Plant Cell Rep
DOI 10.1007/s00299-015-1852-3

ORIGINAL ARTICLE

The sunflower transcription factor HaWRKY76 confers drought and flood tolerance to *Arabidopsis thaliana* plants without yield penalty

Jesica Raineri[1] · Karina F. Ribichich[1] · Raquel L. Chan[1]

Received: 30 June 2015 / Revised: 21 July 2015 / Accepted: 29 July 2015

Abstract

Key message **Arabidopsis transgenic plants expressing the sunflower transcription factor HaWRKY76 exhibit increased yield and tolerance to drought and flood stresses. The genetic construct containing HaWRKY76 is proposed as a potential biotechnological tool to improve crops.**

Abstract Water deficit and water excess are abiotic stress factors that seriously affect crops worldwide. To increase the tolerance to such stresses without causing yield penalty constitutes a major goal for biotechnologists. In this survey, we report that HaWRKY76, a divergent sunflower WRKY transcription factor, is able to confer both dehydration and submergence tolerance to Arabidopsis transgenic plants without yield penalty. The expression pattern of *HaWRKY76* was analyzed in plants grown in standard conditions and under different watering regimes indicating a regulation by water availability. The corresponding cDNA was isolated and cloned under the control of a constitutive promoter and Arabidopsis plants were transformed with this construct. These transgenic plants presented higher biomass, seed production and sucrose content than controls in standard growth conditions. Moreover, they exhibited tolerance to mild drought or flood (complete submergence/waterlogging) stresses as well as the same or increased yield, depending on the stress severity and plant developmental stage, compared with controls. Drought tolerance occurred via an ABA-independent mechanism and induction of stomatal closure. Submergence tolerance can be explained by the carbohydrate (sucrose and starch) preservation achieved through the repression of fermentation pathways. Higher cell membrane stability and chlorenchyma maintenance could be the nexus between tolerance responses in front of both stresses. Altogether, the obtained results indicated that HaWRKY76 can be a potential biotechnological tool to improve crops yield as well as drought and flood tolerances.

Keywords Transcription factor · WRKY · Drought · Submergence · Sunflower · Arabidopsis

Communicated by M. Menossi.

Electronic supplementary material The online version of this article (doi:10.1007/s00299-015-1852-3) contains supplementary material, which is available to authorized users.

✉ Raquel L. Chan
rchan@fbcb.unl.edu.ar

[1] Instituto de Agrobiotecnología del Litoral, CONICET, Universidad Nacional del Litoral, Centro Científico Tecnológico CONICET Santa Fe, Colectora Ruta Nacional No 168 km. 0, Paraje El Pozo, 3000 Santa Fe, Argentina

Introduction

Drought and flood are major abiotic stress factors affecting plants, leading them to trigger physiological and biochemical changes that cause diverse consequences, varying from severe damage to tolerance responses (Fukao et al. 2011; Lakshmanan et al. 2013).

To avoid water deficit, plants count on a series of morphological characteristics and physiological modifications like the cuticle which is able to reduce transpiration, the osmolyte production, the decrease of stomatal conductance that leads to stomata closure, the reduction of leaf surface and the acceleration of leaf senescence (Hirayama and Shinozaki 2010; Kerstiens 1996; Rengel et al. 2012). Drought also triggers the accumulation of reactive oxygen species (ROS), which cause tissue injury and chlorophyll

Published online: 06 August 2015

degradation (Fukao et al. 2011). Unfortunately, these adaptive changes frequently cause detrimental consequences which vary depending on the stress strength and duration, from photosynthesis impairment, biomass and yield reduction in mild stress conditions to plant death when the stress is severe and/or prolonged (Rivero et al. 2007; Skirycz et al. 2011).

On the other hand, plants also react in front of flood threat with a set of strategies to overcome the deleterious effects. Among them, the best established in tolerant cultivars, mainly rice, are the quiescence and the escape strategies (Hattori et al. 2011). Quiescence occurs in cultivars that tolerate complete water coverage, which is possible during just a few days (flash flood). This strategy involves a reduction of carbohydrate consumption to resume it after water draining enabling the growth of new shoots. Conversely, the escape occurs in cultivars partially covered with water that overgrow the water level (deep-water flood). Plants elongate the internodes and sustain certain metabolic activity to continue growing, which can extend during months. Plants triggering the escape strategy share characteristics with those under waterlogging, when the water level stays at the soil line or hardly over there. Examples of these characteristics are the emergence of adventitious roots, the formation of aerenchyma and the hyponastic growth (Bailey-Serres and Voesenek 2008; Nishiuchi et al. 2012). In all of these stressing conditions, submerged tissues have reduced aerobic respiration and photosynthesis because of O_2, CO_2 and light lessening with usual negative consequences in biomass and yield (Bailey-Serres and Voesenek 2008). Upon desubmergence and re-oxygenation, plants must overcome the dehydration that progressively occurs during the stress and thereafter (Setter et al. 2010) as well as the tissue damage caused by ROS (Fukao et al. 2011).

Although drought and flood are apparently opposite, their consequences have common features, so could be the adaptive responses. In fact, in rice there is a crosstalk between drought and submergence/waterlogging tolerance mediated by a member of the group VII of ethylene-responsive transcription factors (ERFs), the submergence tolerance regulator gene, *SUB1A* (Fukao et al. 2011). A similar mechanism could be taking place in *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis), a species having several members of group VII ERFs with distinct roles as sensors in anoxia/hypoxia sensing mechanisms, finally converging in the induction of hypoxia-responsive genes (Gibbs et al. 2011; Licausi et al. 2011; Voesenek and Bailey-Serres 2013).

Non-model species, less explored and able to live in environments with different water regimes, could be valuable genetic sources to study and understand the relationship between both stresses and responses. For this purpose, *Helianthus annuus* L. (common sunflower) is a noteworthy species because it is able to grow in variable agronomic situations and particularly, on soils with different levels of water (FAO 2012; O'Brien et al. 2009; Quartacci and Navari-Izzo 1992).

Transcription factors (TFs) are key regulatory proteins able to induce or repress whole transduction signal pathways. Among plant TFs, those belonging to the WRKY family have been described as regulators of several developmental processes and related with biotic and abiotic stress responses in many plant species including the models Arabidopsis and rice, and also crops such as wheat, maize and sunflower (Chen et al. 2015; Giacomelli et al. 2010; Okay et al. 2014; Raineri et al. 2015; Rushton et al. 2010, 2012). In particular, some WRKY transcription factors have been associated to the drought response, both as positive or as negative regulators (Ding et al. 2014; Gong et al. 2015; Sun and Yu 2015). The response pathways to drought mediated by WRKY TFs were described in some cases as ABA-dependent and in others as ABA-independent (Rushton et al. 2012; Tripathi et al. 2014).

Asteraceae species are characterized by their plasticity (Panero and Funk 2008; Timme et al. 2007). They are present in the majority of biomes, well adapted to diverse soils, extreme climates and topographies (Katinas et al. 2007). Particularly, the *Helianthus* genus suffers an increased speciation rate, which allowed the recent worldwide radiation of the group (Panero and Funk 2008; Timme et al. 2007). Members of the sunflower (or other Asteraceae species) WRKY family have not been characterized so far, with a few exceptions: the sunflower HaWRKY6, involved in the temperature response and regulated by a miRNA (Giacomelli et al. 2012); the chrysanthemum DgWRKY3, involved in salt tolerance (Liu et al. 2013) and the *Artemisia annua* AaWRKY1 which regulates artemisinin biosynthesis (Ma et al. 2009). To unravel how sunflower has adapted to so varied environments, divergent transcription factors are particularly interesting proteins to be functionally characterized. HaWRKY76, a sunflower TF whose biological role remains elusive, clustered in a novel clade of the WRKY family apparently exclusive and diversified in the Asteraceae. This is a sister clade of the IId subgroup, which exhibits at least three structural and potentially functional motifs not found in members of other clades (Giacomelli et al. 2010). Recent surveys allowed to relate members of group IId from Arabidopsis and other plants, such as cotton and grapevine, with osmotic (Liu et al. 2011; Vanderauwera et al. 2012), drought (Yan et al. 2014) and salt tolerance responses (Liu et al. 2011; Vanderauwera et al. 2012; Yan et al. 2014).

To unravel HaWRKY76 function, first its expression pattern in standard or stressing growth conditions was

examined. According to the results, its potential role in water stress responses, both drought and flood, was evaluated. Sunflower resilience to genetic manipulation is still significantly enough to justify the use of a heterologous system. Therefore, Arabidopsis homozygous transgenic lines were obtained and deeply characterized in different conditions of water supply showing enhanced tolerance to drought and flood stresses as well as increased yield under stress and standard growth conditions.

Results

HaWRKY76 is expressed in sunflower seedlings and plantlets and induced after drought and desubmergence treatments

Sunflower specimens at different vegetative stages were sampled to assess *HaWRKY76* expression levels in plants grown in standard conditions and under different water regimes. *HaWRKY76* expression was detected in all the organs of 5-day-old sunflower seedlings grown in soil; its transcripts were around threefold to fourfold higher in roots and hypocotyls than in cotyledons (Fig. 1a). Water stress responses were evaluated in seedlings exposed to severe hydric stress, i.e., a rapid desiccation during 30 min, applied the 5th day. After this treatment, the expression in roots under stress was still higher (12-fold) than in cotyledons (Fig. 1a). To assess the effect of drought in a more advanced developmental stage, watering was completely stopped to 15-day-old plants (four leaves) during additional 15 days. The wilt and damage became evident on the 28th day and the plants died one or 2 days after this day. *HaWRKY76* expression increased in the plantlets by the 14th day after stopping watering (tenfold) concomitantly with the symptoms of dehydration (Fig. 1b). These observations suggested a role of this TF in the response of sunflower plants to drought stress, at least in early developmental stages.

