ScienceAsia 27 (2001) : 93-97

The Solute Accumulation: The Mechanism for Drought Tolerance in RD23 Rice (*Oryza sativa* L) Lines

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Received 27 Apr 2000
Accepted 27 Feb 2001

ABSTRACT Four drought tolerant RD23 rice lines were selected from somaclonal variants arising *in vitro*. They had been selected under the drought condition for 5 generations before the progenies were used for the experiments. After five weeks in the nutrient solution containing 150 g/L PEG6000, the six-week old drought-tolerant seedlings had approximately 4-fold increase in total soluble sugar content, while the original drought sensitive line had only 2.5-fold, when compared with the non-stressed plants. Proline content was also determined in these rice lines. After five weeks of drought treatment, nine-to fifteen-fold increase in proline content was detected in the drought tolerant lines, while the original line had approximately five-fold increase in proline content. These data suggested that the ability to accumulate the solute contributes to better performance in drought-tolerance.

KEYWORDS: *Oryza sativa* L, drought stress, solute accumulation, proline, total soluble sugar.

INTRODUCTION

Plant growth and productivity are negatively affected by water stress and other environmental stress.[1] Genetic improvement of water stress tolerance is important to agricultural plants.

Dehydration tolerance has been investigated using three main approaches in plants: examining tolerant systems, such as seeds and resurrection plants, analyzing mutants from genetic model species, and analyzing the effects of stress on agriculturally relevant plants.[2] The elucidation of how plants tolerate water stress leads to crop improvement in the future.

Water loss from plant tissues under drought conditions results in growth inhibition and in a number of other metabolic and physiological changes. These include abscisic acid (ABA) accumulation,[3] stomatal closure,[4] changes in leaf water potential,[5] the decreased photosynthesis[6] and solute accumulation.[7-8]

Ingram and Bartels[2] proposed that metabolite accumulation is one of the mechanisms for stress tolerance. Several solutes can be accumulated for osmoprotectant, such as proline,[9-11] glycine betaine,[12] and sugars.[5,10,13]

This investigation was to demonstrate that four new drought tolerant rice lines, originated from somaclonal variation *in vitro*, have higher ability in solute accumulation than the original line, which suggest that the solute accumulation is one of the mechanisms for drought tolerance in rice.

MATERIALS AND METHODS

Plant Materials

Five rice (*Oryza sativa* L) lines were used for all experiments. Four of them were drought-tolerant lines, selected from RD23 regenerated seedlings *in vitro*. RD23 rice seedlings were induced for callus formation, then the calli were regenerated and grown to the whole plants, according to Vajrabhaya and Vajrabhaya.[14] Seeds produced from these regenerated lines were used for drought tolerant selection in later generations as indicated below. It was expected that genetic changes resulting from somaclonal variation arising *in vitro* during the plant tissue culture process led to the drought-tolerant character.

The original line, RD23, obtained from Rice Research Institute,Pathumthani, Thailand, was used as a control in all experiments.

Selection for drought tolerant lines

Drought tolerant lines were selected under artificial drought stress condition using polyethylene glycol 6000 (PEG6000). Seven-day old seedlings with 1 centimeter-long coleoptiles were grown in a modified WP nutrient solution[14] containing 150 g/L PEG6000, under 35 mmole.m^{-2}.s^{-1} light intensity at the leaf level, 12/12 h photoperiod, at 31-33°C air temperature for one month. The seedlings were transferred to the freshly prepared nutrient solution every week. Within this growing condition, the original line, RD23 has approximately 3% survival rate, whereas the regenerated lines, with higher survival percentage, were considered as drought-tolerant lines. The seedlings, surviving from the drought stress, then were grown in sand culture using a modified WP nutrient solution[14] in the planting house for seed production. The progenies of drought-tolerant lines were further selected in the drought condition as above for 4 generations. Seedlings of three independent drought-tolerant lines, RD23A1, RD23B1 and RD23C1, and one sibling tolerant line, RD23B2, obtained from the last selection, were used for the experiments.

Proline and total soluble sugar measurement

Free proline was quantified in leaf tissues, according to Bates *et al*[15], and total soluble sugar (TSS) was determined by anthrone method.[10]

Experimental design

The experiment of solute concentration determination was designed for CRD[16] (Completely Randomized Design) with six replicates. Five rice lines, four of which were drought tolerant lines, and a normal original line of RD23, were used for all experiments. Seven-day old seedlings, with 1 cm.-long coleoptiles were subjected to drought-stress condition as used for the selection. Determination of solute accumulation in leaves was done once a week for six weeks. Seedlings grown in the nutrient solution without PEG6000 in the same growing condition were used as a control. After six weeks in the stress condition, the seedlings were then transferred to the normal nutrient solution, and the solute measurement was done after growing in the non-stress condition for one week.

Statistical Analysis

Six replicates were used for each treatment, and two of independent plant extracts were used for each replicate. Each solute concentration was subjected to analysis of variance, and Duncan's New Multiple Range Test (DMRT) was used to test for the mean differences among lines under stress or non-stress condition.

