, including seed germination, vegetative growth, flowering induction, and fruit development (Sun and Gubler, 2004). The *SPY* gene was first identified as a negative regulator in GA signaling, because the mutant displayed a set of GA-treated phenotypes, such as insensitivity to PAC during germination, early flowering, partial male sterility, and pale green color (Jacobsen and Olszewski, 1993; Jacobsen et al., 1996). Although the involvement of *SPY* in GA signaling is indisputable, it has also been recognized that *SPY* is involved in other cellular processes. *spy* mutants also display phenotypes, such as altered leaf phyllotaxy, CK responses, light responses, and reduced hypocotyls and rosette growth, that are not directly related to GA responses (Swain et al., 2001; Tseng et al., 2004; Greenboim-Wainberg et al., 2005). Additionally, double mutation of the *SPY* and *SEC* genes, the only two OGTs in plants, leads to lethality, which may not be caused by GA treatment. These phenomena support the hypothesis that *SPY* has an unidentified function in one or more processes unrelated to GA signaling. Here, we demonstrated that mutation of *spy* led to a strong salt and drought tolerance in plants whose mechanism is independent of GA-DELLA signaling.

*SPY* gene expression was found to be drought stress inducible and slightly responsive to salinity stress. Furthermore, based on microarray analysis, we revealed that under normal growing conditions, in addition to the up-regulation of many GA-responsive genes, the expression of a number of drought-inducible genes was increased; these genes encode LEA proteins, RD20, ABC transporters, and AREB1-like transcription factor (Fig. 5). Under drought conditions, the expression of 16 LEA protein genes was higher in *spy-3* mutants compared with wild-type plants (Supplemental Table S5). LEA proteins function in an unfolded state as ion sinks or water replacement molecules, and that water deficit stress can induce folding and conformational shifts in LEA proteins, so that they act as molecular chaperones to protect important proteins (Goyal et al., 2003; Wise and Tunnacliffe, 2004). Overexpression of DREB1A, constitutively active DREB2A, or an activated form of AREB1 (Maruyama et al., 2004; Fujita et al., 2005; Sakuma et al., 2006) in Arabidopsis resulted in up-regulation of the expression of some LEA genes, and all of these transgenic plants displayed improved drought stress tolerance, further supporting the hypothesis that LEA proteins play a role in plant water deficit tolerance. Direct evidence has shown that overexpression of a rice *OsLea3* gene was able to enhance

plant water deficit stress tolerance (Xiao et al., 2007). The elevated stress-responsive gene expression may confer the stress tolerance on the *spy* mutant.

In addition to the negative regulation of GA responses, the *SPY* gene has been shown to play a positive regulatory role in CK signaling. *spy* mutants were found to be more resistant to exogenously applied CK and exhibited inhibition of the induction of Arabidopsis TYPE A RESPONSE REGULATOR5 (*ARR5*) after CK treatment (Greenboim-Wainberg et al., 2005). Interestingly, according to the results of our microarray analysis, we found that the expression of *CKX3* is increased 15.6- and 3.4-fold relative to the wild type in both normal and dehydrated conditions, respectively, which may cause a decrease in CK level in the *spy-3* mutant (Fig. 5; Werner et al., 2003, 2010). Overexpression of the *CKX3* gene resulted in reduced *ARR5* and *ARR7* promoter activity, a decrease in the total surface area of rosette leaves, and plant growth retardation (Werner et al., 2003; Nishiyama et al., 2011). Similarly, *spy-3* mutant rosette leaves were smaller than those of wild-type plants, despite the fact that GA treatment usually increases the size of these leaves. This morphological phenotype is consistent with the increased expression of *CKX3* in the *spy-3* mutant. Interestingly, recent research has shown that reduction of the CK level via overexpression of the *CKX3* gene in roots can improve plant drought tolerance and leaf mineral enrichment (Werner et al., 2010). Functional analyses of CK-deficient *AtCKX* overexpressors and *atipt* mutants provided direct evidence that CKs negatively regulate salt and drought stress responses in plants (Nishiyama et al., 2011). Moreover, when the CK receptors *AHK2* and/or *AHK3* were disrupted, both the single and double mutants were more tolerant of high-salt and drought stresses (Tran et al., 2007). Taken together, the up-regulation of *CKX3* gene expression might reduce CK signaling in the *spy-3* mutant, contributing to plant salt and drought stress tolerance. Although *CKX3* gene expression was significantly up-regulated in the *spy-3* mutant, it was not down-regulated in the *SPY-OX* plants. This lack of down-regulation probably explains why the stress-tolerant phenotype of *SPY-OX* was not as evident as that of the mutant. No remarkable tolerance to high-salinity stress was observed in the *SPY-OX* plants. The molecular mechanism by which *SPY* mediates CK signaling must be investigated further.

