

PROF. GIUSEPPE FORLANI (Orcid ID : 0000-0003-2598-5718)

Article type : Short Research Paper

handling Editor: Z.B Luo

Stress-driven increase in proline levels, and not proline levels themselves, correlates with the ability to withstand excess salt in a group of 17 Italian rice genotypes

Giuseppe Forlani, Michele Bertazzini & Giovanni Cagnano*

Department of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy

Running head

Proline homeostasis and salt tolerance in rice

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/plb.12916

This article is protected by copyright. All rights reserved.

Accepted Article

Correspondence

Giuseppe Forlani, Department of Life Science and Biotechnology, University of Ferrara, via L. Borsari 46, I-44121 Ferrara, Italy. E-mail: flg@unife.it

Keywords

amino acid homeostasis, free proline level, proline metabolism, rice, salt stress.

* present address: Department of Molecular Biology and Genetics - Crop Genetics and Biotechnology, Aarhus University, Slagelse, Denmark

ABSTRACT

- In most plant species, a rapid increase of free proline content occurs following the exposure to hyperosmotic stress conditions. However, inconsistent results were reported concerning the role of such increase in the plant response to water shortage or excess salt. Therefore, the possibility that proline accumulation may help the cell in withstanding stress conditions, or that it simply represents a stress marker, is still a matter of debate. A possible relationship between proline accumulation and salt tolerance was investigated in a set of 17 Italian rice varieties.
- Rice seedlings were exposed to increasing salt concentrations during germination and early growth. The resulting levels of free proline was measured separately in shoots and roots, and compared to those in untreated controls. Results were related to the

corresponding ability of a given genotype to tolerate stress conditions.

- Neither absolute levels in untreated or salt-stressed seedlings showed a plain relationship with the relative tolerance to salt, estimated as the conductivity values able to reduce growth by 10 or 50%. Conversely, a highly significant correlation was found between the increase of proline levels in shoots and the ability to withstand stress.
- Results strengthen a recent hypothesis assuming that an increase in proline metabolic rates more than the resulting proline content may help the cell to counteract the effects of abiotic stress conditions.

INTRODUCTION

In higher plants, as well as in several microorganisms, increased intracellular free proline content was found in response to a wide variety of abiotic stress conditions, reaching in some species up to 300-fold the level in unstressed cells (Delauney & Verma 1993). On this basis, it was early hypothesized that proline may serve as a compatible osmolyte counteracting water withdrawal from the cell caused by a decreased external water potential. However, in many other species the rise is much less prominent, with concentrations that are only 2-5 fold higher than in controls, and the role of proline in osmotic adjustment has consequently been questioned. Due to the peculiar properties of this imino acid, several other mechanisms have been proposed to explain a beneficial effect of higher proline content under stress. For instance, proline may serve as a reactive oxygen species scavenger or a shuttle for reducing power between cell compartments, may stabilize proteins and membranes (Hayat *et al.* 2012) or act as a signaling molecule to modulate mitochondrial functions, may induce cell death and trigger specific gene expression (Szabados & Saviouré 2010). Lately, it has been

Accepted Article

hypothesized that proline metabolism more than proline content may help the cell to withstand abiotic stress conditions (Kavi Kishor & Sreenivasulu 2014). In fact, the analysis of mutants compromised in either or both proline synthesis and oxidation showed that the activity of both pathways is required for optimal growth at low water potential (Sharma *et al.* 2011). The generation of reactive oxygen species in the mitochondrion during proline oxidation by the activity of proline dehydrogenase seems able to trigger defense pathways and allow the adaptation of cells to a variety of environmental constraints (Ben Rejeb *et al.* 2014). Moreover, Arabidopsis mutants impaired in the first step of either proline synthesis and catabolism showed similar growth reduction under drought despite contrasting free proline levels (Bhaskara *et al.* 2015). Anyhow, further experimental evidence is required to shed more light on these aspects.