Because water deficit and water excess responses are sometimes related, the expression of *HaWRKY76* was evaluated after a submergence treatment. Plantlets (15-day-old) were completely submerged during 11 days and then placed in standard watering conditions for 1 h (desubmergence). After such treatment, sunflower plantlets showed clear symptoms of injury that would end in post-submergence wilting. *HaWRKY76* transcript levels were constant up to the last day of submergence but significantly increased (near tenfold) after 1 h of water restrain (Fig. 1c). This result indicated a role of this TF in the response to dehydration stress that in this case occurs during desubmergence.

Arabidopsis transgenic plants expressing *HaWRKY76* exhibit increased biomass and seed yield

To understand the role of *HaWRKY76* in the water stress response, Arabidopsis homozygous transgenic lines ectopically expressing *HaWRKY76* under the control of the

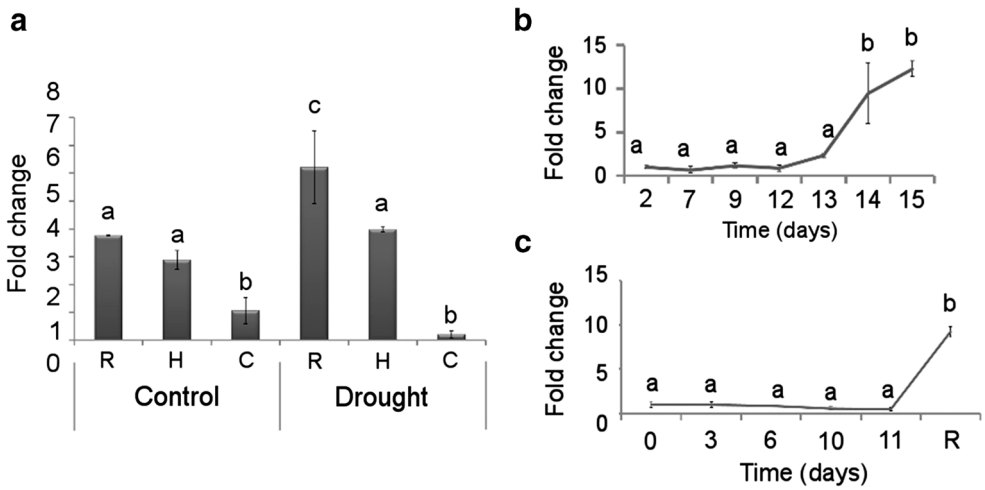

Fig. 1 *HaWRKY76* expression in sunflower: **a** Roots (R), hypocotyls (H) and cotyledons (C) from 5-day-old seedlings well irrigated (control panel) or exposed to severe water stress (drought panel), **b** 15-day-old plantlets (time 0) exposed to severe drought stress or **c** to submergence during 11 days and recovery (R). For **b** and **c**, RNA was extracted from leaves. Transcript levels of *HaWRKY76* were quantified by RT-qPCR, normalized with sunflower *ACTIN* (*ACTIN2 and ACTIN8*), and thereafter with respect to the value measured in the cotyledon sample under control conditions (**a**), in the sample exposed to water stress during 2 days (**b**), or in the beginning of the treatment (**c**), the three arbitrarily assigned a value of one. Two independent experiments were done and *error bars* correspond to standard deviations from three biological replicas in each experiment. An ANOVA test was performed, followed by a Fisher LSD post-hoc test. *Different letters* samples with significant differences ($P < 0.05$)

35S CaMV promoter were obtained and characterized. Three independent lines with different transgene expression levels were chosen for further analyses (Supplementary Fig. S1). To analyze the phenotype of these plants, several assays were carried out on different substrates in non-stressful (standard) conditions.

On agar medium, transgenic seedlings developed longer roots (Fig. 2a). When grown on soil, root diameters from transgenic plants were significantly wider than those of wild-type (WT) ones (Fig. 2b, c) as well as their shoots biomass. Furthermore, two (out of three) of the lines produced more seeds than controls at the end of their life cycle (Fig. 2 d, e), with a yield average increase of 17 %. On sand, transgenic plants also developed higher root biomass (Fig. 2f, g), whereas aerial tissue biomass did not show significant differences between genotypes (data not shown).

HaWRKY76 confers drought and flooding tolerance to Arabidopsis transgenic plants

In temperate climates, it is more likely that mild and not severe drought stress occurs, still negatively affecting growth and yield (Skirycz et al. 2011). To evaluate *HaWRKY76* transgenic plants subjected to mild stress in the vegetative stage, seeds were sown in soil and well watered. Then, the irrigation was limited up to the end of life cycle (see M&M). During this treatment, plants did not die and had the capacity of recovery but leaves lost their turgidity and the plants looked withered. At the end of the cycle, *HaWRKY76* transgenic plants yielded significantly more than their WT controls (Fig. 3a). Drought stress was also applied during the reproductive stage (25-day-old plants); however, when the stress was applied at this developmental stage, transgenics and controls did not exhibit significant differences in yield (Fig. 3b).

Considering the induction of *HaWRKY76* expression in sunflower seedlings after a submergence/desubmergence treatment, the Arabidopsis transgenic plants were evaluated after such stress assay. Twenty-five-day-old plants were submerged during 5 days and then placed in standard conditions until recovery. Yield was measured at the end of the cycle and the results indicated that *HaWRKY76* transgenic plants produced more seeds than their WT counterparts (Fig. 3c). Noteworthy, transgenic plants subjected to 5 days of waterlogging (25-day-old) followed by recovery in standard conditions also exhibited higher yield than the WT genotype (Fig. 3d). Concomitant with the better performance in yield, transgenic plants looked much healthier than WT ones at the end of the reproductive stage (Fig. 3).

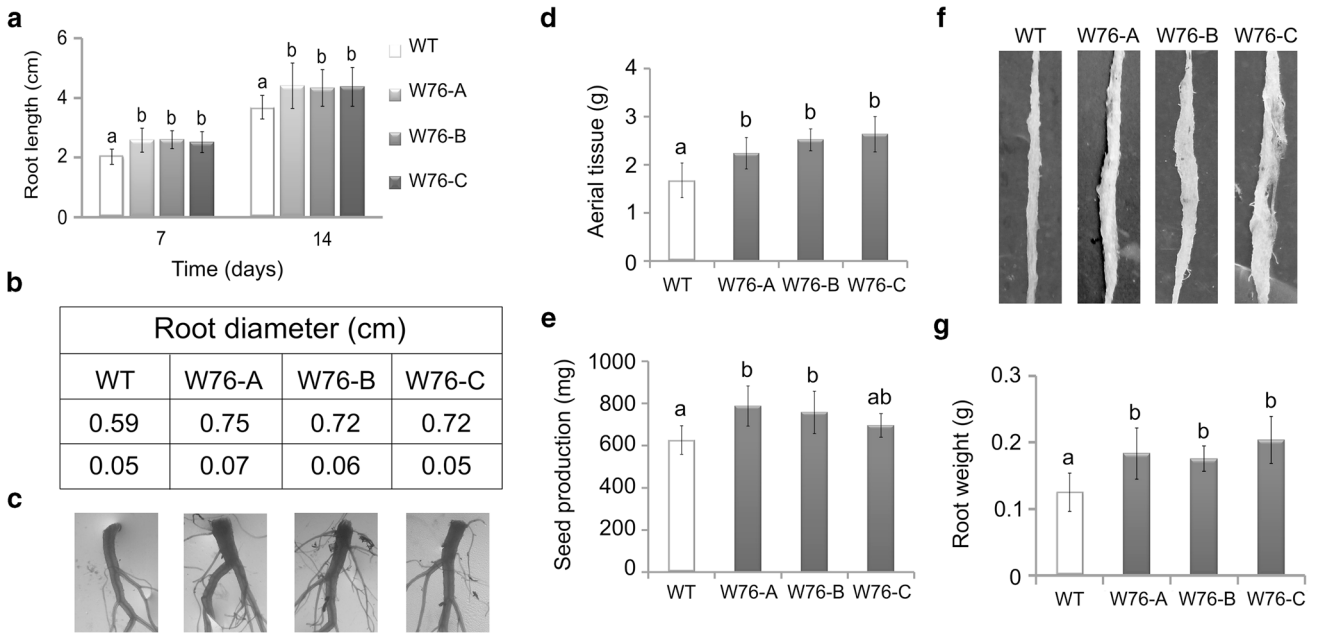

Fig. 2 Phenotypical characteristics of *HaWRKY76* transgenic plants in non-stressful conditions: **a** Roots length from 7- and 14-day-old plants grown on MS agar. *Error bars* correspond to the standard deviations from 20 biological replicas; **b**, **c** roots diameter and illustrative pictures from 35-day-old plants grown on soil; **d**, **e** aerial tissue and harvested seeds from the same plants. *Error bars* correspond to the standard deviations from four biological replicas in each experiment; **f**, **g** illustrative roots pictures and weights from 35-day-old plants grown on sand. *Error bars* correspond to the standard deviations from four biological replicas in each experiment. Three independent experiments were done. W76-A, W76-B, W76-C: three independent *HaWRKY76* transgenic lines. In all cases, ANOVA test was performed, followed by a Fisher LSD post-hoc test. *Different letters* samples which are significantly different from WT ($P < 0.05$)