Because our knowledge concerning the molecular actions of the *SPY* protein has been limited by the inability to reliably detect modified proteins that are direct targets of the *SPY* protein (Olszewski et al., 2010), we generated *SPY-OX* plants and compared transcriptome alterations in *spy-3* versus wild-type plants and *SPY-OX* versus wild-type plants to identify the direct effects of the *SPY* gene on the transcriptome, especially in relation to plant stress response. The *SPY-OX* transgenic plants were morphologically similar to the wild-type plants but were more sensitive to drought stress than the wild-type plants (Fig. 5).

Comparative transcriptomic analyses uncovered genes that showed inverse transcriptional regulation by *SPY* overexpression and *spy-3* mutation under both normal and drought stress conditions (Fig. 6D; Supplemental Tables S9 and S10). The expression of *DREB1E/DDF1* and *SHN1/WIN1* was up-regulated in the *spy-3* mutant but down-regulated in SPY-OX plants. Both genes positively contribute to plant drought or salt tolerance. The *DREB1E/DDF1* gene not only activated stress-responsive gene expression but also enhanced plant tolerance to drought, cold, and heat stresses (Kang et al., 2011). Moreover, the *DREB1E/DDF1* gene can activate *GA2ox7*, a gene encoding a GA-deactivation enzyme, under salinity stress. The expression of this gene may reduce the active GA content in the plant, thus reducing plant growth under stress (Magome et al., 2008). *GA2ox7* gene expression was also consistently decreased 1.68-fold as the *DREB1E/DDF1* gene was reduced in SPY-OX plants. Our results provide evidence that overexpression of the *SPY* gene can suppress the GA response, supporting the hypothesis that *SPY* functions as a negative regulator of GA signaling. The *SPY* gene may not directly regulate the gene activity involved in GA metabolism but may instead regulate transcription factors that control metabolic gene expression. *SHN1/WIN1* is also an AP2/ERF transcription factor, which can activate cuticular wax synthesis, alter cuticle properties, and increase plant drought stress tolerance. *SHN1/WIN1* may act by controlling the expression of *LACS2*, a long-chain acyl-CoA synthetase gene (Aharoni et al., 2004; Kannangara et al., 2007). Recently, plant cuticle composition was found to be essential for osmotic stress response and tolerance. The cuticle not only functions as a physical barrier to minimize water loss but also mediates abiotic stress signaling (Wang et al., 2011). The expression of *SHN1/WIN1* was significantly increased in the *spy-3* mutant and down-regulated in SPY-OX plants, which may result in the different drought-related phenotypes of these two kinds of plants. The expression of *CKX3* and some LEA protein genes was up-regulated by the *spy-3* mutation but not down-regulated by *SPY* overexpression, which probably explains why the SPY-OX plants were not only sensitive to drought but also to salt stress in comparison with wild-type plants (data not shown).

In recent decades, great progress has been made regarding the characterization of GA signal reception and transduction, due largely to the identification of the upstream *GID1* GA receptors and the downstream GA-targeted DELLA proteins (Ueguchi-Tanaka et al., 2005; Harberd et al., 2009). GA promotes plant growth by destroying a class of transcription factors, the DELLA proteins, that function as repressors to restrain plant growth. DELLA proteins are potential candidate targets of *SPY* (Silverstone et al., 1998; Olszewski et al., 2002). The *O*-GlcNAc modification, mediated by *SPY*, was proposed to compete with phosphorylation to modulate DELLA protein abundance and activity in response to GA or environmental signals (Itoh et al., 2005). It has

