Introduction

Iron toxicity is a nutritional disorder that widely affects lowland rice. The time and intensity of its occurrence differs between environments and the the amount of toxic Fe(II) in soil solution (Audebert et al., 2009). After its uptake and translocation, Fe(II) catalyzes reactive oxygen species, which damage cell membranes and structural components (Fang et al, 2001). Rice cultivars differ in their ability to cope with variable Fe(II) situations at different phenological stages as well as in the mechanisms involved (Sahrawat, 2004). Such mechanisms may involve the exclusion of potentially toxic Fe(II) from the root (oxidation power) or the leaf symplast, or the retention, immobilization or detoxification of Fe(II) taken up into plant tissues (Becker and Asch, 2005). The effectiveness of a given tolerance mechanism depends on the intensity and duration of the Fe stress (Asch et al., 2005). Within a given cultivar, the stress tolerance and involved mechanism may change with plant development stage and are likely to be further affected by environmental conditions (temperature, vapor pressure deficit). There is a need to match the diversity of environmental factors of iron toxicity occurrence with genotype- and development stage-specific adaptation mechanisms.

Materials and Methods

A broad-based screening of 25 rice cultivars of different origins (O. *sativa indica* / *japonica*, O. *glaberrima*, *interspecific NERICA*) in hydroponic solution allowed to select ten genotypes contrasting in their tolerance to a defined Fe(II) intensity level (1500 ppm $Fe(II)SO_4 * 7\ H_20$). These cultivars were tested in a novel and variable hydroponic set-up system under several Fe(II) stress intensities (0, 500, 1000, 1500 ppm Fe(II)) and exposure durations (2-6 days), applied at the seedling, vegetative and early reproductive growth stages (4 , 6 and 8-10 weeks old plants), and under conditions of low and high vapor pressure deficit. Tolerance levels were defined based on visual leaf bronzing symptom scoring, root iron plaque formation (quantified after removal with 0,5 M HCl), and iron partitioning (root, stems, leaf) and speciation (Fe(II) and Fe(III). Total iron was determined by atomic absorption spectrometry and active Fe(II) spectrometrically by 2,2-dipyridyl coloration.

Results

Genotypes differed in symptom expression both with time of exposure and at different growth stages under a defined Fe stress (1500 ppm Fe(II)) (*Figure 1*). While all genotypes responded similarly to an increasing duration of Fe(II) stress, highest symptom expression was observed in the sensitive check cultivars IR 31785 (O. *sativa indica*) and Nipponbare (O. *sativa japonica*). With progressing plant development, most cultivars became increasingly tolerant. This was not the case with Nipponbare and the interspecific *sativa x glaberrima* cross WAS 161 that tended to become more sensitive to Fe(II) in the vegetative and reproductive growth stages. Highest tolerance levels were observed in CK73 in the seedling stage and in ITA212 and Pokkali in the vegetative and reproductive stages, respectively. These differential sensitivity trends indicate the likelihood of different and changing tolerance mechanisms.

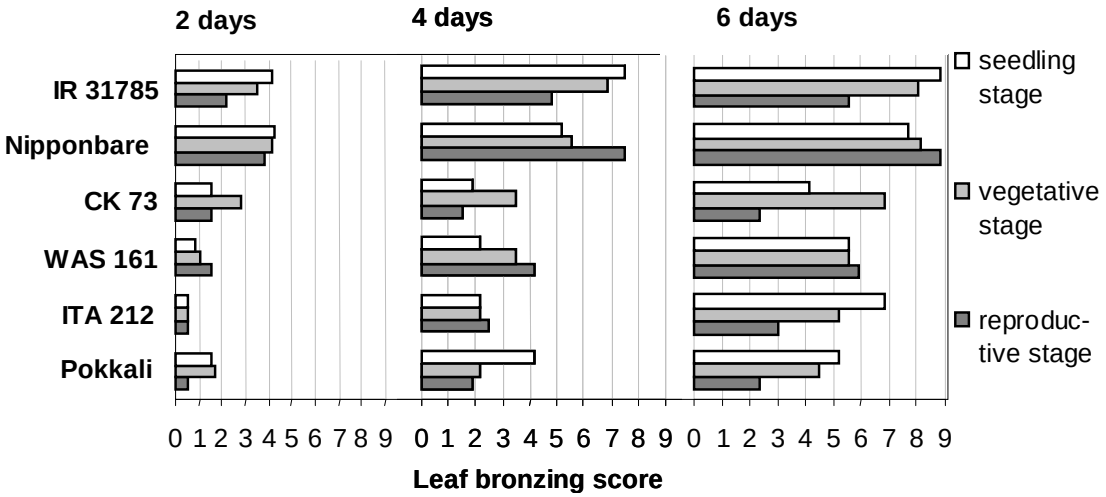

Fig. 1: Symptom score (0=no symptoms, 9=dead) of six selected rice genotypes at different growth stages after 2, 4, 6 days of exposure to Fe(II) stress.