Because of favourable effects of proline accumulation, the possibility that different stress-induced proline levels may contribute to a differential stress tolerance among genotypes of the same plant species has been widely investigated, mainly in crops (*e.g.* de Mezer *et al.* 2014). Indeed, transgenic plants engineered to increase constitutive and stress-induced proline content, either by overexpressing the first enzyme of the biosynthetic pathway or by reducing the catabolic route, showed increased tolerance than wild-type (reviewed in Per *et al.* 2017). However, inconsistent results have been often reported for crop cultivars showing different sensitivity to drought and excess salt. This is the case of rice, a crop that feeds billions of people in the world. In some studies, osmotic stress tolerant rice varieties were shown to accumulate higher proline concentrations under stress than sensitive genotypes (as for instance in Kordrostami *et al.* 2017; Anupama *et al.* 2018; Saini *et al.* 2018), whereas in some other instances the opposite result was found (as in Renuka Devi *et al.* 1996; Demiral & Türkan 2005; Theerakulpisut *et al.* 2005; Chunthaburee *et al.* 2016).

These inconsistent data may depend on either the small number of genotypes that have been considered, or an experimental design comprising genetically distant varieties, where the contribution of proline, if any, is overcome by that of other determinants playing a major role in

providing stress tolerance. Also in the case of a group of six Italian rice genotypes, previously found to differ in susceptibility to moderate saline environments, the increase in proline content did not show a clear relationship with the differential tolerance to salt stress (Bertazzini *et al.* 2018). Here we report the results of a further study on a wider set of Italian rice varieties, in which proline levels in either unstressed or salt-stressed seedlings have been measured, and related to the degree of tolerance to excess salt.

MATERIALS AND METHODS

Plant materials, growth conditions and salt treatments

Rice (*Oryza sativa* L.) genotypes were obtained from the *Unità di Ricerca per la Risiicoltura* (CREA; Vercelli, Italy). Seeds were surface-sterilized and sown in Magenta vessels (6 \times 6 \times 20 cm) filled with 75 mL of agarized (6‰) water, 16 seeds for vessel, and immediately covered with the same volume of a 2X nutrient solution (Bertazzini *et al.* 2018), pH 5.8, containing 2 ml L⁻¹ Plant Preservative Mixture (Plant Cell Technology). Vessels were incubated under a photoperiod of 16 h light (250 μ mol m⁻² sec⁻¹) and 8 h dark at 26 \pm 1°C (day) and 22 \pm 1°C (night) in an incubator equipped with E27 ES 1700 lumen daylight lamps (GE Lighting). The effect of excess salt upon free proline pools was assessed by complementing the nutrient solution with a salt mixture (NaCl, CaCl₂, MgSO₄ and Na₂SO₄ in a 10:1:2:1 molar ratio). The actual value of conductivity in each sample was measured with a conductivity-meter (Hanna HI 8733). The experimental design consisted of a randomized complete block with three replicates. Each block comprised 68 Magenta vessels of 17 genotypes and 3 salt rates (4.7, 8.5 and 15.6 dS m⁻¹), plus untreated controls, whose conductivity (0.72 dS m⁻¹) was well below the damage threshold.

To evaluate the effect of increasing salt concentrations on growth, seeds were sown in Petri dishes filled with agarized (6‰) water, 20 seeds for plate, and covered with the nutrient solution containing or not increasing levels of salts, as described above. The experimental design consisted of a randomized complete block with three replicates. Each block comprised 136 plates of 17 rice genotypes grown in the presence of 7 salt rates (6.6, 8.5, 10.3, 12.1, 13.9, 15.6 and 19.0 dS m⁻¹) plus untreated controls. Exactly one week after sowing, destructive harvest allowed the determination of plant biomass. Roots and shoots were weighted separately for each seedling. Then the material was treated in an oven at 90°C for 48 h for the determination of dry weight. Data were expressed as percent of untreated controls. The conditions causing 50% (IC₅₀) or 10% (IC₁₀) growth inhibition and their confidence intervals were estimated for each cultivar by non-linear regression analysis (variable slope) of whole seedlings values plotted against the logarithm of conductivity, using Prism 6 (version 6.03, GraphPad Software, Inc., USA).

Free proline analysis

For proline determination, seedlings were harvested 14 days after sowing. At the harvest, ten uniformly-grown seedlings were collected for each treatment. Seed residues were discarded, and roots and shoots from each seedling were weighted, resuspended with 2 mL g⁻¹ of a 3% (w/v) solution of 5-sulphosalicylic acid, and extracted with 1 g g⁻¹ quartz sand in a mortar equilibrated on ice. Following centrifugation for 3 min at 14,000 g, proline content was quantified by the acid ninhydrin method, as previously described (Forlani *et al.* 2013). Data were corrected on a dry weight basis, where the dry-to-fresh weight ratio was calculated by treating 10 similarly-grown seedlings in an oven at 90°C for 48 h.