Results and Discussion

Drought tolerant lines have higher survival rates under drought condition than the original line.

Seedlings of drought-tolerant lines and the original line had similar phenotypes when growing in the non-stress condition. However, the drought-tolerant lines showed better characters after one month under drought condition. Most of the control died after one month of drought treatment. Only 3-5% seedlings survived. The fifth generation seedlings of drought tolerant lines, RD23A1, RD23B1, RD23B2, and RD23C1 had drought survival rate of 99, 71, 55, and 64 %, respectively.

Without drought stress, all rice lines showed no difference in solute accumulation, but drought stress induced higher level of solute accumulation in leaves.

Proline and TSS contents in the seedlings grown in the modified WP nutrient solution were

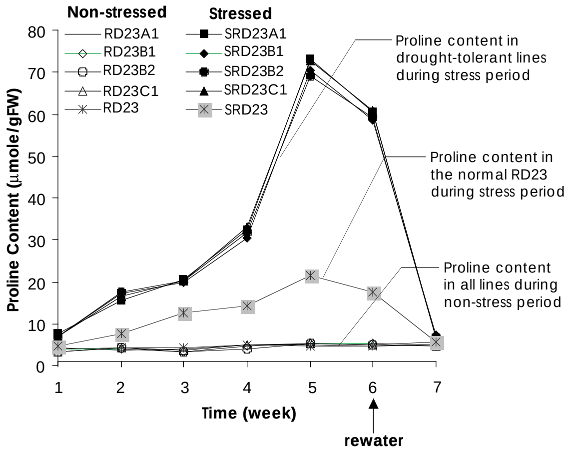

Fig 1. Proline content in stressed and non-stressed rice seedlings. The drought treatment experiment was performed for 6 weeks then the stressed plants were rewatered. After one week in non-stressed condition, the proline content was determined.

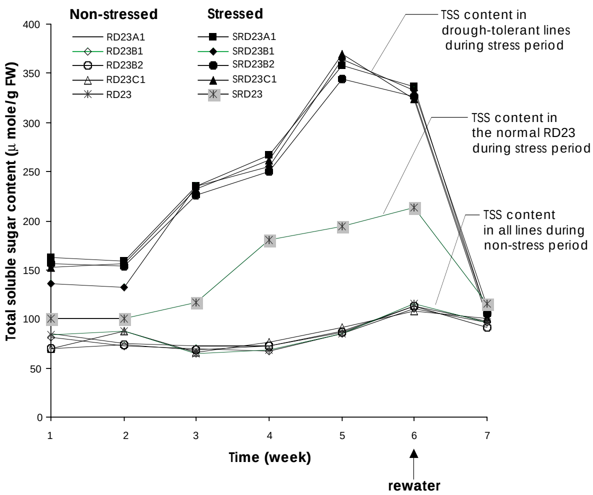

Fig 2. Total soluble sugar content in stressed and non-stressed rice seedlings. The drought treatment experiment was performed for 6 weeks then the stressed plants were rewatered. After one week in non-stressed condition the total soluble sugar content was determined.

determined every week for six weeks. No significant difference of proline accumulation level was detected among lines, grown in the nutrient solution, containing no PEG6000. The similar result was also observed with the TSS content in rice leaves (Fig 1 and Fig 2)

After a week under drought stress, all rice lines accumulated higher proline level than the ones grown in non-stress condition. The data showed that the proline level had increased for 5 weeks and then dropped down in the sixth week (Fig 1). The proline level of normal RD23 was significantly lower than those of the drought-tolerant lines, when plants were grown in the same condition as shown in Table1 and Fig 1. Similar responses was observed when the total soluble sugar level was determined (Table2, and Fig 2).

The solute concentrations were decreased to the normal level, when stressed plants were rewatered.

After six weeks under drought stress, all rice seedlings were transferred to the nutrient solution containing no PEG. One week later, the proline and TSS contents in leaves were measured. It was found that the proline and TSS accumulation levels were similar to what was found in the non-stress plants (Fig 1 and Fig 2 week 7)

Table 1. Proline accumulation level in rice seedlings grown in stress condition for 6 weeks.

Rice Line	Proline level (mmole/g FW)					
	Week1	Week2	Week3	Week4	Week5	Week6
RD23A1	7.62[a]	15.69[a]	20.59[a]	32.52[a]	72.95[a]	60.5[a]
RD23B1	7.1[a]	17.22[a]	19.99[a]	30.31[a]	70.42[a]	58.58[a]
RD23B2	6.9[a]	17.46[a]	20.23[a]	31.87[a]	69.05[a]	59.18[a]
RD23C1	7.1[a]	16.58[a]	20.07[a]	33.16[a]	72.71[a]	60.86[a]
RD23	4.77[b]	7.67[b]	12.6[b]	14.17[b]	21.44[b]	17.54[b]

In column, figures having similar letter do not differ significantly whereas figures bearing dissimilar letters differ significantly (as per DMRT)

Table 2. Total soluble sugar content in rice seedlings grown in stress condition for 6 weeks.