Cultivars strongly differed in the amounts of iron taken up to the plant. Irrespective of the growth stage, most Fe(III) was found in roots, predominantly as external iron in the form of root plaque (Figure 2, lower graph). Apart from the sensitive check (IR31785) where the Fe(III) was actually inside the root, the largest root plaque formers were the tolerant cultivars CK73 and Pokkali. Here, root plaque formation was correlated with reduced iron content in the plants, indicating that the oxidation power of the roots resulted in iron exclusion. Cultivars WAS161 and CK73 showed largest Fe(III) content in stem tissues and accordingly reduced iron levels in leaves. Here, the dumping or immobilization of iron in less active tissues may be the predominant tolerance mechanism. Total leaf iron content tended to increase with plant age but in most cases no significant differences between sensitive and tolerant cultivars were observed. Consequently, total leaf iron did not correlate with symptom scores or iron-induced leaf damage and is not an appropriate indicator for iron toxicity tolerance.

This was very different when considering the active iron fraction (Fe(II)) in the leaves (Figure 2, upper graph), which strongly correlated with the observed leaf bronzing symptoms. Combining both the total and the active iron fraction in leaves may serve as a tool to determine the apoplastic exclusion. Thus, WAS161 showed high total leaf iron (Fe(III)) but little active Fe(II). We hypothesize that the potentially toxic Fe(II) has been oxidized in the apoplast and thus was prevented from entering the symplast. Using WAS161 (high Fe(III)/Fe(II) ratio) and Nipponbare (low Fe(III)/Fe(II) ratio) the hypothesized mechanism of apoplastic exclusion will be further investigated with radioactive isotope studies.

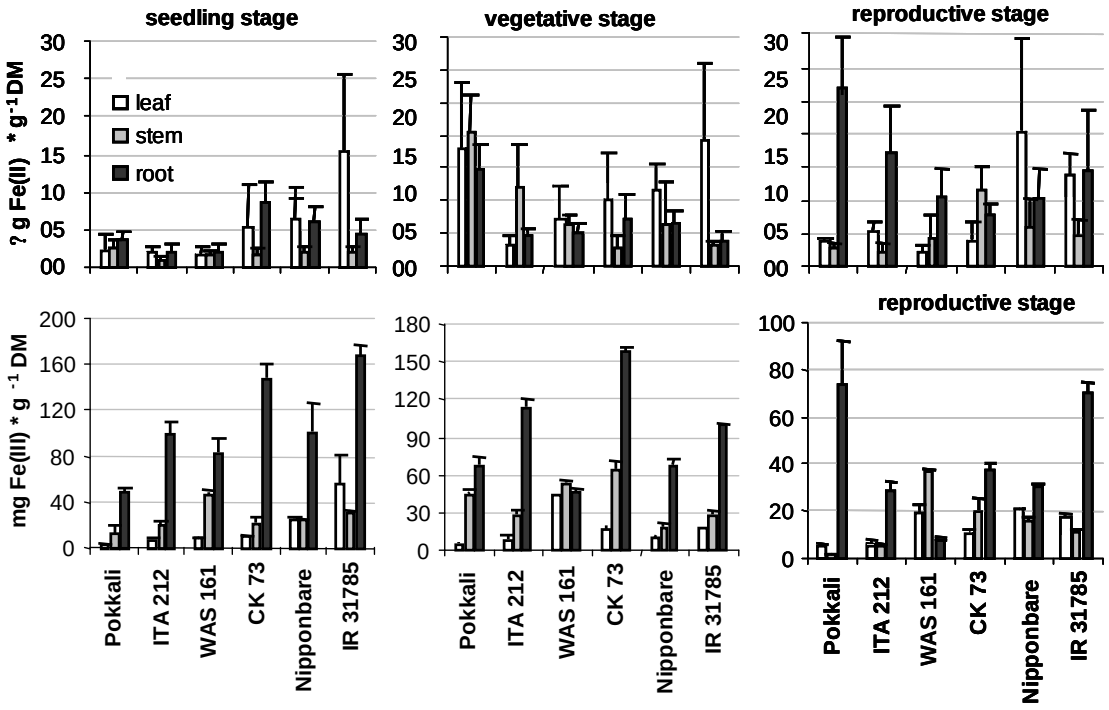

Fig. 2: *Distribution of Fe(II)and total Fe [mg g^{-1} TM] to roots, stems and leaves of six contrasting rice genotypes at different growth stages (1500 ppm Fe(II), 6 d stress period. Bars present means± SD).*

The presented preliminary results indicate that genotypes differentially resist diverse Fe toxic conditions (intensity and duration of Fe(II) exposure). Tolerance levels further differ between development stages of rice. Iron partitioning among organs and iron speciation (ratio of total Fe(III) and active Fe(II)) is seen to provide information on possible tolerance mechanisms. Ongoing work on the effects of temperature and vapor pressure deficit on iron uptake and translocation and further iron speciation in the leaf apoplast and symplast will be presented.

References

Audebert, A., Fofana, M., 2009, *Rice Yield Gap due to Iron Toxicity in West Africa*, J. Agron. Crop Sci. 195, 1, 66-76.

Asch, F., Becker, M., Kpongor, D. S., 2005, *A quick and efficient screen for resistance to iron toxicity in lowland rice*, J. Plant Nutr.Soil Sci. 168, 6, 764-773.

Becker, M., Asch, F., 2005, *Iron toxicity in rice-conditions and management concepts*, J. Plant Nutr. Soil Sci. 168, 4, 558-573.

Fang, W. C., Wang, J. W., Lin, C. C., Kao, C. H., 2001, Iron *induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves*, Plant Growth Regulation 35, 1, 75-80.

Sahrawat, K. L., 2004, *Iron toxicity in wetland rice and the role of other nutrients*, J. Plant Nutr. 27, 8, pp 1471-1504.