Statistical analysis

Anova and correlation analyses were carried out using Prism 6, assuming data were sampled from Gaussian populations (Pearson test).

RESULTS AND DISCUSSION

The addition of increasing levels of a salt mixture mimicking the composition of the soil solution in the field (Grattan *et al.* 2002) induced a progressive increase of free proline content in both roots and shoots of rice seedlings (Fig. 1). From a quantitative point of view the effect was mild, and concentrations in treated seedlings did not exceed 3 times those in controls. However, data were expressed on a dry weight basis, ruling out the possibility that the increase may be simply due to a lower water content of salt-stressed tissues. Quite surprisingly, being in direct contact with the salt-containing substrate, in most instances a marked increase was evident in roots only at the highest salt rate tested, and differences among cultivars were poorly significant (Fig. 1A). On the contrary, at least for some genotypes, levels in shoots showed a substantial increase also at lower conductivity values, and at the highest rate highly significant differences were found among rice varieties (Fig. 1B). On this basis, proline levels were measured in shoots of untreated controls of 17 rice varieties, and in seedlings grown at 15.6 dS m^{-1} , a value corresponding to about 150 mM salt, which caused a significant growth reduction without being lethal. In all cases shoots of treated plantlets contained significantly higher levels of the imino acid, and varieties differed significantly for proline content under both control and stress conditions (Supplementary Fig. S1). Despite the large number of Italian rice cultivars, in many cases little information is available about their relationship, since data are often covered by patents, missing, or incorrectly recorded. However, genetic divergence and relationships were recently assessed based on the polymorphisms of microsatellite fingerprints for 183 Italian varieties, comprising 14 of the 17 cultivars analysed in the present study (all but Centauro,

Salvo and Venere; Cai *et al.* 2013). On this basis, only the cultivars Asia, Gange and Thaibonnet have a close genetic relationship. Interestingly, all these three varieties showed a low proline content under both control and salt stress conditions (Supplementary Fig. S1). Levels were still statistically different ($P = 0.0071$), yet the interaction was not ($P = 0.4309$), suggesting that proline homeostasis responds similarly to salt treatment.

To evaluate whether osmo-induced free proline content in shoots was related to the ability of a given rice variety to withstand salt stress, the effect of increasing salt levels on growth was measured. Once again results (Fig. 2) showed the occurrence of a significant variability among Italian rice cultivars, according to the relatively rich genetic diversity that has been found by molecular analysis (Cai *et al.* 2013). Such a variability was evident mainly at low conductivity values, whereas under more severe conditions the response in terms of growth reduction was similar (Supplementary Fig. S2). This is consistent with the results of a recent screening on a wider set of European rice varieties, showing the occurrence of a differential tolerance to mild salinity stress. However, in that case the effect of only a single, low salt dose (50 mM NaCl, 6 dS m⁻¹) was evaluated (Frouin *et al.* 2018). At least in our case, this result has to be considered as a natural variability, since to the best of our knowledge no cultivar was specifically bred for growing in salt-affected area. This notwithstanding, data are in a good agreement with the current trend of rice cultivar use in salt-affected regions of Northern Italy: according to the available statistics (www.enterisi.it), one of the two genotypes herein showing the highest damage threshold for growth, Baldo, is the most used cultivar in the Delta Po area, accounting for one third of the overall production. The other genotype whose growth appeared less affected by low salt levels, Loto, is on the contrary scarcely cultivated, but this may depend upon its high susceptibility to the blast pathogen (Faivre-Rampant *et al.*, 2011). Considering the group of more closely related varieties, contrary to what found for proline content Asia, Thaibonnet and Gange showed highly different responses, with about 25, 35 and 60% growth reduction at 8.5 dS m⁻¹, respectively. However, a similar difference is not unexpected for a multigenic trait.