Rice Line	Total soluble sugar level (mmole/g FW)					
	Week1	Week2	Week3	Week4	Week5	Week6
RD23A1	162.37[a]	158.59[a]	234.35[a]	266.38[a]	358.53[a]	337.07[a]
RD23B1	135.80[a]	131.44[a]	234.93[a]	255.86[a]	364.17[a]	332.71[a]
RD23B2	155.75[a]	153.25[a]	225.80[a]	250.62[a]	343.82[a]	326.90[a]
RD23C1	152.32[a]	156.50[a]	232.49[a]	261.79[a]	369.63[a]	323.82[a]
RD23	100.11[b]	100.05[b]	117.14[b]	179.93[b]	194.17[b]	213.13[b]

In column, figures having similar letter do not differ significantly whereas figures bearing dissimilar letters differ significantly (as per DMRT)

Table 3. Proline and total soluble sugar contents in rice leaf tissues after the seedlings were grown in the non-stress and stress conditions for 5 weeks.

Rice lines	Proline content (µmole/g FW)		Total soluble sugar content (µmole/g FW)	
	non stress	stress	non stress	stress
RD23C1	5.18[a]	72.71[b]	90.98[a]	369.63[b]
RD23B2	5.30[a]	69.05[b]	86.21[a]	343.82[b]
RD23B1	5.26[a]	70.42[b]	85.63[a]	364.17[b]
RD23A1	4.81[a]	72.95[b]	87.61[a]	358.53[b]
RD23	4.61[a]	21.44[a]	85.22[a]	194.17[a]

In column, figures having similar letter do not differ significantly whereas figures bearing dissimilar letters differ significantly (as per DMRT)

The proline and total soluble sugar accumulation during drought stress in rice seedlings was similar to the response in other species; for example wheat (*Triticum durum*)[9], sorghum (*Sorghum bicolor*)[5], alfalfa (*Medicago sativa*)[10], and cassava *(Manihot esculenta*).[17]

The drought-tolerant lines accumulated up to fifteen fold of proline content after five week of dehydration stress while the original line, RD23 increased approximately five folds (Table3).

Drought tolerant lines TSS accumulation ability were also better than the normal line's. Approximately 4 fold increasing in TSS content was detected in the tolerant lines while only 2.5 fold accumulation was observed in the original line (Table3).

This data suggest that the drought tolerance, at least in these rice lines, is correlated with the proline and total soluble sugar accumulation ability. Not only playing role in the osmotic adjustment during the water stress, the proline accumulation is also considered to be involved in the protection of the enzymes[18] and cellular structure[19] and to act as a free radical scavenger.[20]

Discussion

Although, several studies have shown that proline accumulation during stress plays an important role as the protective agent, the salt tolerance in rice does not show the positive correlation with proline accumulation.[21] It was shown that the salt sensitive cultivar, Kong Pao, had the higher proline accumulation level than the salt tolerant cultivar, Nona Bokra during salt stress condition. However, it cannot be ruled out the protective role of proline under salt stress in other plant species according to the increase in salt resistant character in the transgenic tobacco with the overexpression of Δ^1-pyrroline-5-carboxylate synthetase, which had the high proline level.[22]

A large number of genes which confer several mechanisms for water stress tolerance have been shown[2] and the molecular study of these genes are in progress in some model plant species, for example *Arabidopsis thaliana* L.[23] Therefore the understanding of drought-tolerant gene expression will likely lead to the improvement of the agricultural crop in the future.

Conclusion

At present we obtain the drought tolerant rice lines, some of which is highly tolerant to drought stress at seedling stage. It was clearly shown that all of our drought tolerant lines had improved the solute accumulation ability during drought condition, in correlation with the improvement of drought tolerant character in these lines.

As these drought tolerant lines originated from the same original, RD23, they all should have the same genetic background, except the mutated gene(s) that are responsible for the drought tolerant character. However, it cannot be ruled out the possibility that there may be some other hidden mutated genes occurred during *in vitro* culture.

A number of genes responsible for drought tolerant characters can be determined by conventional methods by analyzing the segregation of drought tolerant levels in the progenies, parents of which have different ability in drought tolerance. On the other hand, molecular cloning of the drought tolerant genes from the cDNA or genomic libraries of these drought tolerant lines is also possible.

Based on this studies, the time point of drought tolerant expression in the drought tolerant lines and the mechanisms we learned from this research will gear us to the right direction of drought tolerant gene cloning and would lead to its application in the next step.

Acknowledgements

This research was supported by Thailand Research Fund (1998-1999). We are grateful to Prof Dr Thavorn Vajrabhaya and Assoc Prof Dr Preeda Boon-Long for critical reading of the manuscript. Further thanks are due to anonymous referee for the useful comments which made the considerable improvement of this article.

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