

IDENTIFICATION OF MOLECULAR MARKERS (RAPD) AND DETERMINATION
OF THE PROLINE CONTENT AND ANTIOXIDANT ACTIVITIES FOR SALT
TOLERANCE IN RICE

By

MOHAMED ISMAIL SITHY SAFEENA ✓

Thesis

Submitted in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in the

POSTGRADUATE INSTITUTE OF AGRICULTURE

of the

UNIVERSITY OF PERADENIYA

PERADENIYA

OCTOBER 2004

557213 ✓

C 633.18
S12



557213

AGRICULTURE LIBRARY
UNIVERSITY OF PERADENIYA

ABSTRACT

Rice crops are subjected to various stress conditions that reduce the yield and quality of grain produced. Salinity response in rice is one such condition, showing multigenic nature and intra-crop variability. The present study attempts to identify molecular markers and determine the biochemical and physiological aspects of salt tolerance in rice.

Seventy-two randomly selected rice accessions were initially screened physiologically for salt tolerance in a greenhouse using a salt solution of 12 dS m^{-1} . They were identified into three major groups as highly tolerant (22 varieties), moderately tolerant (21 varieties) and sensitive (29 varieties).

The PCR conditions were perfected in order to obtain more elucidative RAPD profiles. Rice varieties showing difference to salt tolerance were initially screened for DNA polymorphism using 60 RAPD primers. Of these, 10 primers were selected and used to screen all 72-rice varieties. The selected ten primers produced 132 bands and the most observable bands (80%) were within the range of 0.2 Kb – 3.0 Kb. The most common bands were observed in the range of 1.60 Kb - 1.69 Kb.

The primers OPS3 and OPAK10 produced specific bands in highly tolerant varieties at 510 bp and 470 bp, respectively. The band OPS3₅₁₀ was present very distinctly in 17 out of 22 highly tolerant varieties and was absent in all moderately tolerant and

sensitive varieties. OPAK10₄₇₀ was present in 5 highly tolerant and 3 moderately tolerant varieties. Interestingly, both bands were present in proven highly tolerant varieties and absent in known sensitive varieties. The band OPS20₁₉₅₀ was observed in highly tolerant and moderately tolerant varieties. This polymorphic band was not considered to be a marker for salt tolerant since it was observed with two sensitive varieties as well. OPAK18₉₅₀ was common in moderately tolerant varieties than in other groups of varieties. Among the polymorphic bands observed, OPS3₅₁₀ was unique to highly tolerant varieties and, therefore, it was considered as a putative molecular marker for salt tolerance.

Three dendrograms were constructed based on RAPD banding patterns generated respectively, by selected ten primers, four best primers (OPS3, OPS20, OPAK10, OPAK18) and just OPS3. Neither the dendrogram based on ten primers nor the one with just OPS3 was suitable to group rice varieties for salinity response. The dendrogram generated with four best primers identified salinity response clearly among the three groups of varieties (highly tolerant, moderately tolerant and sensitive). It is suggested that by using the above four primers, rice varieties could be screened for salt tolerance. All dendrograms could separate the wild rice (*O. rufipogon*) distinctly from *O. sativa* varieties.

The biochemical and physiological aspects of this study were focused to investigate the effect of salinity on the parameters associated with photosynthesis (transpiration rate, stomatal conductance, leaf area, photosynthesis rate and chlorophyll content) and to determine the proline accumulation and antioxidant enzyme activity in four rice

varieties (Nona Bokra, Pokkali, At 353 and IR 28) at three growth stages at EC= 0, 4, 6 and 8 dS m⁻¹ in order to understand the metabolic adjustments and biochemical modification made by the rice plant during seedling, tillering, and flowering stages under high salinity.

A drastic decline in the rate of transpiration was observed when salinity was imposed. IR 28 maintained the lowest rate of transpiration, which ranged from 3%-28% at 4 and 8 dS m⁻¹ respectively. Nona Bokra and Pokkali were able to maintain a significantly high rate of transpiration. The reduction in the rate of transpiration was accompanied by a simultaneous increase in stomatal diffusive resistance with increasing salt levels. The highest stomatal diffusive resistance was observed at EC= 6 and 8 dS m⁻¹ compared to 4 dS m⁻¹. The stomatal diffusive resistance was increased in IR 28 and At 353 by 208% and 107%, respectively, compared to the control during the experimental period.

The induction of salt stress significantly decreased leaf area by 48-58% at 8 dS m⁻¹. The exposure of plant to salinity decreased the photosynthesis rate significantly in all varieties at all sampling dates. The photosynthesis rate was reduced by 14-70% at 8 dS m⁻¹ in all varieties. Total leaf chlorophyll content also declined by 9-64% in all varieties. The reduction of photosynthesis rate under high salinity resulted in a reduction of plant survival by 28-50% during the experiment at EC= 4, 6, 8 dS m⁻¹.

The proline accumulation was higher in leaves than in roots. At tillering stage, most varieties accumulated higher amounts of proline in leaves compared to other stages.

It was found that accumulation of proline at seedling stage might be used as a physiological marker (index) of salt tolerance. Only Nona Bokra showed 71% increase of proline at the flowering stage at the highest salt level while Pokkali, At 353 and IR 28 showed a significant reduction at this stage. The pattern of change in the root proline was the same as that of leaf proline.

The possible involvement of activated oxygen species in the mechanism of damage by salt stress was studied during the experiment. At all growth stages, highly tolerant varieties had higher super oxide dismutase (SOD) activity than moderately tolerant and/ or sensitive varieties. The constitutive level of catalase (CAT) at seedling stages was significantly higher in Pokkali and Nona Bokra (62% and 107% respectively) compared to their counterparts. The salt treatment resulted in an increase of peroxidase (POD) activity in At 353 (174%) and in IR 28 (116%) compared to Nona Bokra at 6dS m^{-1} . With the increasing salt levels, the concentration of malonidialdehyde (MDA) was increased in the sensitive and moderately tolerant varieties at all growth stages.

The study showed that high salinity had severe impact on stomatal regulation and transpiration rate. Also, induced salinity significantly decreased the leaf area, photosynthesis rate and chlorophyll content. The study on antioxidant enzymes activity indicates that free radical-mediated damage of membranes may play an important role in the cellular toxicity of salt in rice and that highly tolerant varieties exhibit a protection mechanism against increased radical production by maintaining the specific activity of antioxidant enzymes.