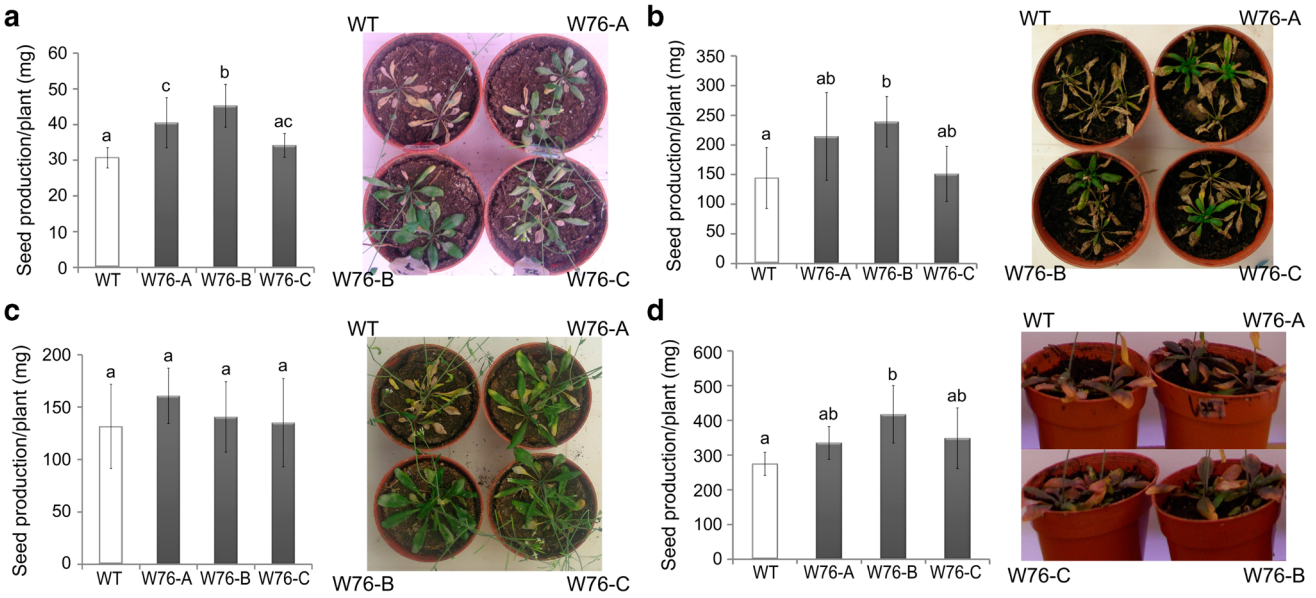

Fig. 3 *HaWRKY76* transgenic plants exhibit equal or higher yield than WT after water deficit or water excess treatments: **a**, **b** Seeds production of transgenic (W76-A, W76-B, W76-C) and WT plants subjected to mild water deficit in the vegetative (**a**) or the reproductive (**b**) stage. **c**, **d** Seeds production of transgenic (W76-A, W76-B, W76-C) and WT plants in the reproductive stage subjected to submergence (**c**) or waterlogging (**d**). Two or three plants per pot were assayed as shown in the illustrative photographs on the *right* of each graph. Three experiments were done and *error bars* correspond to standard deviations from four biological replicas in each experiment. ANOVA test was performed, followed by a Fisher LSD post-hoc test. *Different letters* samples which are significantly different from WT ($P < 0.05$). Photographs were taken during the stress treatment (**a**, **b**) or 5 days after the stress treatment (**c**, **d**)

Leaves' adaptations in *HaWRKY6* transgenic Arabidopsis plants would explain the associated drought tolerance

To disclose the mechanisms involved in tolerance to mild drought stress, leaf water loss and plant water consumption were checked in WT and transgenic plants. For water loss assessment, 25-day-old plants were either well watered or not watered at all during 5 days. Then, leaves were detached and weighted at the times indicated in the figure (Fig. 4). Transgenic leaves lost less weight than controls and the difference was early noticed (40 min after leaves removal, Fig. 4a, b). For water consumption, the pot weight of plants subjected to drought stress was registered during all the period (until harvest) and taken as water expenditure. Two out of three transgenic lines consumed significantly less water than controls (Fig. 4c). Additionally, the chlorophyll content in stress conditions was measured once and 2 weeks after starting with the stress treatment and it was higher in transgenic leaves than in the WT counterparts but only at the end of the cycle. This last result indicated that transgenic plants entered the senescence stage with a slight delay (Fig. 4d). One of the consequences of water stress is a functional failure of cell membranes as permeability selectors. For this reason, conductivity changes produced by cell ion leakage were also evaluated and are shown in Fig. 4e. As expected after the differential behavior in front of drought stress, transgenic plants suffered lower cell membrane damage than the WT counterparts (Fig. 4e). Altogether these results suggested that the physiological tolerance observed in *HaWRKY76* transgenic plants occurs via the induction of stomatal closure, higher cell membrane stability and a delay in the drought-induced senescence.

Assessment of transcript levels of ABA-related genes in *HaWRKY76* transgenic plants suggests that drought tolerance occurs via an ABA-independent mechanism

There are several genes, mainly related with ABA biosynthesis and signaling, that became drought markers because it is well documented that their expression is regulated by water deficit or osmotic stress (de Bruxelles et al. 1996; Shinozaki and Yamaguchi-Shinozaki 2000; Xiong et al. 1999). To unravel the signaling that could conduct to the biochemical and physiological changes observed in *HaWRKY76* transgenic plants, expression of ABA synthesis-related genes (*ABA1* and *ABA2*), negative and positive regulators of ABA signaling (*ABI1* and *ABI2*, *ABI3* and *ABI5*, respectively), other ABA-responsive genes such as *RD29B*, *RAB18*, *EM6* and *ADH* (ABA-dependent), *RD29A*, *COR47* and *COR15A* (ABA-independent) were investigated. No significant differences in the expression

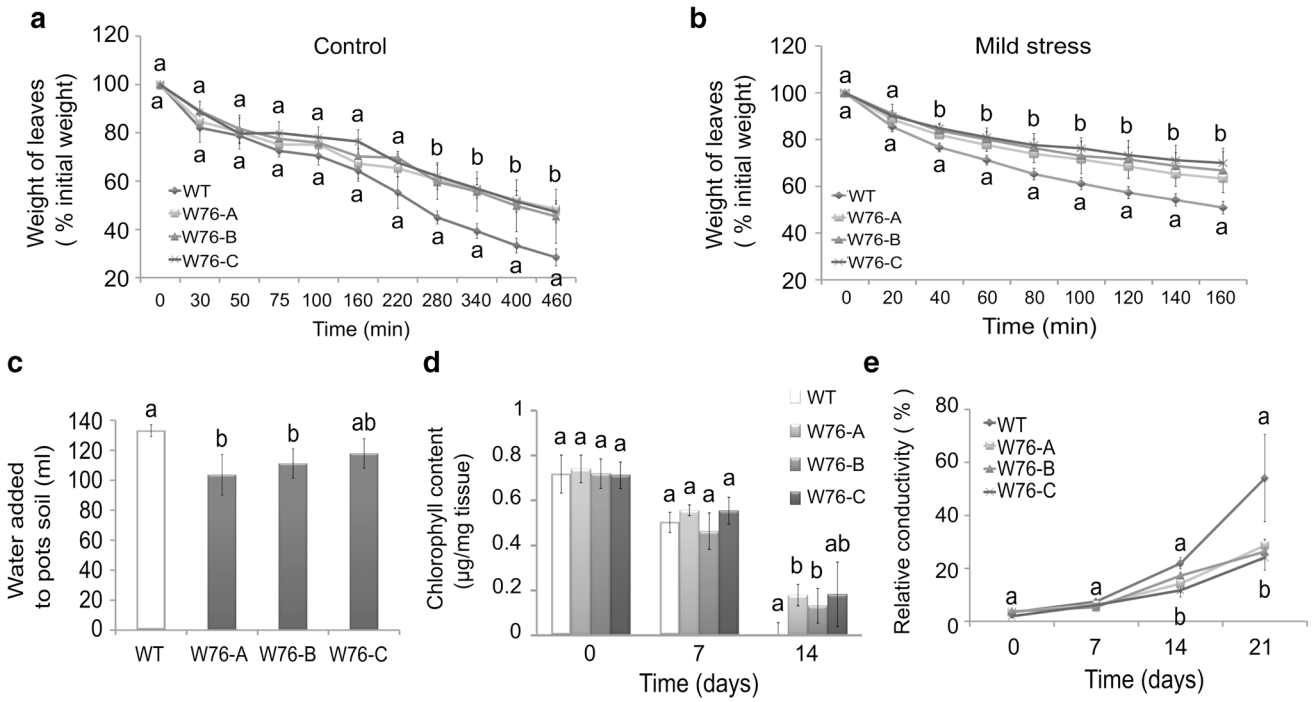

Fig. 4 *HaWRKY76* transgenic plants consume and lose less water than WT: **a**, **b** Water loss in 25-day-old detached leaves from well-irrigated (**a**) or mild drought-stressed plants (**b**). Leaves weight is expressed as the % of the weight registered at time 0, taken as 100 %. *Error bars* correspond to standard deviations from four biological replicas. (c) Total water added (ml) to mild stressed 25-day-old plants during the treatment. *Error bars* correspond to standard deviations from 10 biological replicas. **d** Chlorophyll content and **e** conductivity of stressed leaves, both related to that measured in WT. W76-A, W76-B, W76-C represent three independent HaWRKY76 transgenic lines. Three independent experiments were done and *error bars* correspond to standard deviations from three or four biological replicas in each experiment. ANOVA test was conducted in all the cases, followed by a Fisher LSD post-hoc test. *Different letters* samples with significant differences ($P < 0.05$)

levels between 25-day-old transgenic and WT leaves grown in stress conditions were detected for *ABA1*, *ABA2*, *COR47* and *COR15A*, whereas the others were less induced in the transgenic genotypes (Fig. 5). Moreover, expression levels of the same genes were evaluated in WT and transgenic plants subjected to ABA treatments. Corroborating the latter observation, the regulation of these genes by ABA was not significantly different between transgenic and WT plants, with the exception of *ABI3* and *ABI5* whose levels were higher in transgenic plants in response to ABA (Fig. S2). In view of these observations, i.e., a similar regulation of ABA-related genes in WT and transgenics after ABA treatments, an ABA-independent response to confer drought tolerance is more likely triggered in *HaWRKY76* transgenic plants.