been proposed that salt stress inhibits plant growth by reducing endogenous bioactive GA levels, leading to the accumulation of DELLAs (Achard et al., 2006). Studies have demonstrated that the growth restraint conferred by DELLA proteins is beneficial and promotes plant survival under severe salinity stress conditions. A quadruple DELLA mutant lacking *GAI*, *RGA*, *RGL1*, and *RGL2* has been found to be less tolerant to severe salt stress and to exhibit suppression of the salt tolerance conferred by *gal-3* (Achard et al., 2006). Unexpectedly, in various *spy* mutants, the level of *RGA* protein was consistently higher than in wild-type plants, although *spy* mutants displayed GA-treated phenotypes (Silverstone et al., 2007). Higher levels of DELLA proteins in the *spy* mutants indicate that the absence of the *O*-GlcNAc modification might enhance the stability of the proteins but likely reduces their activity in terms of GA signaling. It is also possible that abundant DELLA proteins might confer plant stress tolerance on the *spy-3* mutant. Additionally, many biotic-responsive genes were down-regulated in the 2-h-dehydrated *spy-3* mutant (Supplemental Fig. S3B; Supplemental Table S4). Whether the mutant is more susceptible to biotic attack is unknown.

Research on *O*-GlcNAc modification is more advanced in mammalian cells than in plants. *O*-GlcNAc modification modulates signaling by influencing gene expression, protein degradation, and trafficking in cells and also plays roles in nutrient sensing, cell cycle progression, and stress response (Hart et al., 2007). In mammalian cells, a rapid and global increase in *O*-GlcNAc-ylation on many proteins upon exposure to stress stimuli was found, indicating a positive role for OGTs (Hart et al., 2007). Here, we characterized a negative role for *SPY*, a plant OGT that plays a negative role in plant survival under severe salinity and drought stresses because the *spy* mutants were more tolerant to these stresses. Unlike mammals and insects, plants have two distinct genes encoding OGTs, *SPY* and *SEC*, and both genes have unique and overlapping roles (Olszewski et al., 2010). The activity of a single OGT gene is essential for cell viability in mammals (Hart et al., 2007). In plants, the simultaneous deletion of the *SPY* and *SEC* genes is embryo lethal, whereas single mutations of each gene produce different phenotypes. A phylogenetic analysis of all eukaryotic OGT genes found that mammalian OGTs are SEC-like but not *SPY*-like proteins (Olszewski et al., 2010). The gene function of *SEC* in plant stress and/or hormone response and the relationship between these two gene functions merit further investigation.

## MATERIALS AND METHODS

### Plant Materials and Transgenic Plant Construction

*Arabidopsis* (*Arabidopsis thaliana*) plants were grown, transformed, and treated as described previously (Qin et al., 2008). *spy-1* and *spy-3* mutant seeds were obtained from the Arabidopsis Biological Resource Center (catalog nos. CS6266 and CS6268, respectively). *spy-8* and *spy-12* mutant seeds were kindly provided by Dr. Tai-ping Sun. The full-length coding sequence of the *SPY* gene

was constructed downstream of the 35S promoter with an enhancer sequence in the pGKX vector (Qin et al., 2008) and transformed into Col plants for the purpose of overexpressing this gene.

## RNA Gel-Blot and Real-Time RT-PCR Analyses

Total RNA was isolated with the RNAiso (Takara) reagent from 3-week-old plants. RNA gel-blot analysis was performed as described previously (Yamaguchi-Shinozaki and Shinozaki, 1994). A *SPY*-specific probe was prepared by PCR amplification of the C-terminal end of the *SPY* gene from nucleotide 2,003 to the final nucleotide. For real-time RT-PCR analysis, 1  $\mu$ g of total RNA was used for the first-strand cDNA synthesis using SuperScript III transcriptase (Invitrogen). The resultant cDNA solution was diluted five times, and a 1- $\mu$ L solution was used as the template in subsequent real-time PCR. PCR was performed in a 10- $\mu$ L volume using the ABI7500 system and SYBR Green I mixture (Takara). Each reaction was performed in triplicate to obtain the average and SD for the expression level of each gene.

## Microarray Analysis and Statistical Analysis

Genome-wide expression studies with Arabidopsis 44K Oligo Microarrays (Agilent Technologies) were performed using 3-week-old wild-type and *spy-3* plants. Gene expression was compared between wild-type and *spy-3* plants under both unstressed and 2-h dehydration stress conditions. For each sample (pooled from six plants), 200 ng of total RNA was isolated using the RNAiso reagent (Takara) and used for the analysis. Biological replication was performed by analyzing the samples obtained from two independent treatments (for both the control and 2-h dehydration stress conditions). For each experiment, two slides were analyzed to perform a Cy3 and Cy5 dye swap, and a total of eight slides were hybridized for the four experiments. Statistical analysis of the microarray data to integrate and normalize each spot's signal intensity was performed using the Lowess method with the Feature Extraction 9.5 program (Agilent Technologies). The statistical analysis was carried out using ArrayAssist software (Stratagene). The Welch *t* test was used as a parametric test, and the Benjamini and Hochberg false discovery rate for multiple testing corrections was used with a threshold set at  $P < 0.05$  to identify reliable genes. Following this statistical analysis, we generated lists of the genes showing significant differences in expression between wild-type and *spy-3* plants under normal conditions and after exposure to a 2-h dehydration stress. Of the genes that were considered to show significant expression differences based on the previous tests, those that exhibited an absolute fold change greater than 2.0 were selected. Such genes were considered to be up-regulated in *spy-3* plants relative to wild-type plants. All of the microarray data are available at <http://www.ebi.ac.uk/arrayexpress/> under the accession numbers E-MEXP-3171 and E-MEXP-3362.

