Phenotyping of a worldwide *japonica* rice collection under salt stress treatment for association study

Elena Baldoni, Gabriele Orasen, Moez Maghrebi, Michele Pesenti, Alessandro Abruzzese, Fabio F. Nocito, Gian A. Sacchi

Dipartimento di Scienze Agrarie e Ambientali - Università degli Studi di Milano, via Celoria 2, 20133 Milano, ITALY

BACKGROUND

Salt stress is one of the environmental constraints that affect crop cultivation worldwide, since more than 800 Mha of land throughout the world suffer from salinization problems (Xiong and Zhu 2001). Among cereals, rice (*Oryza sativa* L.) is one of the most sensitive to salt stress, although cultivars can differ in their response to salinity (Horie et al. 2012, Munns and Tester 2008). In Europe, due to scarce water availability and the rise in sea levels, there is a clear tendency toward salinization in the river deltas where rice is grown (Yáñez 2010). In addition, the biotic stress of the Apple snail species from genus *Pomacea* is a growing problem for rice cultivation in Europe. Apple snail eats the sown seeds and the rice plantlets, completely destroying paddy fields (Halwart 1994, Cowie 2002, Joshi 2006). Recently, apple snails have been detected in the Ebro river delta (Spain), and now it represents an important problem for rive production also in Europe. To date, the only effective measure to combat apple snail is the flooding of infested paddy fields with sea water. This treatment successfully destroys apple snail infestations, nevertheless residual salt concentrations negatively affected rice productivity (Moreso 2014). Thus, the identification of European elite rice varieties tolerant to salt stress and the dissection of salt stress tolerance mechanisms are of high interest for European rice breeding. Genome Wide Association Study (GWAS) is a modern approach to study natural variation in crop plants and to discover important traits for crop breeding (Huang and Han 2014). In these studies, the importance to perform a precise phenotyping is a critical point, especially for the dissection of complex traits such as the tolerance to abiotic stresses.

MATERIALS AND METHODS

A phenotyping activity has been planned to study the natural variation of a worldwide *japonica* rice collection in response to salt stress. A greenhouse experiment is in progress on 281 cultivars subjected to salt stress (fig. 1). Plants were sowed on 16th May 2016. The greenhouse is placed in Tavazzano (Lodi, ITALY; 45.333449, 9.427294). The pots containing paddy soil were placed in a bigger bucket, containing water to mimic submerged condition of the rice plants (fig. 2). Five plants were placed in each pot. Two biological replicates were used. For each replicate, the control pot (non-saline soil) was placed close to the treated pot (saline soil). For high-salinity treatment, salted water has been poured onto the soil, to reach a soil electrical conductivity of 3-5 dS/cm^2 (fig. 2). In addition to the 281 *japonica* genotypes, 5 *indica* genotypes were used as control, since their phenotype of sensitiveness/tolerance in response to salt stress is known (table 1). The measurement of different physiological traits has been performed and collected data are in analysis (fig. 3).

Fig. 1: *japonica* rice collection in greenhouse at 4th-leaf stage.

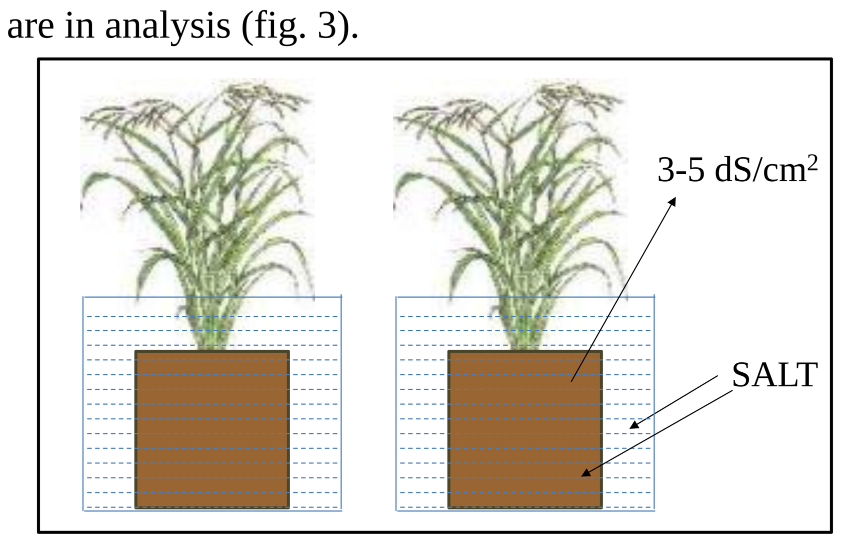

Fig. 2: scheme of the high-salinity treatment.