Carbohydrate balance exhibits significant differences between *HaWRKY6* transgenic and WT plants after submergence

As a response to flooding stress, diverse plant species display changes in carbon use as one of the strategies to overcome such stress (Fukao and Xiong 2013; Sasidharan et al. 2013). Carbohydrate partitioning was evaluated in 25-day-old plants before and after 5 days of a submergence treatment. At the beginning of the treatment, soluble glucose levels were roughly equal in transgenic and WT plants, whereas sucrose was higher in transgenic plants and starch did not show differences between genotypes (Fig. 6). During the submergence treatment, all these carbohydrates decreased in both WT and transgenics, but at different extents. Glucose and starch levels diminished less in the transgenic plants than in the WT ones, whereas sucrose did not show significant differences between lineages (Fig. 6a–c boxes). Sucrose and starch final levels were also higher in the transgenics than in the WT (Fig. 6b, c). On the other hand, no significant changes between genotypes were observed in biomass or stem elongation indicating that starch was unlikely used for growth (Supplemental Fig. S3a, b). Just 1 day after desubmergence, even though carbohydrate content had decreased in all the plants during the stressing treatment, the transgenics looked better than the non-transgenic ones (Supplemental Fig. S3c). Additionally, glucose and sucrose contents increased up to roughly the same levels in all the genotypes, whereas starch decreased only in the *HaWRKY76* transgenic plants (Fig. S4a–c)

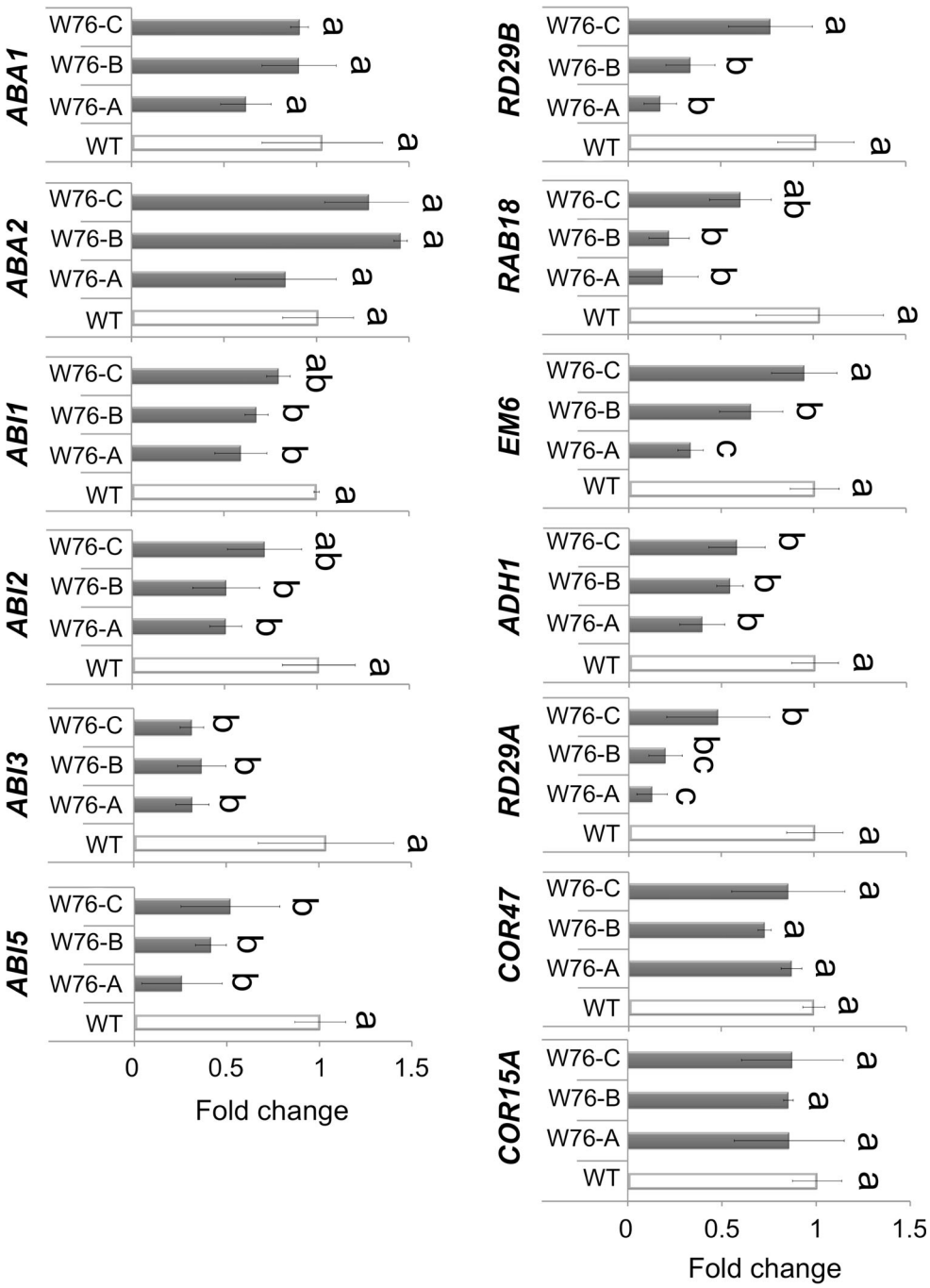

Fig. 5 Expression levels of ABA-signaling-related genes: Genes related to ABA synthesis: *ABA1*, *ABA2*, ABA signaling: *ABI1*, *ABI2*, *ABI3*, *ABI5*, ABA-dependent: *RD29B*, *RAB18*, *EM6*, *ADH* and ABA-independent pathways: *RD29A*, *COR47* and COR15A in *HaWRKY76* transgenic (W76-A, W76-B, W76-C) and WT plants. Transcript levels were quantified by RT-qPCR, normalized with *ACTIN2/8*, and thereafter with respect to the lower expression sample, arbitrarily assigned a value of one. *Error bars* correspond to standard deviations from three biological replicas. Accession numbers of the evaluated genes are listed in Table S1

After a submergence treatment *HaWRKY76* transgenic plants exhibit less ROS and carbohydrate metabolism-related-genes transcripts

Submergence damage on plants is mainly caused by the oxidative stress triggered by hypoxia, which induces adaptive responses such as oxidant impairment and increase of fermentation pathways (Blokhina and Fagerstedt 2010). To assess the response of the transgenic plants to oxidative stress, superoxide production as the main ROS indicator and the status of fermentation genes were evaluated at the end of a submergence treatment. Upon 5 days of submergence, *HaWRKY76* leaves produced less superoxide than WT (Fig. 7a, b). On the other hand, expression levels of some key genes involved in sucrose cleavage (*SUS1* and *SUS4*), and ethanol and amino acid fermentation (*PDC1*, *PDC2*, *ADH*, *ASP2*, *ATT1*) were quantified. After 3 days of submergence, *SUS1* and *ADH* (from the ethanol pathway) expressions were repressed, whereas *ATT1* (from the alanine pathway) was induced in two of three

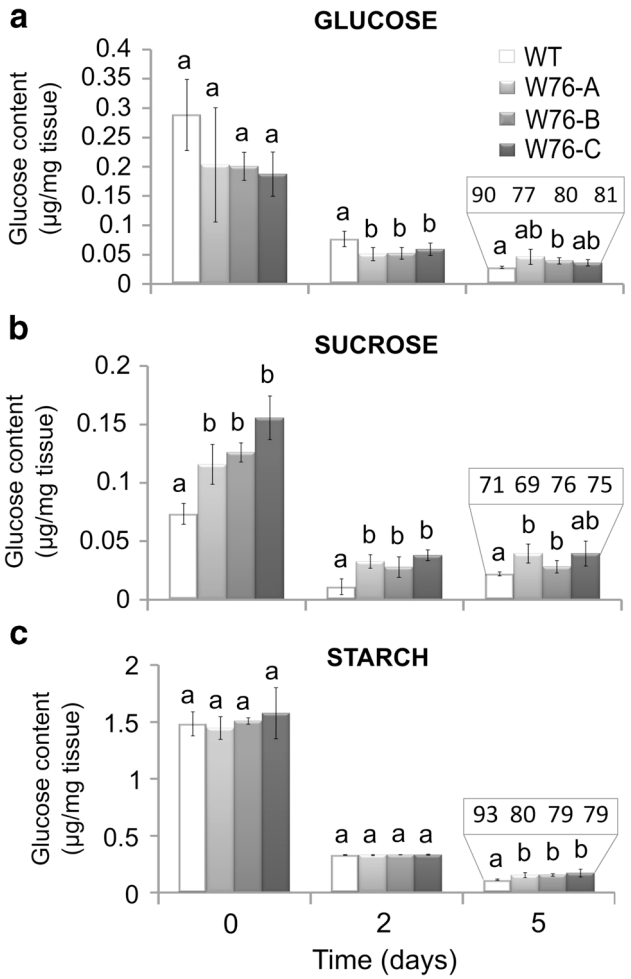

Fig. 6 Carbohydrate partitioning in transgenic and WT plants during a submergence treatment: Glucose, sucrose and starch contents were evaluated at the indicated times in transgenic (W76-A, W76-B, W76-C) and WT plants (25-day-old) subjected to submergence during 5 days. Numbers in boxes represent the percentages of each carbon source effectively consumed taking the content at the beginning of the treatment as 100 %. Three independent experiments were done and *error bars* correspond to standard deviations from four biological replicas in each experiment. ANOVA test was conducted in all the cases, followed by a Fisher LSD post-hoc test. *Different letters* samples with significant differences ($P < 0.05$)

transgenic lineages compared with WT. The other genes did not show differences between genotypes after this treatment (Fig. S5).

