Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco

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Stress perception and signal transduction leading to tolerance involve a complex interplay of different gene products. We describe here the isolation and characterization of an intronless gene (*OSISAP1*) from rice encoding a zinc-finger protein that is induced after different types of stresses, namely cold, desiccation, salt, submergence, and heavy metals as well as injury. The gene is also induced by stress hormone abscisic acid. Overexpression of the gene in transgenic tobacco conferred tolerance to cold, dehydration, and salt stress at the seed-germination/seedling stage as reflected by the percentage of germination/green seedlings, the fresh weight of seedlings, and their developmental pattern. Thus, *OSISAP1* seems to be an important determinant of stress response in plants.

The development and survival of plants is constantly challenged by changes in environmental conditions. To tide over these adversities, plants elicit complex physiological and molecular responses. Stress is perceived and transduced through a chain of signaling molecules that ultimately affect regulatory elements of stress-inducible genes to initiate the synthesis of different classes of proteins including transcription factors, enzymes, molecular chaperons, ion channels, and transporters or alter their activities. Such cascading events controlled by a battery of genes and their intricate regulation help the system to overcome the unfavorable conditions. According to some estimates, plants possess somewhere between 25,000 and 55,000 genes (1–6). Many of these are “housekeeping” genes that are expressed in all of the tissues, whereas others are organ-specific or regulated by environmental cues. To understand the process of development of plants and their response to environmental stresses, it is imperative to know the function of crucial genes and their regulation during different phases of the life cycle (7, 8). Conventional mutation genetics and cloning of the corresponding genes as well as new approaches such as differential screening, subtractive hybridization, differential display, and microarray analysis, along with reverse genetics, have been used to clone such genes and define their function (9–11).

Rice is the most important food crop as well as a model monocot system (12–14). However, the production of rice should increase by 60% in the next 25 years to keep pace with the growing world population. Minimization of the loss caused by biotic and abiotic environmental factors can not only help improve net production but extend rice cultivation in marginal and noncultivable lands (9, 15). Functional genomics in rice is thus an important area of research whereby the function of new genes involved in plant development and survival is defined. Discovery of genes involved in environmental stress responses provides new targets for genetic engineering of rice and other crops for better tolerance/resistance. In our laboratory, we are interested in isolating previously uncharacterized genes of rice and defining their functions. Different screening strategies were used to isolate genes from elite *indica* rice (*Oryza sativa* L. var. Pusa Basmati-1) that are expressed in an organ-specific manner or are induced by stress. In this communication, we describe the characteristics of a gene encoding a zinc-finger protein that is

induced by several stresses. Overexpression of the gene, *OSISAP1* (*O. sativa* subspecies *indica* stress-associated protein gene), in tobacco leads to stress tolerance as assessed at the seedling stage.

Materials and Methods

Plant Materials and Treatments. Rice (*O. sativa* subsp. *indica* var. Pusa Basmati-1) seeds were treated and grown as described (16). After 7 days of growth, the seedlings were transferred to 100-ml beakers containing cotton soaked in water, and for treatments, water containing the desired solute was used. Sodium chloride was added at a final concentration of 200 mM. Abscisic acid (ABA; Sigma) was dissolved in DMSO to make a stock of 10 mM and was diluted further in water. Injury to the seedlings was inflicted by clipping the leaf margins at ≈ 1 -cm intervals. For cold stress, the seedlings were maintained at $8 \pm 1^\circ\text{C}$. Desiccation was simulated by drying the plants on tissue paper and keeping them wrapped in dry tissue paper for the desired time. Seedlings growing in a 100-ml beaker were kept submerged under water in a 2-liter glass beaker. Cut leaves of 9-day-old seedlings were treated with 5, 10, and 15 mM benzyl alcohol at 25°C for 3 h, and then the treatment was continued at $8 \pm 1^\circ\text{C}$ for 48 h (17). For control, cut leaves were kept in water at 25°C for 3 h and then at $8 \pm 1^\circ\text{C}$ for 48 h. For treatment with DMSO, cut leaves of seedlings were kept at 25°C for 6 h in the presence of 2%, 4%, and 6% DMSO, and control was kept at 25°C for 6 h in water.