Genotypes	Salt Response
IR29	sensitive
IR64	sensitive
FL478 (IR29 X Pokkali)	tolerant
IR64-Saltol	tolerant
Pokkali*	tolerant

Table 1: reference genotypes used for phenotyping.
*Assayed only for germination.

GERMINATION RATE	STRESS SYMPTOMS	TRANSPIRATION	GROWTH	LEAF FLUORESCENCE	CHLOROPHYLL CONTENT	STERILITY
count of emerged seeds	visual salt injury (IRRI SES)	thermal imaging	pocket LAI	fluorimeter	dualex	count of empty seeds

GERMINATION	VEGETATIVE STAGE	VEGETATIVE STAGE	FLOWERING	FLOWERING	FLOWERING	RIPENING

Fig. 3: list of the measured physiological traits. For each trait, the used method and the considered growth stage are listed.

PRELIMINARY RESULTS

The analysis of physiological data is still in progress. A preliminary evaluation of germination and plant height data highlighted that most genotypes exhibited an intermediate phenotype in response to salt stress (figs. 4 and 5). Few varieties resulted very sensitive to salt stress, showing very low germination (0-15%) under salt stress (fig. 4) or lower growth (height increase; from -50% to -20%) in treated plants compared to control ones (fig. 5). On the other hand, few genotypes resulted very tolerant to salt stress, showing high germination (65-85%) under salt stress (fig. 4) or a higher growth (up to +20%) in treated plants compared to control ones (fig. 5). Figure 6 shows the average heights of six representative genotypes during the vegetative stage. Three genotypes (IR29, Maioral, Zena) resulted very sensitive to salt stress for this trait, showing a great difference between control and treated plants, whereas the other three genotypes (Eurosis, IR64-Saltol, Sirio) resulted tolerant to salt stress, showing a similar growth in control and treated plants.

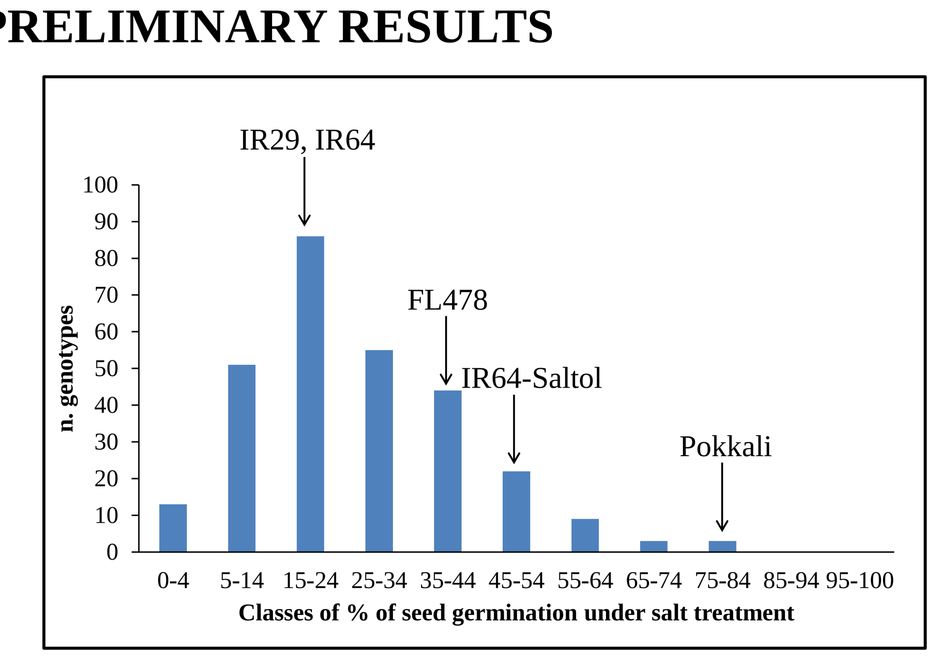

Fig. 4: distribution of the 281 rice genotypes in classes of germination.