Transgenic plants exhibit more lysigenous aerenchyma and higher membrane stability than their controls triggering tolerance to waterlogging and partial submergence

Because some plant species can tolerate flooding of different depth, transgenic and WT plants were assessed for waterlogging and partial submergence stresses. The assays

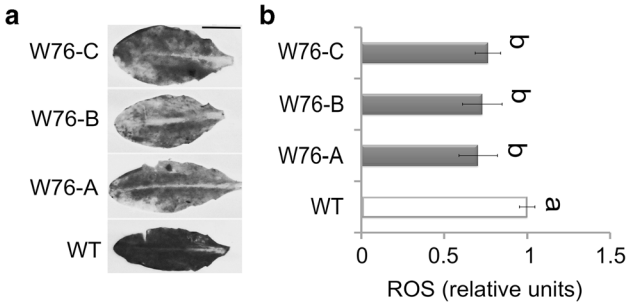

Fig. 7 Transgenic *HaWRKY76* leaves produced less ROS than WT after a submergence treatment: **a** Illustrative photographs of NBT stained 25-day-old fully expanded leaves after 5 days of submergence, **b** quantitative estimation of superoxide production. In **a**, the *scale bar* represents 1 cm. In **b**, quantification was done by densitometry, normalizing the intensities of transgenic leaves with those of WT arbitrarily assigned a value of one. Two independent experiments were done and *error bars* correspond to standard deviations from three biological replicas in each experiment. ANOVA test was conducted in all the cases, followed by a Fisher LSD post-hoc test. *Different letters* samples with significant differences ($P < 0.05$)

were carried out with various water levels (schematized in Supplemental Fig. S6). As a waterlogging stress acclimation response, 5 days after the treatment, 25-day-old *HaWRKY76* rosette leaves suffered less cell membrane damage compared to WT, as reflected by their lower ion leakage (Fig. 8a). On the other hand, *HaWRKY76* transgenic plants developed longer stems (Fig. 8b), and more and wider areas of lysigenous aerenchyma in the stems, the cortex and the pith cavity, than WT, when they were partially submerged (Fig. 8c, d).

Discussion

Crops yield is seriously affected by water imbalance, either by excess or deficit. The adaptation of plants to such situations depends on multiple factors and each species has evolved different capabilities to deal with the changing environment. Sunflower is considered as a rather water stress-tolerant species (Rengel et al. 2012). The identification of genes able to sense and trigger adaptive responses in front of water instability and the comprehension of how these genes act in both, water deficit and excess responses, will increase our knowledge and the possibility to develop technologies to improve crops. Genes encoding transcription factors are key candidates for this purpose.

Sunflower exhibits phylogenetically divergent transcription factors (Arce et al. 2011; Giacomelli et al. 2010). Examples of such sunflower transcription factors shown as potential biotechnological tools to improve crops are HaHB4 and HaHB11, all belonging to the homeodomain-leucine zipper I (Chan et al. 2010, 2013). Remarkably,

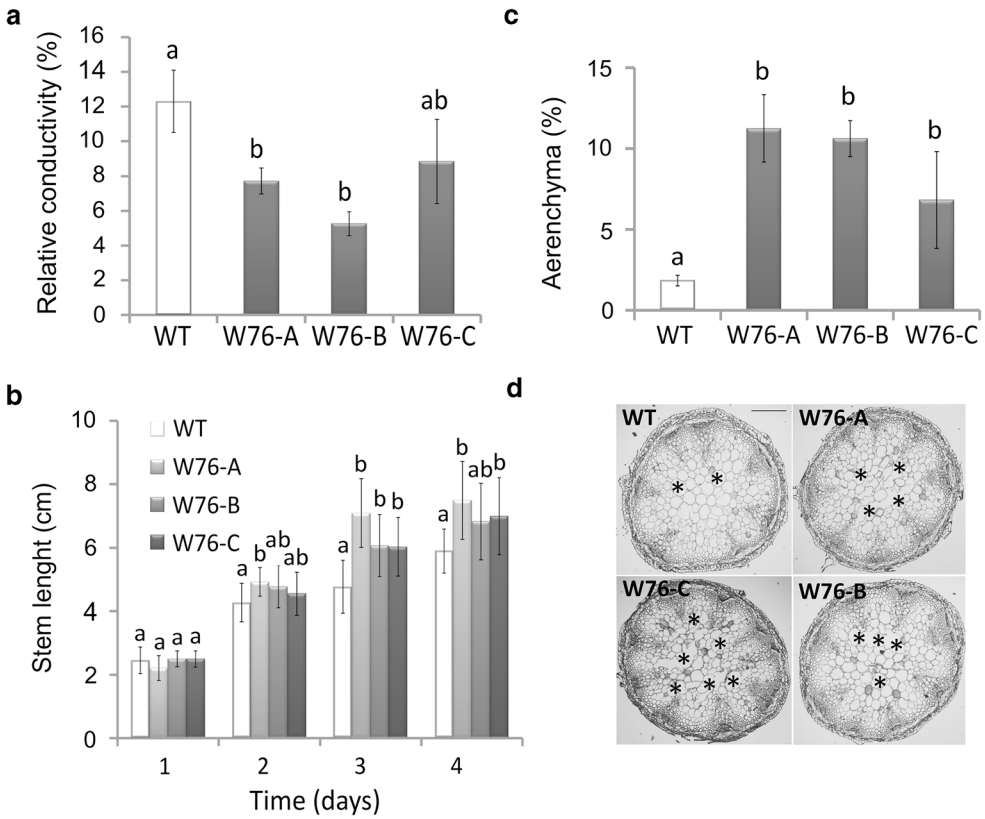

Fig. 8 Waterlogging and partial submergence differentially affect transgenic and WT stems and leaves: **a** Conductivity of waterlogging-stressed leaves (25-day-old plants) related to their total conductivity, **b** stem length measured during 4 days of partial submergence, **c** percentage of stem aerenchyma areas expressed as % of the whole section area, and **d** stem transversal sections of WT and transgenic (W76-A, W76-B, W76-C) plants after partial submergence. In **d**, *asterisks* indicate lysigenous aerenchyma areas and the *scale bar* represents 200 μm. Two independent experiments were done and *error bars* correspond to standard deviations from three biological replicas. ANOVA test was conducted in all the cases, followed by a Fisher LSD post-hoc test. *Different letters* samples with significant differences ($P < 0.05$)

HaWRKY76 also diverges from the WRKY II d clade, exhibiting a WKKY domain instead of the canonical WRKY (Giacomelli et al. 2010). In this work, it is shown that this divergent TF is regulated by both deficit and excess of water. This regulation was the first clue leading to investigate the role of *HaWRKY76* in water stress responses.

Plants transformed with HaWRKY76 exhibited an overall better performance than controls in both standard growth conditions or subjected to water imbalance. In non-stressful conditions, transgenic plants exhibited larger shoot and root biomass, longer roots and higher yield. Subjected to water stress, they also presented higher yield than WT, although variable depending on the kind of stress and the developmental stage in which the stress was suffered. In this sense, growth of drought-stressed plants slowed early and, as a consequence, shoots size, branching and yield diminished drastically in all the genotypes. Flood was less stressful than drought although the water depth differentially affected the plants' performance, being submergence a more harmful treatment than waterlogging. Completely covered plants arrested growth and carbon sources consumption and, during the recovery phase, part of the resources went to produce new stems replacing the dead ones. Conversely, waterlogged plants mainly sustained their aerial structure with better consequences in yield than the submerged plants. On the other hand, the phenotype impacts of the transgene were chiefly related to its relative expression level, except by the seed production. In that case, the performances of the highest and lowest expression lineages were similar and closer to the WT than the intermediate expression lineage was. Maybe, the transgene expression level has a lower impact in such more complex phenotype than in simpler ones.

How HaWRKY76 transgenic plants adapt themselves better than controls to so different stressing, even opposite, factors without yield penalty? Figure 9 schematizes the physiological and molecular changes tested, tempting to explain such performance.

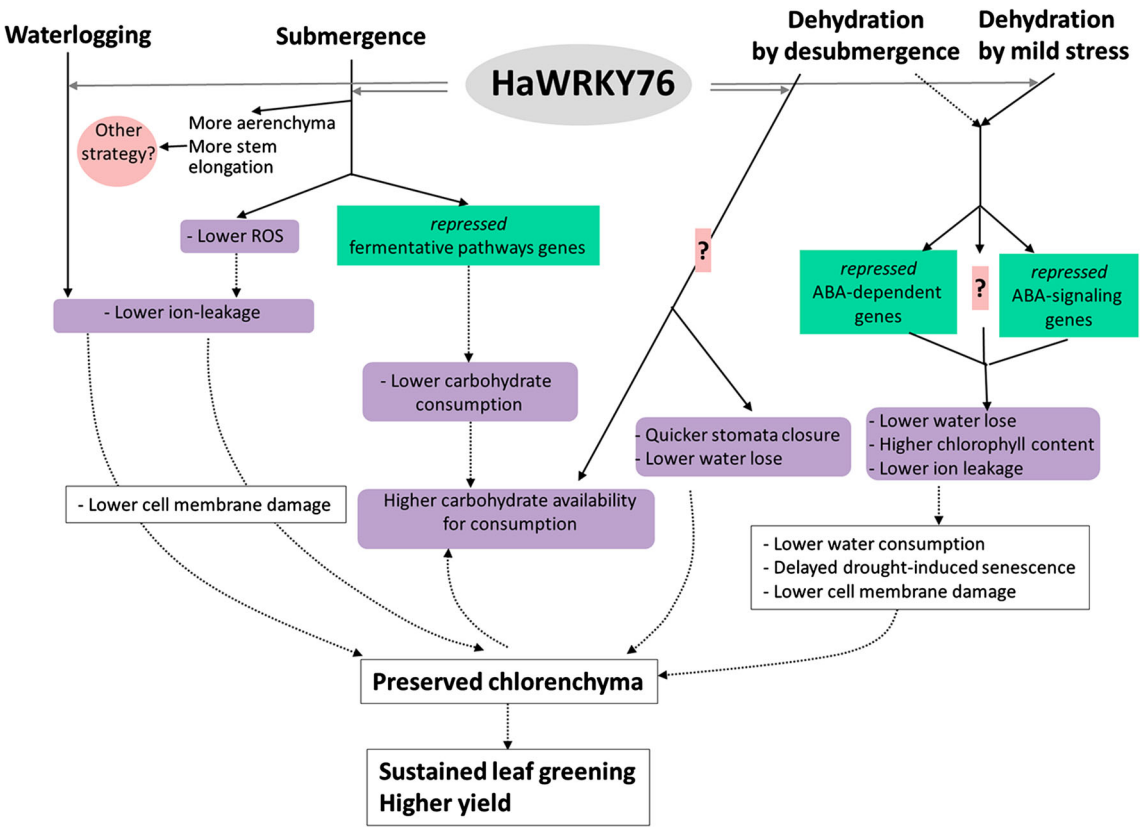

Fig. 9 Proposed model for HaWRKY76 function: *Gray arrows* direct or indirect HaWRKY76 action. *Black* wide and continuous *arrows* indicate biochemical or physiological changes registered in Arabidopsis plants expressing *HaWRKY76*. *Dotted arrows* probable consequences of these changes. Quotation marks (?) suggest unknown factor/s that may regulate positive downstream responses

Under water deficit, transgenic plants exhibited a faster stomata closure, higher chlorophyll concentration and less membrane detriment than their controls, whereas under water excess they had more sucrose at the end of the illuminated period to be consumed during the night. These higher sucrose levels would be the result of the already higher sucrose content observed in the transgenics in standard conditions followed by a roughly equal consumption during the stress period in both genotypes. At the same time, starch is less consumed and chlorenchyma is preserved through reducing ROS and increasing membrane stability, which could contribute to sustain high sucrose levels even with higher consumption. The maintenance of the photosynthetic tissue integrity seems to be the main contact point between both responses, to water deficit and to water excess.