Cloning of *OSISAP1*. A cDNA library from roots of 7-day-old rice seedlings was prepared in Lambda ZAP Express vector (Stratagene). A large number of random clones were picked from the library, and single-clone excision was performed to obtain recombinant pBK-CMV phagemid vector per manufacturer instructions. These clones were used as radiolabeled probes and hybridized to total RNA isolated from different parts of the rice plant. Such differential screening revealed clones that were expressing at variable levels in different parts of the rice plant. The transcript for *OSISAP1* was detected at a much higher level in the root and prepollination stage spikelet, as compared with shoot, and hence was used for differential screening during stress. Analysis of the expression profile during stress revealed that *OSISAP1* transcript was induced in response to several stresses. To isolate the genomic clone of *OSISAP1*, a rice genomic DNA library was prepared in λ DASH II vector (Stratagene) by using *Mbo*I-digested genomic DNA. The cDNA was used to generate a radiolabeled probe and screen the library (18). Positive plaques were purified by three rounds of screening, and a 5.5-kb subcloned fragment (from a recombinant phage

Abbreviations: *OSISAP1*, *Oryza sativa* subspecies *indica* stress-associated protein gene; ABA, abscisic acid.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF140722 (*OSISAP1* cDNA) and AY137590 (genomic clone)].

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clone) containing the gene was sequenced. The transcription start site was determined by primer extension analysis using a primer designed encompassing the translation start site (18).

RNA Blot Analysis. After RNA extraction, RNA blot analysis was carried out with 20 μ g of total RNA (16, 19). The [α^{32} -P]dATP-labeled *OSISAP1* cDNA was used as a probe. Hybridization was detected by autoradiography. Ethidium-bromide-stained rRNA bands from identical samples served as control for total RNA quantity and quality.

Transformation of Tobacco. To overexpress rice *OSISAP1* in tobacco (*Nicotiana tabacum* var. Xanthi), the cDNA was cloned in pBI121 (CLONTECH) by replacing the *gus* gene. The resulting vector, pBISAPc, has the *CaMV35S* promoter driving expression of *OSISAP1* cDNA. This construct then was mobilized into *Agrobacterium tumefaciens* strain LBA4404 by chemical transformation. *Agrobacterium*-mediated transformation of tobacco was carried out per standard protocol (20). The integration of transgene in different lines was confirmed by Southern blot analysis, and for RNA blot analysis, RNA was isolated from leaves to evaluate the expression of the introduced gene. *OSISAP1* cDNA was used as a radiolabeled probe for both Southern and RNA blot analysis.

Analysis of Transgenics for Abiotic Stress Tolerance. Wild-type (WT) and T1 seeds of transgenic tobacco were surface-sterilized with 70% ethanol in a microcentrifuge tube for 30 s with constant agitation under a laminar flow hood. After the treatment, ethanol was removed by using a pipette. The seeds were immersed in 2% (vol/vol) sodium hypochlorite solution containing a drop of Tween 20 for 5 min, agitated occasionally by tapping the microcentrifuge tube, and subsequently washed at least six times with autoclaved Milli-Q water. Before salt and cold stress, the seeds were grown in medium containing half-strength Murashige and Skoog medium without sucrose and organic ingredients (MSH) (21) for 16 days in a culture room maintained at $25 \pm 2^\circ\text{C}$ under a 16-h light/8-h dark cycle, and each rack was illuminated with light ($50\text{--}100 \mu\text{mol}\cdot\text{m}^{-2}$ per s) provided by three white fluorescent tubes (Philips Champion 40W/54) and one yellow fluorescent tube (Philips Trulight 36W/82). The 16-day-old seedlings were transferred to fresh MSH and allowed to grow for another 5 days before stress treatment was given. For salt stress, seedlings were transferred aseptically onto eight layers of tissue paper soaked with 250 mM NaCl solution in MSH. The Petri plates were sealed with Parafilm to prevent evaporation. They were allowed to grow for 4 days under culture-room condition as described above. Subsequently, the seedlings were washed briefly in sterile Milli-Q water, with excess water soaked on sterile tissue paper, and allowed to grow on MSH under