## Salt Stress Tolerance Test

For the plant seedling tolerance test, seeds were sown on germination medium (GM) agar plates and poststratified at 4°C for 4 d. After growing on GM plates for 7 d, the plants were transferred onto 0.5 $\times$  Murashige and Skoog (MS) agar plates containing 200 mM NaCl. The plates were maintained at 22°C under a 16-h-light/8-h-dark cycle until visual symptoms could be observed and photographed. For the plant salt tolerance test at the germination stage, the seeds were sown on MS agar plates with 150 mM NaCl and poststratified at 4°C for 4 d. Then, they were transferred to 22°C under a 16-h-light/8-h-dark cycle until visual symptoms could be observed and photographed.

## Drought Stress Tolerance Test

Briefly, 3-week-old plants grown on GM agar plates were transferred to soil. After 1 week of conditioning, the plants were subjected to a water-withholding treatment for approximately 14 d, after which the most obvious differences were observed between the wild-type and mutant plants. The plant survival rate was recorded after 1 week of reinitiated watering and recovery. For the GA treatment, Col plants were transferred to soil after 3 d of germination on GM agar plates and sprayed with a 50  $\mu$ M GA<sub>3</sub> solution or water every other day for 3 weeks. The water supply was then cut off for approximately 2 weeks for the dehydration treatment. To ensure that the pots in which Col and *spy-3* plants were growing contained equal water levels during the dehydration stress treatment, the plants were transferred into pots containing the same amount of soil saturated by water. The initial weight of

each pot was controlled, and the total weight of each pot was measured daily after 12 d of dehydration stress, so as to calculate and record the water content of each pot daily. Watering was resumed when the average total weights of the pots containing Col and *spy-3* were comparable. For the plant water loss assay, the aerial parts of 2-week-old *spy-1*, *spy-3*, and Col plants were placed in empty petri dishes, and the plant weights were periodically recorded. For each assay, the average and SD were calculated from four plants. The leaf electrolyte leakage was determined as described by Qin et al. (2004).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number NM\_111987 (*SPINDLY*).

## Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** *spy-8* and *spy-12* mutants were more tolerant to salt stress during seed germination.

**Supplemental Figure S2.** Heat map of transcriptomic analysis of normally growing *spy-3* versus Col plants.

**Supplemental Figure S3.** Heat map of transcriptomic analysis of 2-h-dehydrated *spy-3* versus Col plants.

**Supplemental Table S1.** A total of 506 up-regulated and 287 down-regulated genes in 0-h *spy-3* plants.

**Supplemental Table S2.** A total of 59 drought stress-inducible genes up-regulated in 0-h-dehydrated *spy-3* plants.

**Supplemental Table S3.** A total of 114 GA-up-regulated genes in *spy-3* plants.

**Supplemental Table S4.** A total of 612 up-regulated and 720 down-regulated genes in 2-h-dehydrated *spy-3* plants.

**Supplemental Table S5.** A total of 98 drought-inducible genes up-regulated in 2-h-dehydrated *spy-3* plants.

**Supplemental Table S6.** Drought tolerance test of SPY-OX plants.

**Supplemental Table S7.** Up- or down-regulated genes in SPY-OX plants under normal growing conditions.

**Supplemental Table S8.** Up- or down-regulated genes in SPY-OX plants after 2 h of dehydration stress.

**Supplemental Table S9.** Genes up-regulated in the *spy-3* mutant but down-regulated in SPY-OX plants under normal growing conditions.

**Supplemental Table S10.** Genes up-regulated in the *spy-3* mutant but down-regulated in SPY-OX plants under 2-h dehydration conditions.

**Supplemental Table S11.** Primer list for qRT-PCR.

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