The mechanisms by which HaWRKY76 confers enhanced tolerance to drought and submergence seem to be different from other well described in the literature. In this sense, a series of genes that became markers of drought, osmotic stress and/or induced by ABA (de Bruxelles et al. 1996; Shinozaki and Yamaguchi-Shinozaki 2000; Xiong et al. 1999) were down-regulated or did not present transcriptional changes at all in *HaWRKY76* transgenic plants. Moreover, after ABA treatments, transcript levels of these genes were induced in WT and transgenic plants at similar extents. In the same sense, other authors also reported complex scenarios regarding these genes. *ABI1* and *ABI2* as well as *ABI5*, considered as negative and positive signal regulators, respectively, are repressed, induced or unchanged in different plants described as drought-tolerant or drought-sensitive (Jung et al. 2008; Kim et al. 2014; Luo et al. 2013). The diverse treatments applied by different research groups varying in the levels of water restriction and plant developmental stages also make complicated the comparisons and analyses. On the other hand, the crosstalk between adaptive responses may be triggering alternative signaling ways that differentially modulate gene expression. In this sense, it is known that sucrose availability and partitioning regulate stomata aperture, affecting drought tolerance and yield (Albacete et al. 2014), which could be playing a complex crosstalk with ABA signaling. Indeed, during desubmergence, when dehydration also occurs, sucrose levels increased but in all the genotypes. These high sucrose levels would have their origin in the overall scarce photosynthesis, altered by the

previous stress. Although chlorenchyma would be preserved in transgenic plants, these plants would be more affected at the beginning of desubmergence by the prevalence of the mechanisms against dehydration as acceleration of stomata closure (Fig. S7). Altogether, the results support the hypothesis of a quiescence-like strategy triggered by HaWRKY76 transgenic plants to deal with submergence stress.

It is well documented in the literature that hypoxia induces the early expression of genes involved in alcohol fermentation and sucrose cleavage such as pyruvate decarboxylase (*PDC1*), alcohol dehydrogenase (*ADH*) and sucrose synthase (*SUS1*) encoding genes (Johnson et al. 1994; Komatsu et al. 2011; Loreti et al. 2005; Sachs et al. 1980). The induction of *PDC1*, *PDC2, ADH, SUS1, SUS4* and other genes of the anoxic transcriptome was evident up to the first 24 h and, after that, a huge declination was reported (Koch et al. 2000; Loreti et al. 2005). Notably, in *HaWRKY76* transgenic plants, the expression of these genes was repressed or unchanged after 3 days of treatment fitting with a late hypoxia scenario.

In anoxia conditions, sucrose produces fluctuations in gene expression profiles. In this sense, it was reported that, *SUS1* and *SUS4* transcripts showed lower induction by anoxia after the addition of exogenous sucrose, even at the very beginning of the treatment (Loreti et al. 2005), whereas in seedlings grown in non-stressful conditions, neither *SUS1* nor *SUS4* expression was regulated by this carbohydrate (Baud et al. 2004). These reports suggested that the regulation of both genes might not be related to anoxia but to the sugar starvation that is a consequence of anoxia (Loreti et al. 2005). The higher sucrose level displayed in *HaWRKY76* transgenic plants could explain why *PDC1*, *ADH* and *SUS1* were repressed or unchanged in these plants and conditions. After few days of submergence, sucrose levels in *HaWRKY76* transgenic plants declined, but were still higher than in WT, concomitantly with the repression of *SUS1*. Regarding *PDC1* and *ADH*, it was reported that in the presence of exogenous sucrose, the expression of these genes continued to be induced after 24 h anoxia and after that remained higher than in standard conditions (Loreti et al. 2005). In *HaWRKY76* transgenic plants, these genes were repressed or unchanged, which can be explained if residual PDC1 and ADH activities in *HaWRKY76* transgenic plants were enough to a minimum of carbon flow through an alternative fermentative pathway. This hypothesis has support in two reports: Ismond et al. (2003) showed that knock-down *pdc1* mutants were phenotypically similar to WT when subjected to hypoxia and Ellis et al. (1999) informed that *adh* null mutants did not exhibit anoxia tolerance in roots but only in shoots (Ellis et al. 1999). Moreover, ROS (hydrogen peroxide) were necessary for the induction of *ADH* expression in Arabidopsis (Baxter-Burrell et al. 2002) and in *HaWRKY76* leaves, the production of ROS (superoxide) was lower than in WT, in accordance with lower *ADH* levels. Finally, the expression of *ATT1*, the gene encoding the first enzyme in the alanine fermentation pathway, was slightly increased in two of three *HaWRKY76* lines, which could indicate low alanine production.

HaWRKY76 transgenic plants also showed tolerance to waterlogging and partial submergence. Moreover, transgenic plants that suffered waterlogging stress rendered higher yield than WT. The physiological mechanisms explaining such performance were the higher membrane stability in leaves and the increase in lysigenous aerenchyma observed in stems. The increase in aerenchyma would contribute to distribute the oxygen from an advantageous source, the stems, to a depleted sink, the roots (Armstrong 1979; Manzur et al. 2009; Nishiuchi et al. 2012). These plants also elongated their stems significantly more than WT after partial submergence, a typical trait of the escape tolerance strategy, which suggests a large versatility in the adaptive responses depending on the environmental conditions.

To the best of our knowledge there are two genes reported until now as able to confer tolerance to both drought and flood, without plant growth or yield detriment. Both are TFs encoding genes: *SUB1A,* a rice ERF transcription factor (Fukao et al. 2011) and *HaHB11,* a sunflower HD-Zip type I TF (Chan et al. 2013). Both genes *SUB1A* and *HaHB11* display the quiescent strategy to confer flood tolerance, as it is proposed here for HaWRKY76.

In light of the findings described here, it is sounded to propose HaWRKY76 as a potential biotechnological tool to improve tolerance of crops to abiotic stresses, and to suggest that the physiological and molecular mechanisms triggered by this transcription factor to confer drought and flooding tolerance do not fit completely with the previously described.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana Heynh. ecotype Columbia (Col-0) as well as the transgenic lines (W76-A, W76-B and W76-C) were grown on soil at 22–24 °C under long-day photoperiods at an irradiance of approximately 150 μE m^{-2} s^{-1} in 8 cm diameter × 7 cm height pots, in a growth chamber. For root phenotype assays, seeds were also sown on Petri dishes with Murashige and Skoog medium, 0.8 % agar, or directly on sand in 15 cm diameter x 25 cm height pots, one to three plants per pot depending on the experiment

and as indicated in each figure. Seeds in Petri dishes were placed at 4 °C for 2 days and then transferred to the growth chamber.

Helianthus annuus L. (sunflower CF31, Advanta) seeds were grown on soil in 8 cm diameter × 7 cm height pots, one plant per pot, and placed in the same growth chamber

All the pots were well irrigated with 0.5 × Hoagland solution except those subjected to stress treatments. At the periods indicated in the Figures, organ fragments were sampled and frozen with liquid nitrogen until evaluation.

Morphological and yield assessments

In plants grown on agar, root length was measured with a ruler. In 35-day-old plants grown on sand, root diameter was measured with a micrometer gauge at the hypocotyl boundary. In 35-day-old-plants grown in sand or in soil, the complete root system and shoot were collected and weighted. Seeds from plants grown on soil in standard or stress conditions were harvested at the end of the life cycle.

Cloning and plant transformation

The coding sequence of *HaWRKY76* (Accession Number HaT13l007971; http://www.heliagene.org) was amplified (5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 58 °C and 2 min at 72 °C in a MJ Research apparatus) using total RNA extracted from leaves as template and HaW76_F and HaW76_R oligonucleotides (Table S1). The amplification product was cloned in the pBI121 plasmid (Jefferson et al. 1987) under the control of the *35S CaMV* promoter. *Agrobacterium tumefaciens* strain LBA4404 was transformed with this construct and used to obtain transgenic Arabidopsis plants by the floral dip procedure (Clough and Bent 1998). Ten positive independent lines were used to select homozygous plants and three of them (T3 homozygous lines) presenting different transgene expression levels were chosen for further analysis. WT plants were used as negative controls.

RNA isolation and RT-qPCR

RNA was isolated using Trizol® reagent following the manufacturer' associated protocol (Invitrogen™). RNA was quantified using a NanoPhotometer ™ (Implen) and its purity was checked through absorbance ratios (A260/280>1.8; A260/230>2.0). DNA contamination was assessed by PCR using the gene reference primers (Table S1). Reverse transcription was done using 1 µg RNA and M-MLV reverse transcriptase (Promega™) according to manufacturer's instructions. Quantitative PCR was basically carried out as previously described by Giacomelli et al. (Giacomelli et al. 2010) using specific designed oligonucleotides (Table S1). The PCR cycle was applied 40 times as follows: 95 °C for 16 s, 60 °C for 16 s and 72 °C for 16 s, with a previous denaturation step at 95 °C for 2 min. RT samples without template and N° RT samples were used as negative controls for each pair of primers. Amplicons sizes were not longer than 216 bp. Expression levels for all the genes were quantified and normalized by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001), checking the melting profiles to discharge primer dimers. *ACTIN2* and *ACTIN8* were used as internal controls.