culture-room conditions for recovery. For cold treatment, Petri plates containing 21-day-old seedlings were transferred to a cold chamber maintained at $8 \pm 1^\circ\text{C}$ for 15 days. The plates then were transferred back to culture-room conditions for recovery of seedlings. For dehydration stress, the seeds were germinated on 0.3 and 0.4 M mannitol, and observations were recorded over a period of 8 days. The contribution of kanamycin-sensitive (segregating, nontransgenic) seedlings that did not harbor the transgene was excluded from the total number of seedlings analyzed for each line to assess stress tolerance. The T1 progeny of lines SAPc L9 and SAPc L8 did not show segregation for kanamycin resistance, whereas lines SAPc L11, SAPc L22, and SAPc L43 showed 20.9%, 19.3%, and 23% kanamycin-sensitive seedlings, respectively. Based on these values, the effect of kanamycin-sensitive seedlings was excluded for calculating the percentage green seedlings after salt stress. All experiments were repeated at least twice, and data in the form of the mean of two experiments with absolute variation are given.

Results

A stress-associated protein 1 gene (*OSISAP1*) from rice was cloned by differential screening of a cDNA library during an attempt to unravel the activity of genes that control stress tolerance. It was shown to confer cold, dehydration, and salt stress tolerance in transgenic tobacco.

Analysis of *OSISAP1*. The cDNA of *OSISAP1* is of 844 bp, including a 19-bp poly(A) tail, and codes for a protein of 164 aa with a predicted molecular mass of 17.6 kDa (GenBank accession no. AF140722). The database search using the amino acid sequence as query showed homology to several zinc-finger proteins including the human and mouse PRK1-associated protein AWP1 (22), PVPR3 (23), human and mouse zinc-finger proteins ZNF216 (24), *Xenopus* ubiquitin-like fusion proteins (25), and the ascidian posterior end mark (PEM6) protein (26). All these proteins showed homology to *OSISAP1* in the AN1-type zinc-finger region, which is present at the C terminus of the protein, stretching between amino acids 101 and 163. It has a consensus sequence of $\text{Cx}_2\text{--}_4\text{Cx}_9\text{--}_{12}\text{Cx}_2\text{Cx}_4\text{Cx}_2\text{Hx}_5\text{HxC}$, where x represents any amino acid. However, several other amino acids were found to be invariant in this domain. The conserved cysteine and histidine residues may help form a zinc finger (indicated in bold type in Fig. 1a). Toward the N terminus, there are four cysteine residues (at amino acids 22, 26, 38, and 41) that are conserved between *OSISAP1*, AWP1, and ZNF216 (Fig. 1b). This region is similar to the A20 (an inhibitor of cell death)-like zinc fingers, which mediate self-association in A20 (27). *OSISAP1* also has $\approx 51\%$ identity over a stretch of 41 aa (56–96) to the human transcription factor NF κ B p65 subunit consensus sequence (28).

Fig. 1. Comparison of deduced amino acid sequence from *OSISAP1* and other zinc-finger proteins. Conserved cysteine and histidine residues are indicated in bold type. Conservation of amino acids at the C-terminal AN1-type zinc-finger (a) and the N-terminal A20 type zinc-finger (b) of *OSISAP1* vis-à-vis PVPR3 (*P. vulgaris* pathogenesis-related 3 protein; ref. 23), AWP1 (*Homo sapiens* protein associated with PRK1; ref. 22), mZNF216 (*Mus musculus* zinc-finger protein 216; ref. 24), hZNF216 (*H. sapiens* zinc-finger protein 216; ref. 24), PEM6 (*Ciona savignyi* posterior end mark 6 protein; ref. 26), and XLULFP (*Xenopus laevis* ubiquitin-like fusion protein; ref. 25).

The homology is toward the C terminus of the human protein, between amino acids 370 and 410.