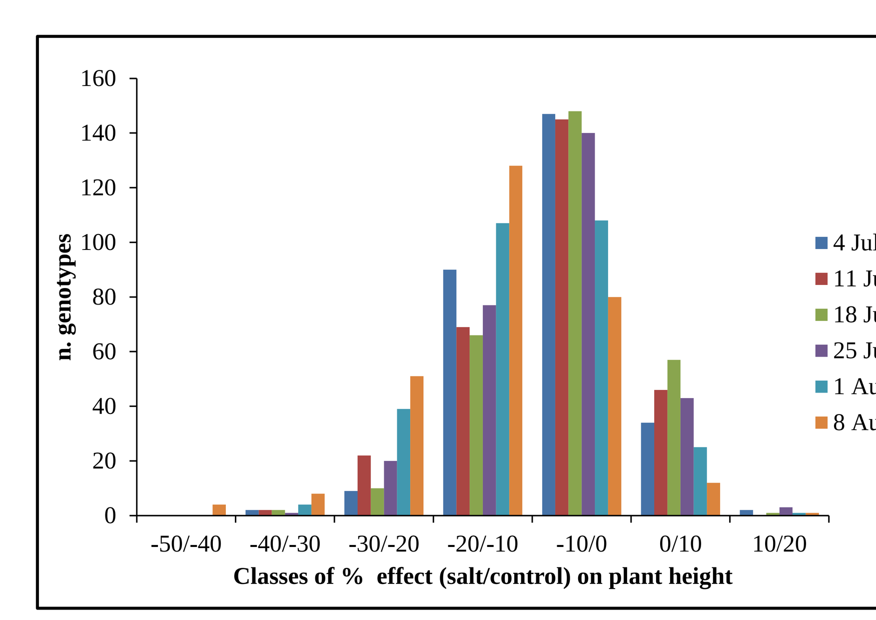

Fig. 5: distribution of the 281 rice genotypes in classes of percentage effect of salt treatment (salt *vs* control) on plant height.

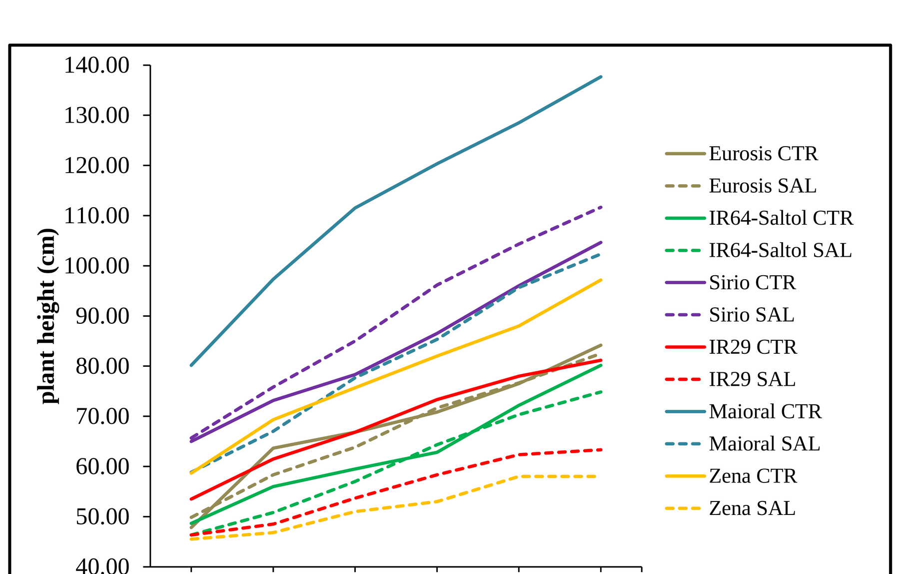

Fig. 6: height of 6 representative genotypes in control or salt conditions during the vegetative stage.

Infrared thermal imaging is a very useful tool for the screening of large numbers of genotypes differing for stomatal traits, specifically for those related to salt tolerance. Indeed, a relationship between direct measurements of stomatal conductance and leaf temperature of plants grown in high-salinity conditions has been described, indicating that thermography accurately reflects the physiological status of salt-stressed plants (James and Sirault 2012). In figure 7, an example of thermal imaging of rice plants during tillering stage, using a semi-automated long-wave infrared cameras system, is shown. Differences in leaf temperature are highlighted by a difference in leaf colour. Control plant (left) showed a dark blue colour, while salt-stressed plant (right) showed a light blue colour, indicating a higher leaf temperature (ca +1°C) and therefore a lower transpiration. Thermo-pictures will be analyzed with a dedicated image analysis software, to measure the differences in temperature.