Stress tolerance assays

Severe hydric stress was applied to sunflower seedlings (5-day-old) and plants in the 4-leaf vegetative stage, named plantlets (15-day-old). Seedlings were exposed to a rapid desiccation on a filter paper during 30 min and watering was completely stopped during 15 days for plantlets.

Mild drought stress was applied to Arabidopsis plants in the vegetative (starting when seedlings were 2 days old) and the reproductive stage (starting when plants were 25 days old). For the stress during the vegetative stage, seeds were sown in soil saturated with 0.5 × Hoagland solution and thereafter irrigated with 2 ml every 2 days up to the reproductive stage. Then, irrigation was increased to 5–7 ml every day until the end of the life cycle. For the stress during the reproductive stage, irrigation was stopped during 3 days and thereafter a variable volume of solution was added every 3 days, enough to achieve 70 % of the water-saturated pot weight.

Submergence experiments were conducted on 15-day-old sunflower plantlets and 25-day-old Arabidopsis plants. Distinct periods of submergence were applied; sunflower plantlets were subjected to 11 days of submergence (water level at several cm above the ground) followed by one hour of desubmergence, while Arabidopsis plants were subjected to 5 days of treatment followed by 1 day of desubmergence.

Waterlogging and partial submergence stress assays were carried out with 15-day-old Arabidopsis plants, leading the level of water up to a maximum of 5 mm above the ground (tolerance and yield assays), or 1 cm above the rosette leaves (anatomical studies), or 2–4 cm below the basal portion of the main inflorescence during all of the assay (stem elongation assays).

ABA treatments

Six-day-old *HaWRKY76* transgenic (W76-A) and WT plantlets were germinated and grown on a mesh with Murashige and Skoog 0.5 × medium, 0.8 % agar. Thereafter, the mesh with the plantlets was transferred to a new plate with the same medium supplemented with ABA (0,

0.5, 1 or 2 μM) for 24 h. The plants were frozen in liquid nitrogen before RNA extraction and analysis.

Leaf water loss and plant water consumption assays

Detached leaves from non-stressed and stressed 30-day-old Arabidopsis plants (5 days after the beginning of the stress treatment) were placed in a controlled environment (22–24 °C, around 150 μE m^{-2} s^{-1}) and their weights were taken at the times indicated in the corresponding Figure. Pot weight loss from mild stressed plants was registered as water consumption.

Cell membrane stability assay

The ion leakage technique was carried out basically as described by Sukumaran and Weiser (1972) and adapted by Cabello and Chan (2012). Leaves were detached at three different times after the beginning of the drought treatment and used as samples for the indicated procedure.

Chlorophyll content calculation

Chlorophyll was essentially quantified following the method described by Chory et al. (1994). Leaves of well-watered and stressed Arabidopsis plants were detached at two times as indicated in the corresponding Figure.

Carbohydrate quantification

Glucose, sucrose and starch were quantified in 25-day-old Arabidopsis plants before and after 5 days of submergence. Around 50–80 mg of rosette leaves was pulverized in a mortar with a pestle and liquid nitrogen, and then mixed with 700 μl of homogenizing buffer (5,4 mM sodium phosphate pH 7.5, 62.5 % methanol, 26.8 % chloroform, 0.1 mM EDTA). After 30 min in ice, the samples were mixed with 300 μl of double distilled water, vigorously agitated and centrifuged during 5 min at 4 °C and 15,000 *g*. After that, the upper phase (total soluble sugars content) was evaporated to dryness at 37 °C, and the sediment was resuspended in 100 μl of double distilled water and used for glucose and sucrose determinations (sample 1). The interphase (starch content) was washed with absolute ethanol, dried at 70 °C during 1 h and resuspended in 250 μl of 0.1 N of sodium hydroxide; the resuspension was neutralized with 75 μl of 0.5 N of acetic acid, pH 5.1 and used for starch determination (sample 2). The lower phase was discarded.

Glucose was quantified for both, glucose and sucrose quantification, with a colorimetric enzymatic assay (SB®, Santa Fe, Argentina) based on Trinder's glucose oxidase method (Trinder 1969). A half of the sample 1 was used for determination of glucose concentration as μg glucose/mg leaf sample (Gluc1). The other half was incubated with 71 U of invertase (Sigma-Aldrich) during 1 h at 37 °C and also used for determination of glucose content that includes soluble glucose from the sample plus soluble glucose from sucrose hydrolysis (Gluc2). Sucrose concentration was then determined as Gluc2–Gluc1.

A total of 50 μl of sample 3 was incubated overnight at 37 °C with 2.5 U of amyloglucosidase (Roche) that completely hydrolyzes the starch, releasing glucose. Glucose was quantified as described above and considered as starch concentration.

ROS assay

Superoxide production in Arabidopsis leaves after a desubmergence treatment (31-day-old) was determined using the inhibition of the nitroblue tetrazolium (NBT) reduction staining method. Detached leaves were immediately vacuum-infiltrated during 15 min with 0.1 mg ml^{-1} NBT (Sigma-Aldrich) in a buffer containing 25 mM Hepes pH 7.6 and 0.05 % Tritón X-100. Thereafter, samples were incubated during 1 h at 37 °C in the dark and boiled in 80 % ethanol for 10 min to remove pigments. Finally, the stained leaves were scanned and densitometry was calculated using ImageJ software (Schneider et al. 2012).

Anatomical studies

Arabidopsis plants subjected to partial submergence were investigated for aerenchyma formation. Segments of stems (0.5 cm from the first internode) were fixed 48 h in 3.7 % formaldehyde, 5 % acetic acid, 47.5 % ethanol at room temperature, dehydrated through ethanol series, and embedded in Histoplast (Biopack™). Sections of 10 μm thick were mounted on slides coated with 50 mg/ml poly-D-Lys (Sigma™) in 10 mm Tris–HCl, pH 8.0, and dried during 7 days at 37 °C. After removing the paraffin with xylene, sections were rehydrated by ethanol series, stained with 0.1 % toluidine blue and mounted on synthetic Canadian balsam (Biopack™). The sections were photographed.

Stem elongation assay

Arabidopsis plants subjected to partial submergence were evaluated for stem elongation during the treatment. Lengths of the stems were registered daily for the periods indicated in the corresponding Figure.

Stomata measurements. Four leaves from different transgenic and WT 31-old-day plants (25-day-old plants after 5 days of submergence plus 1 day of desubmergence) were collected. Peeling was practiced with a razor blade;

abaxial epidermis was glued over a glass slide and observed with a microscope. Pictures of at least five different areas per leaf were taken and representative stomata are shown. Around 40–60 stomata for each genotype were evaluated.

Statistical analysis

To compare the effects of the applied treatments one-way Analysis of variance (ANOVA) was done, followed by a Fisher LSD post-hoc test. Previously, normality and homogeneity of variances were checked by a Shapiro–Wilk's test and a Bartlett's test, respectively.

Relative expression levels of *PDC1*, *ADH* and *SUS1*. Transcript levels were quantified by RT-qPCR, normalized with housekeeping transcript levels of *ACT2/8*, and thereafter with respect to the higher expression line, arbitrarily assigned a value of one. Two independent experiments were done and error bars correspond to standard deviations from three biological replicas in each experiment. ANOVA test was conducted in all the cases, followed by a Fisher LSD post-hoc test. Different letters indicate samples with significant differences ($P < 0.05$). Accession numbers of the evaluated genes are listed in Table S1.

Author contribution statement JR performed the experimental assays and contributed to the Figures design. KFR performed experimental assays and participated in the data analysis as well as in the MS writing. RLC conceived this study, coordinated the experiments and drafted the MS. All the authors read and approved the MS.

Acknowledgments This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT-PAE 37100 and PICT 2011 850), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2011 11420100100278) and Universidad Nacional del Litoral (UNL, CAID 2011 50120110100399 and 50120110100349). KFR and RLC are members of CONICET and UNL; JR is a Fellow of CONICET and member of UNL. We thank Dr. Julieta Cabello and Dr. Gabriel Céccoli (IAL–CONICET UNL) for their valorous technical advice.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Albacete AA, Martinez-Andujar C, Perez-Alfocea F (2014) Hormonal and metabolic regulation of source-sink relations under salinity and drought: from plant survival to crop yield stability. Biotechnol Adv 32:12–30

Arce AL, Raineri J, Capella M, Cabello JV, Chan RL (2011) Uncharacterized conserved motifs outside the HD-Zip domain in HD-Zip subfamily I transcription factors; a potential source of functional diversity. BMC Plant Biol 11:42

Armstrong W (1979) Aeration in higher plants. In: Woolhouse HWW (ed) Advances in botanical research, vol 7. Academic Press, USA, pp 225–332

Bailey-Serres J, Voesenek L (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol 59:313–339

Baud S, Vaultier MN, Rochat C (2004) Structure and expression profile of the sucrose synthase multigene family in Arabidopsis. J Exp Bot 55:397–409

Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J (2002) RopGAP4-dependent Rop GTPase rheostat control of Arabidopsis oxygen deprivation tolerance. Science 296:2026–2028

Blokhina O, Fagerstedt KV (2010) Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. Physiol Plant 138:447–462

Cabello JV, Chan RL (2012) The homologous homeodomain-leucine zipper transcription factors HaHB1 and AtHB13 confer tolerance to drought and salinity stresses via the induction of proteins that stabilize membranes. Plant Biotechnol J 10:815–825

Chan RL, Gonzalez DH, Dezar CA, Gago GM (2010) Transcription Factor Gene Induced By Water Deficit Conditions And Abscisic Acid From Helianthus Annuus, Promoter And Transgenic Plants. Patent WO2004/099365

Chan RL, Cabello JV, Giacomelli JI (2013) HaHB11 provides improved plant yield and tolerance to abiotic stress. Patent WO2013116750 (A1)

Chen Q, Liu Z, Wang B, Wang X, Lai J, Tian F (2015) Transcriptome sequencing reveals the roles of transcription factors in modulating genotype by nitrogen interaction in maize. Plant Cell Rep. doi:10.1007/s00299-015-1822-9