Genes showing homology to the conserved region encoding the zinc finger of *OSISAP1* have been annotated in other plant species such as *Arabidopsis* and *Prunus*. The *OSISAP1* sequence showed maximum homology (79% identity) to PVPR3 (*Phaseolus vulgaris* pathogenesis-related protein) zinc-finger domain. The protein has no signatures of signal sequence and is possibly a soluble intracellular protein. The amino acid sequence has a potential protein kinase C phosphorylation site (at position 30) and an *N*-myristoylation site (at position 132).

OSISAP1 is single-copy gene as determined by Southern blot analysis. However, when the *indica* rice database hosted at the Center for Genomics and Bioinformatics (University of Beijing) was searched with the *OSISAP1*-coding region, contig 46636 showed 98% homology, representing essentially the same gene, and contig 10325 showed only 36% homology. A search of the database for similar gene(s) in *japonica* rice revealed a gene with 99% homology (bacterial artificial chromosome OSJNBa0046G16, GenBank accession no. AC108756, chromosome 9) and another gene showing 36% homology on chromosome 8 (bacterial artificial chromosome OSJNBa0025J22, GenBank accession no. AP005245). It remains to be seen whether Pusa Basmati-1 also has a gene with lesser homology that was not detected under the stringency conditions used for Southern hybridization. The coding region of the genomic clone of *OSISAP1* is continuous, without an intron (GenBank accession no. AY137590). The start site of transcription was mapped to a G nucleotide 126 bp upstream of ATG. When the genomic region upstream of the transcription start site was analyzed *in silico*, several cis-acting elements involved in stress-responsive gene expression such as C-repeat/dehydration-responsive element, ABA-responsive element, heat shock element, wound-responsive element, GT box, ethylene-responsive element, and GC motifs were identified (29–31).

Stress-Induced Expression of *OSISAP1*. The gene is induced under several abiotic stresses. The transcript levels increased to a very high level within 1 h after cold treatment to the seedlings (Fig. 2a). The level continued to increase until 3 h, remained at elevated levels until 12 h, and declined thereafter. The cold-induced membrane rigidification is considered to be the primary event in cold perception by plants (17, 32). The event of membrane rigidification at low temperature can be prevented by benzyl alcohol, which acts as a membrane fluidizer, or may be simulated at room temperature by treatment with a membrane rigidifier, DMSO. The cold-induced accumulation of *OSISAP1* transcript was dramatically reduced by the inhibitor of membrane rigidification, i.e., benzyl alcohol (Fig. 2b). On the other hand, treatment of seedlings with membrane rigidifier DMSO significantly increased *OSISAP1* expression at room temperature (Fig. 2b). However, with higher concentrations of DMSO, induction was relatively less, possibly because of the toxic nature of the chemical. In the case of salt stress, transcript levels peaked as early as 15 min and declined after 1 h (Fig. 2c). However, even at 24 h, the mRNA level was more than that of the control. A similar pattern of transcript accumulation was observed for desiccation stress (Fig. 2c). In the case of submergence stress, a different induction kinetics was observed (Fig. 2d). The gene was induced strongly within 3 h, after which its mRNA level declined dramatically to the level of the control. However, the mRNA level peaked again at 12 h and stayed high until 36 h, showing a decline thereafter. The gene was found also to be responsive to different heavy metals (Fig. 2e). Essentially, treatment with copper, cadmium, manganese, or zinc salts led to a significant increase in transcript abundance by 3 h, and at 6 h a decline was evident. Calcium and lithium salts only had a marginal effect on the mRNA level. The gene also was responsive to mechanical

Fig. 2. Expression pattern of *OSISAP1* after different stresses to rice seedlings: cold (a), chemical modulators of membrane fluidity (b), salt and desiccation (c), submergence (d), heavy metals (e), mechanical wounding (f), and ABA (g). C, WT rice control without exposure to stress. *OSISAP1* cDNA was used as a radiolabeled probe for Northern hybridization in all cases. The lower panels in all cases show ethidium bromide-stained rRNA for equivalent loading and RNA quality.

injury (Fig. 2f). The gene responded to ABA at concentrations as low as 1 μ M (Fig. 2g); the expression of *OSISAP1* peaked 30 min after treatment of seedlings with 1 μ M ABA. However, with increasing concentration of ABA, the steady-state transcript level was maintained for a longer duration.