Regression analysis of data allowed for each genotype the estimate of the concentration inhibiting growth by 50% (IC_{50}), and the threshold over which salts become inhibitory (IC_{10} , damage threshold). Proline content was related to either parameter. When considering absolute levels in untreated or salt-stressed seedlings, no significant correlations were found (Fig 3A-D), though higher R^2 values were evident with proline in treated seedlings (Fig. 3CD). However, when the mean increase of free proline concentration in treated seedlings with respect to untreated controls of the same rice variety was considered, a highly significant correlation was found (Fig. 3EF). A higher correlation coefficient with IC_{10} than with IC_{50} values suggests that the increase in proline levels may play a role mainly in the response to mild salt stress conditions, whereas at higher conductivity values other mechanism(s) may overwhelm it in providing differential tolerance. Because the increase in free proline, and not its homeostatic levels under either normo-osmotic or salt stress conditions, correlates with the relative tolerance to salt stress, the protective effect could be more related to proline metabolism than to a possible role of the imino acid as compatible osmolyte, radical scavenger or membrane/enzyme protectant. This would be consistent with an increasing array of data showing the activation under stress of genes in both the anabolic and the catabolic pathways (Kaplan *et al.* 2007; Sharma & Verslues 2010; Senthil-Kumar & Mysore 2012; Rizzi *et al.* 2015). This would cause an apparently futile cycle between glutamate and proline (Miller *et al.* 2009; Lv *et al.* 2011), which may play a critical role for plant survival under stress through maintainance of the cellular redox balance, regulation of $NADP^+/NADPH$ ratio and enhancement of the oxidative pentose phosphate pathway (Hayat *et al.* 2012, Kavi Kishor & Sreenivasulu 2014). Different roles might be played in different tissues, as increased proline synthesis from glutamate was shown to mitigate $NADP^+$ overreduction in photosynthetic tissues under drought thereby avoiding redox imbalance, whereas catabolism in the root apex was found essential to continued growth at low water potential (Sharma *et al.* 2011). Moreover, enhanced proline oxidation in the mitochondrion leads to the generation of reactive oxygen species (Cecchini *et al.* 2011, Servet *et al.* 2012), which in turn act as second messengers in various signalling cascades and induce the expression of defense

Accepted Article

pathways conferring tolerance to either abiotic or biotic stress conditions (Miller *et al.* 2011, Ben Rejeb *et al.* 2014). Whatever the mechanism(s) and although such hypothesis still awaits full confirmation, for instance through demonstration of the occurrence of P5C transporters across the mitochondrial membrane, it would explain why even small changes in proline homeostasis are able to contribute to the plant response to hyperosmotic/drought stress.

CONCLUSIONS

The results herein reported show the lack of correlation between either basal or stress-induced proline levels and the ability of a group of Italian rice genotypes to withstand salt stress. Conversely, a significant relationship was found between the increase of proline homeostatic levels under stress and stress tolerance. Such information could allow reconsidering previous data, and explain their apparent inconsistency.

ACKNOWLEDGEMENTS

This work was supported by AGER Foundation in the frame of the Risinnova project, grant # 2010-2369.

SUPPORTING INFORMATIONS

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Effect of salt treatment on free proline levels in shoots of rice seedlings. Seeds were sown in the absence or in the presence of salts (NaCl, CaCl₂, MgSO₄ and Na₂SO₄ in a 10:1:2:1 molar ratio) so as to obtain a conductivity value of 15.6 dS m⁻¹. Shoots were extracted when untreated controls (0.72 dS m⁻¹) had fully developed the third leaf (about two weeks after sowing). Data were expressed on a dry weight basis, and are mean ± SE over 10 independent replications. The analysis of variance was carried out using Prism 6 (version 6.03, GraphPad Software, Inc., USA).

Figure S2. Response variability of rice seedling growth to salt treatments. Seeds were sown in the absence or in the presence of salts (NaCl, CaCl₂, MgSO₄ and Na₂SO₄ in a 10:1:2:1 molar ratio) so as to obtain a given conductivity value, as indicated. Growth was evaluated one week after sowing. Data were expressed on a dry weight basis, refer to whole seedlings, are mean ± SE over 28 to 60 replications (depending on germination rate at different conductivity values), and are shown as a whisker-and-box plot. The analysis of variance was carried out using Prism 6 (version 6.03, GraphPad Software, Inc., USA).

REFERENCES

Anupama A., Bhugra S., Lall B., Chaudhury S., Chugh A. (2018) Assessing the correlation of genotypic and phenotypic responses of indica rice varieties under drought stress. *Plant Physiology and Biochemistry* **127**, 343–354.