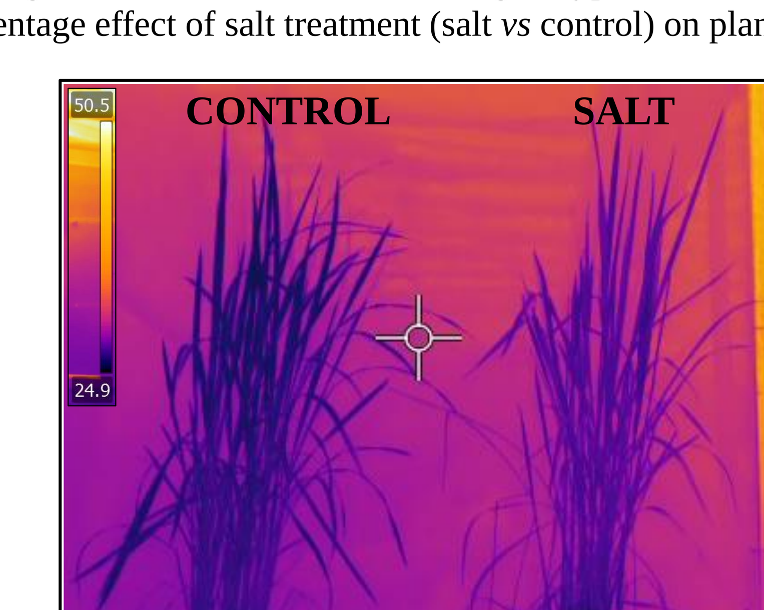

Fig. 7: thermal imaging of control (left) and salt-treated (right) plants of the same genotype

The evaluation of the physiological measurements is still in progress. The obtained results will give a comprehensive view of the physiological response of the 281 *japonica* rice genotypes to salt treatment, since the considered traits are related to different cellular processes and to different physiological stages of rice plants. This phenotyping will be repeated next year. The obtained phenotypic data will be used for a GWAS, using genotypic data derived from a Genotyping by Sequencing (for more information, see the poster “GWAS of cadmium and arsenic contents in the grain of rice plants grown under different field water managements”, Orasen et al.) to identify *loci* involved in salt tolerance.

REFERENCES

Cowie RH. *Apple snails (Ampullariidae) as agricultural pests: their biology, impacts and management*, in *Molluscs as crop pests*, G.M. Baker, Editor. 2002, CABI Publishing: Wallingford. Pp. 145–192.
Halwart M. *The golden apple snail Pomacea canaliculata in Asian rice farming systems: Present impact and future threat.* International Journal of Pest Management 1994, 40(2):199-206.
Horie T, Karahara I, Katsuhara M. *Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants.* Rice 2012, 5(1):1-18.
Huang X. Han B. *Natural Variations and Genome-Wide Association Studies in Crop Plants.* Annu. Rev. Plant Biol. 2014, 65:531–51.
James RA, Sirault XR. *Infrared thermography in plant phenotyping for salinity tolerance*. Methods Mol .Biol. 2012, 913:173-89.
Joshi RC, Sebastian LS. *Global advances in ecology and management of golden apple snails*. 2006, Nueva Ecija: Philippine Rice Research Institute.
Moreso L. *Comença a retrocedir la plaga del cargol poma*. El Punt Avuí +, 2014.
Munns R, Tester M. *Mechanisms of salinity tolerance.* Annu Rev Plant Biol 2008, 59:651-81.
Xiong L, Zhu JK. *Abiotic stress signal transduction in plants: Molecular and genetic perspectives*. Physiol. Plantarum 2001, 112:152-166.
Yáñez MV. *Cambio climático en el Mediterráneo español*. 2010: Inst. Español de Oceanografía.

ACKNOWLEDGEMENT

We acknowledge the support of NEURICE project (New commercial European RICE (*Oryza sativa*) harbouring salt tolerance alleles to protect the rice sector against climate change and apple snail (*Pomacea insularum*) invasion, Grant Agreement n. 678168).