Abiotic Stress Tolerance of Transgenic Tobacco Seedlings Harboring *OSISAP1*. Five independent transgenic lines (SAPcL8, SAPcL9, SAPcL11, SAPcL22, and SAPcL43) of tobacco harboring the pBISAPc, as confirmed by Southern blot analysis (data not shown), and constitutively expressing *OSISAP1* (Fig. 3) were analyzed for stress tolerance in the T1 generation.

Transgenic lines were analyzed for cold tolerance in the T1 generation. The measurement of fresh weight of seedlings after a 15-day recovery period revealed that the stressed transgenic seedlings gained 67–92% fresh weight, whereas untransformed (WT) could only gain 35% fresh weight in comparison with unstressed seedlings (Fig. 4a). Additionally, the fresh weight of transgenic seedlings was always better than WT seedlings at low

Fig. 3. Expression of *OSISAP1* in leaves of untransformed (UT) and transgenic (SAPcL8, SAPcL9, SAPcL11, SAPcL22, and SAPcL43) tobacco. Other details are similar to those described for Fig. 2.

Fig. 4. Effect of cold stress on tobacco seedlings from WT and *OSISAP1*-overexpressing transgenic lines (SAPcL8, SAPcL9, SAPcL11, SAPcL22, and SAPcL43). (a) Twenty-one-day-old seedlings were grown at $8 \pm 1^\circ\text{C}$ for 15 days and transferred to culture-room conditions. Fresh weight was recorded for cold-stressed seedlings after 15 days of cold-stress recovery. Fresh weights of unstressed seedlings of the same age also were recorded and designated as control. Absolute variation of two experiments is shown at the top of each bar. (b) Twenty-one-day-old seedlings of untransformed and *OSISAP1*-overexpressing lines were cold-stressed at $8 \pm 1^\circ\text{C}$ for 15 days and then transferred back to MSH for recovery. Photographs of representative seedlings of WT and five transgenic lines were taken after 15 days of recovery.

temperature. Phenotypically, the transgenic seedlings appeared healthier than the WT after the stress treatment and recovery (Fig. 4b). In the case of transgenic seedlings, the third or fourth

leaf had already emerged, whereas in WT, only the first two leaves could be observed.

Evaluation of *OSISAP1*-overexpressing lines for dehydration-stress tolerance revealed that the percentage germination of WT was much less compared with transgenics over an 8-day period. On day 8 of exposure to 0.3 M mannitol, only 60% of WT germinated, whereas $\geq 90\%$ germination was seen in all transgenic lines except line 22, which showed 69% germination (Fig. 5a). When germinated on 0.4 M mannitol, only 41% germination was seen in WT, whereas transgenics showed between 69% and 92% germination (Fig. 5b). Also, the fresh weight of stressed transgenics was much higher compared with that of WT, as reflected on quantitative estimation (Fig. 5c) and visual inspection (Fig. 5d).

For determining the effect of *OSISAP1* overexpression on the salt tolerance of transgenic tobacco, transgenic and WT seedlings were grown as described in *Materials and Methods*. No significant difference was observed in the fresh weight of WT and transgenic seedlings after the fourth day of exposure to stress (Table 1, day 0 of recovery period). However, highly significant improvement in fresh weight gain of seedlings from transgenic lines under stress was observed over a recovery period of 12 days (Table 1). Over a similar period, fresh weight gain in WT seedlings under stress was minimal. After 8 days of recovery from salt stress, although chlorosis was apparent in both the WT and transgenic lines, it was much less in transgenic seedlings because they retained more chlorophyll and thus green color (Fig. 6a). The appearance of transgenic seedlings was much better than the WT after stress recovery, and they resumed vegetative growth faster (Fig. 6b).