Chory J, Reinecke D, Sim S, Washburn T, Brenner M (1994) A role for cytokinins in de-etiolation in Arabidopsis (det mutants have an altered response to cytokinins). Plant Physiol 104:339–347

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16:735–743

de Bruxelles GL, Peacock WJ, Dennis ES, Dolferus R (1996) Abscisic acid induces the alcohol dehydrogenase gene in Arabidopsis. Plant Physiol 111:381–391

Ding ZJ, Yan JY, Xu XY, Yu DQ, Li GX, Zhang SQ, Zheng SJ (2014) Transcription factor WRKY46 regulates osmotic stress responses and stomatal movement independently in Arabidopsis. Plant J 79:13–27

Ellis MH, Dennis ES, Peacock WJ (1999) Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. Plant Physiol 119:57–64

FAO (2012) Sunflower: a ray of hope for flood-affected farmers in Sindh. Accessed December 2014 http://www.fao.org/emergencies/fao-in-action/stories/stories-detail/en/c/152907/

Fukao T, Xiong L (2013) Genetic mechanisms conferring adaptation to submergence and drought in rice: simple or complex? Curr Opin Plant Biol 16:196–204

Fukao T, Yeung E, Bailey-Serres J (2011) The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell 23:412–427

Giacomelli JI, Ribichich KF, Dezar CA, Chan RL (2010) Expression analyses indicate the involvement of sunflower WRKY transcription factors in stress responses, and phylogenetic reconstructions reveal the existence of a novel clade in the Asteraceae. Plant Sci 178:398–410

Giacomelli JI, Weigel D, Chan RL, Manavella PA (2012) Role of recently evolved miRNA regulation of sunflower HaWRKY6 in response to temperature damage. New Phytol 195:766–773

Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J,

Holdsworth MJ (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature 479:415–418

Gong X, Zhang J, Hu J, Wang W, Wu H, Zhang Q, Liu JH (2015) FcWRKY70, a WRKY protein of Fortunella crassifolia, functions in drought tolerance and modulates putrescine synthesis by regulating arginine decarboxylase gene. Plant Cell Environ. doi:10.1111/pce.12539

Hattori Y, Nagai K, Ashikari M (2011) Rice growth adapting to deepwater. Curr Opin Plant Biol 14:100–105

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052

Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG (2003) Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. Plant Physiol 132:1292–1302

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Johnson JR, Cobb BG, Drew MC (1994) Hypoxic Induction of Anoxia Tolerance in Roots of Adh1 Null Zea mays L. Plant Physiol 105:61–67

Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, Nahm BH, Choi YD, Cheong JJ (2008) Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol 146:623–635

Katinas L, Gutiérrez D, Grossi MA, Crisci JV (2007) Panorama de la familia Asteraceae (Compositae) en la Republica Argentina. Bol Soc Argent Bot 42:113–129

Kerstiens G (1996) Cuticular water permeability and its physiological significance. J Exp Bot 47:1813–1832

Kim EY, Seo YS, Park KY, Kim SJ, Kim WT (2014) Overexpression of CaDSR6 increases tolerance to drought and salt stresses in transgenic Arabidopsis plants. Gene 552:146–154

Koch KE, Ying Z, Wu Y, Avigne WT (2000) Multiple paths of sugar-sensing and a sugar/oxygen overlap for genes of sucrose and ethanol metabolism. J Exp Bot 51:417–427

Komatsu S, Deschamps T, Hiraga S, Kato M, Chiba M, Hashiguchi A, Tougou M, Shimamura S, Yasue H (2011) Characterization of a novel flooding stress-responsive alcohol dehydrogenase expressed in soybean roots. Plant Mol Biol 77:309–322

Lakshmanan M, Zhang Z, Mohanty B, Kwon JY, Choi HY, Nam HJ, Kim DI, Lee DY (2013) Elucidating rice cell metabolism under flooding and drought stresses using flux-based modeling and analysis. Plant Physiol 162:2140–2150

Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LA, Perata P, van Dongen JT (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature 479:419–422

Liu H, Yang W, Liu D, Han Y, Zhang A, Li S (2011) Ectopic expression of a grapevine transcription factor VvWRKY11 contributes to osmotic stress tolerance in Arabidopsis. Mol Biol Rep 38:417–427

Liu QL, Zhong M, Li S, Pan YZ, Jiang BB, Jia Y, Zhang HQ (2013) Overexpression of a chrysanthemum transcription factor gene, DgWRKY3, in tobacco enhances tolerance to salt stress. Plant Physiol Biochem 69:27–33

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25:402–408

Loreti E, Poggi A, Novi G, Alpi A, Perata P (2005) A genome-wide analysis of the effects of sucrose on gene expression in Arabidopsis seedlings under anoxia. Plant Physiol 137:1130–1138

Luo X, Bai X, Sun X, Zhu D, Liu B, Ji W, Cai H, Cao L, Wu J, Hu M, Liu X, Tang L, Zhu Y (2013) Expression of wild soybean WRKY20 in Arabidopsis enhances drought tolerance and regulates ABA signalling. J Exp Bot 64:2155–2169

Ma D, Pu G, Lei C, Ma L, Wang H, Guo Y, Chen J, Du Z, Wang H, Li G, Ye H, Liu B (2009) Isolation and characterization of AaWRKY1, an Artemisia annua transcription factor that regulates the amorpha-4,11-diene synthase gene, a key gene of artemisinin biosynthesis. Plant Cell Physiol 50:2146–2161

Manzur ME, Grimoldi AA, Insausti P, Striker GG (2009) Escape from water or remain quiescent? Lotus tenuis changes its strategy depending on depth of submergence. Ann Bot 104:1163–1169

Nishiuchi S, Yamauchi T, Takahashi H, Kotula L, Nakazono M (2012) Mechanisms for coping with submergence and waterlogging in rice. Rice 5:1–14

O'Brien D, Belshe D, Meyer R, Falk J, Olson B (2009) High Plains Sunflower Production Handbook. K-State Research and Extension Publication, MF-2384

Okay S, Derelli E, Unver T (2014) Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. Mol Genet Genomics 289:765–781

Panero JL, Funk VA (2008) The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Mol Phylogenet Evol 47:757–782

Quartacci MF, Navari-Izzo F (1992) Water stress and free radical mediated changes in sunflower seedlings. J Plant Physiol 139:621–625

Raineri J, Wang S, Peleg Z, Blumwald E, Chan RL (2015) The rice transcription factor OsWRKY47 is a positive regulator of the response to water deficit stress. Plant Mol Biol 88:401–413. doi:10.1007/s11103-015-0329-7

Rengel D, Arribat S, Maury P, Martin-Magniette ML, Hourlier T, Laporte M, Varès D, Carrère S, Grieu P, Balzergue S, Gouzy J, Vincourt P, Langlade NB (2012) A gene-phenotype network based on genetic variability for drought responses reveals key physiological processes in controlled and natural environments. PLoS One 7:e45249

Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci USA 104:19631–19636

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258

Rushton DL, Tripathi P, Rabara RC, Lin J, Ringler P, Boken AK, Langum TJ, Smidt L, Boomsma DD, Emme NJ, Chen X, Finer JJ, Shen QJ, Rushton PJ (2012) WRKY transcription factors: key components in abscisic acid signalling. Plant Biotechnol J 10:2–11

Sachs MM, Freeling M, Okimoto R (1980) The anaerobic proteins of maize. Cell 20:761–767

Sasidharan R, Mustroph A, Boonman A, Akman M, Ammerlaan AM, Breit T, Schranz ME, Voesenek LA, van Tienderen PH (2013) Root transcript profiling of two Rorippa species reveals gene clusters associated with extreme submergence tolerance. Plant Physiol 163:1277–1292

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Setter TL, Bhekasut P, Greenway H (2010) Desiccation of leaves after de-submergence is one cause for intolerance to complete submergence of the rice cultivar IR 42. Funct Plant Biol 37:1096–1104

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Skirycz A, Vandenbroucke K, Clauw P, Maleux K, De Meyer B, Dhondt S, Pucci A, Gonzalez N, Hoeberichts F, Tognetti VB, Galbiati M, Tonelli C, Van Breusegem F, Vuylsteke M, Inzé D (2011) Survival and growth of Arabidopsis plants given limited water are not equal. Nat Biotechnol 29:212–214

Sukumaran N, Weiser C (1972) Freezing injury in potato leaves. Plant Physiol 50:564–567

Sun Y, Yu D (2015) Activated expression of AtWRKY53 negatively regulates drought tolerance by mediating stomatal movement. Plant Cell Rep 34:1295–1306 doi:10.1007/s00299-015-1787-8

Timme RE, Simpson BB, Randal Linder C (2007) High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. Am J Bot 94:1837–1852

Trinder P (1969) Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol 22:246

Tripathi P, Rabara RC, Rushton PJ (2014) A systems biology perspective on the role of WRKY transcription factors in drought responses in plants. Planta 239:255–266

Vanderauwera S, Vandenbroucke K, Inzé A, van de Cotte B, Mühlenbock P, De Rycke R, Naouar N, Van Gaever T, Van Montagu MC, Van Breusegem F (2012) AtWRKY15 perturbation abolishes the mitochondrial stress response that steers osmotic stress tolerance in Arabidopsis. Proc Natl Acad Sci USA 109:20113–20118

Voesenek LA, Bailey-Serres J (2013) Flooding tolerance: O_2 sensing and survival strategies. Curr Opin Plant Biol 16:647–653

Xiong L, Ishitani M, Lee H, Zhu JK (1999) HOS5-a negative regulator of osmotic stress-induced gene expression in Arabidopsis thaliana. Plant J 19:569–578

Yan H, Jia H, Chen X, Hao L, An H, Guo X (2014) The cotton WRKY transcription factor GhWRKY17 functions in drought and salt stress in transgenic Nicotiana benthamiana through ABA signaling and the modulation of reactive oxygen species production. Plant Cell Physiol 55:2060–2076