- Ben Rejeb K., Abdelly C., Savouré A. (2014) How reactive oxygen species and proline face stress together. *Plant Physiology and Biochemistry* **80**, 278–284.
- Bertazzini M., Sacchi G.A., Forlani G. (2018) A differential tolerance to mild salt stress conditions among six Italian rice genotypes does not rely on Na⁺ exclusion from shoots. *Journal of Plant Physiology* **226**, 145–153.
- Bhaskara G.B., Yang T.H., Verslues P.E. (2015) Dynamic proline metabolism: importance and regulation in water limited environments. *Frontiers in Plant Science* **6**, 484.
- Cai X., Fan J., Jiang Z., Basso B., Sala F., Spada A., Grassi F., Lu B.-R. (2013) The puzzle of Italian rice origin and evolution: determining genetic divergence and affinity of rice germplasm from Italy and Asia. *PLoS ONE* **8**, e80351.
- Cecchini N.M., Monteoliva M.I., Alvarez M.E. (2011) Proline dehydrogenase contributes to pathogen defense in Arabidopsis. *Plant Physiology* **155**, 1947–1959.
- Chunthaburee S., Dongsansuk A., Sanitchon J., Pattanagul W., Theerakulpisut P. (2016) Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi Journal of Biological Sciences* **23**, 467–477.
- Delauney A.J., Verma D.P. (1993) Proline biosynthesis and osmoregulation in plants. *Plant Journal* **4**, 215–223.
- de Mezer M., Turska-Taraska A., Kaczmarek Z., Glowacka K., Swarczewicz B., Rorat T. (2014) Differential physiological and molecular response of barley genotypes to water deficit. *Plant Physiology and Biochemistry* **80**, 234–248.
- Demiral T., Türkan İ. (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany* **53**, 247–257.
- Fabro G., Kovacs I., Pavet V., Szabados L., Alvarez M.E. (2004) Proline accumulation and *AtP5CS2*

gene activation are induced by plant–pathogen incompatible interactions in Arabidopsis. *Molecular Plant Microbe Interactions* **17**, 343–350.

Faivre-Rampant O., Bruschi G., Abbruscato P., Cavigliolo S., Picco A.M., Borgo L., Lupotto E., Piffanelli P. (2011) Assessment of genetic diversity in Italian rice germplasm related to agronomic traits and blast resistance (*Magnaporthe oryzae*). *Molecular Breeding* **27**, 233–246.

Forlani G., Berlicki Ł., Duò M., Dziędziola G., Giberti S., Bertazzini M., Kafarski P. (2013) Synthesis and evaluation of effective inhibitors of plant δ^1 -pyrroline-5-carboxylate reductase. *Journal of Agricultural and Food Chemistry* **61**, 6792–6798.

Frouin J., Languillaume A., Mas J., Mieulet D., Boissard A., Labeyrie A., Bettembourg M., Bureau C., Lorenzini E., Portefaix M., Turquay P., Vernet A., Périn C., Ahmadi N., Courtois B. (2018) Tolerance to mild salinity stress in japonica rice: A genome-wide association mapping study highlights calcium signaling and metabolism genes. *PLoS One* **13**, e0190964.

Grattan S.R., Zeng L., Shannon M.C., Roberts S.R. (2002) Rice is more sensitive to salinity than previously thought. *California Agriculture* **56**, 189–195.

Hayat S., Hayat Q., Alyemeni M.N., Wani A.S., Pichtel J., Ahmad A. (2012) Role of proline under changing environments: a review. *Plant Signaling and Behavior* **7**, 1456–1466.

Kaplan F., Kopka J., Sung D.Y., Zhao W., Popp M., Porat R., Guy C.L. (2007) Transcript and metabolite profiling during cold acclimation of Arabidopsis reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *Plant Journal* **50**, 967–981.

Kavi Kishor P.B., Sreenivasulu N. (2014) Is proline accumulation *per se* correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell and the Environment* **37**, 300–311.

Kordrostami M., Rabiei B., Hassani Kumleh H. (2017) Biochemical, physiological and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage. *Physiology and*

Molecular Biology of Plants **23**, 529–544.

Lv W.T., Lin B., Zhang M., Hua X.J. (2011) Proline accumulation is inhibitory to Arabidopsis seedlings during heat stress. *Plant Physiology* **156**, 1921–1933.