Discussion

A large number of genes in plants are induced after exposure to various abiotic stresses (33). These genes function in various ways to confer stress tolerance to plants (29, 30, 34, 35). The expression patterns of these genes are governed by the need of the system, i.e., genes that are required during early phases of stress

Fig. 5. Effect of dehydration stress on tobacco seedlings from WT and *OSISAP1*-overexpressing transgenic lines (SAPcL8, SAPcL9, SAPcL11, SAPcL22, and SAPcL43). Seeds were germinated on 0.3 M (a) and 0.4 M (b) mannitol. (c) Relative fresh weight of 8-day-old seedlings germinated on 0.3 or 0.4 M mannitol. The fresh weight is shown relative to the fresh weight of unstressed seedlings. Absolute variation of two experiments is shown in a–c. (d) Representative seedlings of WT and five transgenic lines taken after 8 days of germination on 0.3 M (Upper) and 0.4 M (Lower) mannitol.

Table 1. Effect of salt stress (250 mM NaCl for 4 days) on fresh weight (mg per seedling) of 21-day-old seedlings of WT and *OSISAP1* overexpressing transgenic tobacco during the recovery period (0, 4, 8, and 12 days)

Identity	Day 0		Day 4		Day 8		Day 12	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
WT	4.2 (0.25)	3.6 (0.35)	4.6 (0.10)	1.9 (0.17)	6.3 (0.08)	3.8 (0.07)	7.2 (0.19)	4.2 (0.11)
SAPcL8	4.2 (0.40)	4.2 (0.25)	4.4 (0.40)	4.5 (0.32)	6.9 (0.13)	7.3 (1.06)	7.0 (0.06)	8.3 (0.24)
SAPcL9	3.1 (0.10)	3.3 (0.15)	3.5 (0.20)	3.2 (0.25)	6.0 (0.22)	7.3 (0.37)	7.4 (0.17)	7.1 (0.09)
SAPcL11	3.6 (0.35)	3.7 (0.26)	3.8 (0.35)	3.9 (0.17)	6.9 (0.03)	6.2 (0.53)	7.9 (0.04)	7.2 (0.25)
SAPcL22	3.7 (0.20)	3.4 (0.10)	3.5 (0.55)	3.6 (0.11)	5.6 (0.27)	6.1 (0.42)	6.8 (0.10)	6.8 (0.14)
SAPcL43	3.2 (0.25)	3.0 (0.05)	3.4 (0.35)	3.1 (0.37)	4.9 (0.04)	5.4 (0.72)	6.4 (0.17)	6.6 (0.30)

Values represent the mean of two observations of 10 seedlings ($\pm$ absolute variation).

response are induced soon after stress, whereas those required during homeostasis and recovery are induced later. Two rice varieties, Pokkali (salt-tolerant) and IR 24 (salt-sensitive) were compared for their gene-expression profiles after salt stress, and several genes were found to be up- or down-regulated. A noticeable difference between the two varieties was the timing of expression of similar genes. After salt stress, the expression profile in Pokkali after 15 min of treatment was similar to that of IR 29 after 1 h, which can explain the sensitivity of IR 29 to salt stress (36). Because *OSISAP1* is induced to high levels on exposure to stress, its product might be required early after stress. Such early induction of gene expression has also been observed for desiccation and salt induction of *RD29* (37, 38), desiccation induction of *COR47* and *ERD10* (39, 40), and salt induction in the case of *OSLEA3* (41), as well as desiccation, salt, or ABA induction of *RD22* (42). The functions of these genes are not well defined. Some genes with known function are also induced to high levels early after stress. The genes for calcium-dependent protein kinases (*AtCDPK1*, *AtCDPK2*, and *OsCDPK7*) are induced rapidly by drought and salt stress (43, 44),

and cold induction of *CBF* genes for transcription factors (45, 46) has been observed. Similarly, a receptor-like protein kinase is induced rapidly by ABA, dehydration, high salt, and cold (47). A MYC-related DNA-binding transcription factor is induced early after dehydration, salt stress, and ABA (42). The dehydration-responsive element-binding transcription factor *DREB2* is induced within 10 min after dehydration and salt stress (48), whereas a subset of *Arabidopsis* Cys₂/His₂-type zinc-finger transcription factors are induced early after desiccation, salt, or ABA treatment (49). The *OSISAP1* might not be a transcription factor, because it lacks any typical nuclear localization signal. However, it may use its zinc-finger domain for protein-protein interaction to pick-a-back for such a purpose. From the nature of early induction, the *OSISAP1* gene product might act as well early in the signal transduction pathway of stress response.

Certain stress-induced proteins already have been shown to impart stress tolerance, although their functions remain to be defined. The *COR15a* gene from *Arabidopsis* is induced after cold stress, and its overexpression in transgenic *Arabidopsis* leads to increased freezing tolerance of chloroplasts as well as protoplasts (50). It still is not known how *COR15a* stabilizes membranes against freeze-induced damages. It is speculated that *COR15a* defers the incidence of freeze-induced transition to hexagonal II phase but has no effect on expansion-induced lysis (51). Similarly, overexpression of the barley gene *HVA1* (a group III late embryogenesis abundant protein) could confer stress tolerance to transgenic rice (52, 53), yet its mode of action is not known. These examples provide a target for improving stress tolerance of crop plants and give an opportunity to understand the function of previously uncharacterized genes in an organismal, cellular, or subcellular context. *OSISAP1* is also a stress-inducible gene predicted to encode a zinc-finger protein in rice. Overexpression of this gene in transgenic tobacco leads to an increase in stress tolerance, as determined by cold-, dehydration-, and salt-tolerance assays. It was found that the fresh weight, retention of green color, and leaf development as well as percentage germination were much better in transgenic lines, as compared with WT tobacco, under stress and recovery conditions. However, it would be of interest to know whether stress tolerance in tobacco is either due to ectopic expression or the natural function of *OSISAP1*.

The *OSISAP1* protein has homology to a mammalian protein A20 (54). The zinc-finger domain of A20 is required for dimerization (27), and it inhibits the tumor necrosis factor-induced apoptosis through inhibition of NF- κ B-mediated gene expression (55–62). High-salinity stress leads to inhibition of cell division and acceleration of cell death (35), whereas chilling stress leads to wilting and chlorosis of tissues and electrolyte leakage (63). All of these ultimately would lead to cell death. Overexpression of *OSISAP1* can help avoid stress-associated injuries such as chlorosis and cell death in transgenic plants and better recovery from stress. Thus, although in two different systems and with only a zinc-finger common

Fig. 6. Effect of salt stress on tobacco seedlings from WT and T1 progenies of transgenic lines (SAPcL8, SAPcL9, SAPcL11, SAPcL22, and SAPcL43) overexpressing *OSISAP1*. (a) Twenty-one-day-old seedlings were salt-stressed in 250 mM NaCl for 4 days and then transferred back to MSH for recovery. After 8 days of recovery, seedlings of WT and transgenic lines that showed no apparent signs of chlorosis were counted. Absolute variation in two experiments is shown at the top of each bar. (b) Twenty-one-day-old seedlings were stressed with 250 mM NaCl for 4 days and then transferred back to MSH for recovery. Photographs of representative seedlings of WT and five transgenic lines were taken after 8 days of recovery.

between the two, *OSISAP1* and *A20* have similar effects when overexpressed. The protein *OSISAP1* also shows high homology of its second C-terminal zinc-finger domain with the AN1 type of zinc-finger proteins. These proteins, *PEM6*, *ZNF216*, *XLULFP*, and *PVPR3*, have no defined function. The *OSISAP1* protein is hydrophilic, and this property also may contribute to the increased stress tolerance in transgenic plants as reported for other such proteins (35, 51, 64). As shown by the present investigation, overexpression of several other genes has been found to provide stress tolerance to seedlings (44, 65–71).

In conclusion, this study has characterized a zinc-finger protein gene from rice and unraveled a determinant of abiotic stress tolerance that may be used to engineer stress tolerance in other crop plants. It also has not escaped our notice that the protein may have function in other stresses that have been shown to induce its expression.

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