Miller G., Honig A., Stein H., Suzuki N., Mittler R., Zilberstein A. (2009) Unraveling δ^1 -pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. *Journal of Biological Chemistry* **284**, 26482–26492.

Miller G., Suzuki N., Ciftci-Yilmaz S., Mittler R. (2011) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell & the Environment* **33**, 453–467.

Per T.S., Khan N.A., Reddy P.S., Masood A., Hasanuzzaman M., Khan M.I.R., Anjum N.A. (2017) Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. *Plant Physiology and Biochemistry* **115**, 126–140.

Renuka Devi P.S., Sabu A., Sheeja T.E., Nambisan P. (1996) Proline accumulation and salt tolerance in rice. In: *Recent Advances in Biotechnological Applications of Plant Tissue and Cell Culture*, Ravishankar G.A., Venkataraman L. Eds. Oxford and IBH Publishing Co., New Delhi, India, pp: 410–414.

Rizzi Y.S., Monteoliva M.I., Fabro G., Grosso C.L., Laróvere L.E., Alvarez M.E. (2015) P5CDH affects the pathways contributing to Pro synthesis after ProDH activation by biotic and abiotic stress conditions. *Frontiers in Plant Science* **6**, 572.

Saini S., Kaur N., Pati P.K. (2018) Reactive oxygen species dynamics in roots of salt sensitive and salt tolerant cultivars of rice. *Analytical Biochemistry* **550**, 99–108.

Senthil-Kumar M., Mysore K.S. (2012) Ornithine- δ -aminotransferase and proline dehydrogenase genes play a role in non-host disease resistance by regulating pyrroline-5-carboxylate metabolism-induced hypersensitive response. *Plant Cell and the Environment* **35**, 1329–1343.

- Servet C., Ghelis T., Richard L., Zilberstein A., Savoure A. (2012) Proline dehydrogenase: a key enzyme in controlling cellular homeostasis. *Frontiers in Bioscience* **17**, 607–620.
- Sharma S., Verslues P.E. (2010) Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. *Plant Cell and the Environment* **33**, 1838–1851.
- Sharma S., Villamor J.G., Verslues P.E. (2011) Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. *Plant Physiology* **157**, 292–304.
- Szabados L., Savouré A. (2010) Proline: a multifunctional amino acid. *Trends in Plant Science* **15**, 89–97.
- Theerakulpisut P., Bunnag S., Kong–Ngern K. (2005) Genetic diversity, salinity tolerance and physiological responses to NaCl of six rice (*Oryza sativa* L.) cultivars. *Asian Journal of Plant Science* **4**, 562–573.

Captions to figures

Fig. 1. Effect of increasing conductivity values on free proline levels in roots (panel A) and shoots (panel B) of rice seedlings. Seeds of five selected genotypes, as indicated, were sown directly in the medium containing various salt concentrations, and extracted when untreated controls (0.72 dS m^{-1}) reached the three-leaf stage. Data were normalized to dry weight, and are mean \pm SD over 5 independent replicates.

Fig. 2. Effect of increasing salt concentrations on the growth of rice seedlings. Exactly one week after sowing, destructive harvest allowed the determination of plant biomass. Roots and shoots were weighted separately for each seedling. Data were expressed as percent of

untreated controls, refer to the plant dry weight, and are mean \pm SE over 28 to 60 replications, depending on germination rate at different conductivity values. The conditions causing 50% (IC₅₀) or 10% (IC₁₀) growth inhibition and their confidence intervals were estimated for each cultivar by non-linear regression analysis of whole seedlings values plotted against the logarithm of conductivity.

Fig. 3. Relationship between proline levels in shoot tissues of unstressed (panels A and B) and salt-stressed (C and D) rice seedlings and the relative tolerance to excess salt of a given genotype, expressed as the conductivity value causing 10% (A, C and E) or 50% (B, D and F) growth inhibition. The net increase in proline concentration is also plotted (E and F). Seeds of 17 rice cultivars were sown directly in the medium containing or not 200 mM NaCl, 20 mM CaCl₂, 40 mM MgSO₄ and 20 mM Na₂SO₄, resulting in a conductivity value of 15.6 dS m⁻¹, and extracted when untreated controls reached the three-leaf stage. Results were normalized to dry weight, and are mean \pm SE over 10 independent replicates. Data were subjected to statistical analysis using Pearson test of correlation (two-tailed).





