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The Solute Accumulation : The Mechanism for Drought Tolerance in New Thai Rice (*Oryza sativa* L.) Cultivars.,

(Montakarn Vajarabhaya, Thavorn Vajarabhaya, Warunya Kumpun and Supachitra Chadchawan) ซึ่งจะจัดขึ้นระหว่างวันที่ 1-7 สิงหาคม 2542 ณ เมือง St.Louis ประเทศสหรัฐอเมริกา และกำลังอยู่ในระหว่างดำเนินการจัดทำเอกสารเพื่อ submit ต่อ วารสาร Plant Cell Report

ภาคผนวก

The Solute Accumulation: The Mechanism for Drought Tolerance in New Thai Rice (*Oryza sativa* L.) Cultivars.

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Abstract

Four new Thai rice cultivars were selected from somaclonal variation *in vitro*. They had been selected under the drought pressure for 5 generations before the progenies were used for the experiments. After five weeks in the nutrient solution containing 150g/L PEG, the six week-old drought tolerant seedlings performed approximately 4 fold increasing in sugar content, while the original drought sensitive line had only 2.5 fold increasing, when compared to the non-stressed plants. Proline content was also determined in these rice lines. When the seedlings were grown under the drought condition for five weeks, nine to fifteen fold increasing in proline content was detected in the drought tolerant lines, while the original line showed about five fold increasing in proline content. These data suggested that the better ability in solute accumulation contributed to the better performance in drought tolerance of these new Thai rice varieties.

Introduction

Plant growth and productivity are negatively affected by water stress and other environmental stress (Boyer, 1982). 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 (Ingram and Bartels, 1996). The elucidation of how plants tolerate to 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 ABA accumulation (Mansfield and McAinsh, 1995), stomatal closure (MacRobbie, 1991), changes in leaf water potential (Premachandra et al, 1995), the decreased photosynthesis (Hsiao, 1973) and solute accumulation (Skriver and Mundy, 1990; Bray, 1991).

It was proposed that metabolite accumulation is one of the mechanisms for stress tolerance (Ingram and Bartels, 1996). Several solutes can be accumulated for osmoprotectant, such as proline (Mattioni et al, 1997; Irigoyen et al, 1992; Griousse et al, 1992), glycine betaine (Bohnert et al, 1995). And sugars (Premachadra et al, 1995; Pelah et al. 1997; Irigoyen et al. 1992).

Here, we demonstrated that four new drought tolerant rice lines, originated from somaclonal variation *in vitro*, have better ability in solute accumulation than the original line. Thus, it leads to their drought tolerant property.

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 plants were induced for callus formation, then the calli were regenerated to the whole plants *in vitro*. Seeds produced from these *in vitro* regenerated lines were used for drought tolerant selection in later generations. It was expected that genetic changes resulted from somaclonal variation *in vitro* during the plant tissue culture process led to the drought tolerant character.

The original line, RD23, obtained from Rice Department, 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 the modified WP (Vajarabhaya and Vajarabhaya, 1991) nutrient solution, containing 150 g/L PEG6000, under 1500lux light intensity, 12/12 photoperiod, at temperature of 31-33 degree Celsius for one month. Within this growing condition, the original line, RD23 has approximately 3% survival, while the regenerated lines, having higher percent survival, were considered as drought tolerant lines. The seedlings, survived from the drought stress, then were sand grown and supplemented with modified WP nutrient solution, in the greenhouse for seed production. The progeny of each drought tolerant line was further selected in the drought condition as above for 4 generations. Seedlings of three independent drought tolerant lines, and one sibling tolerant line, RD23A1, RD23B1, RD23C1, and 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 (1973), and total soluble sugar (TSS) was determined by anthrone method (Irigoyen et al, 1992).

Experimental design

The solute concentration determination experiment was designed for Factorial in CRD (Factorial in Completely Randomized Design) with six replicates. Five rice lines, which were four of the drought tolerant lines, and the original line RD23, were used for testing. Seven-day old seedlings, with 1 cm. long coleoptiles were subjected to drought stressed 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 condition were used as controls. After six weeks in the stressed condition, seedlings were transferred to the normal nutrient solution, and the solute measurement was done after growing in the non-stressed condition for a week.

Statistical Analysis

Each solute concentration was subjected to analysis of varie4nce, and Duncan's New Multiple Range Test (DMRT) was used to test for differences among lines under stress or non-stress condition.

Results

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

Seedlings of drought tolerant lines and the original line, RD23, are similar when growing in the non-stress condition. However, drought tolerant lines showed the better characters after a month under drought stress as shown in Fig.1. Most of the RD23 died after one month of drought treatment. Only 3-5% seedlings survived with green leaves. The fifth generation seedlings of drought tolerant lines, RD23A1, RD23B1, RD23B2, and RD23C1 had drought survival percentage of 99, 71, 55, and 64, respectively.

Without drought stress, all rice lines showed no difference in solute accumulation.

After one week-old seedlings were subjected to the modified WP nutrient solution without PEG, proline and TSS levels were determined every week for six weeks. The results were shown in Table1 and 2. No significant difference of proline accumulation level was detected among lines. The similar result was also observed with the TSS level data.

Table1 Proline accumulation level (mole/g fresh weight) in rice seedlings grown in non stress condition for 6 weeks

Rice Line	Proline level (mole/g fresh weight)					
	Week1	Week2	Week3	Week4	Week5	Week6
RD23A1	4.37	3.53	4.13	4.81	4.81	4.77
RD23B1	4.01	3.97	3.20	4.73	5.26	5.26
RD23B2	3.45	4.45	3.37	4.13	5.30	5.09
RD23C1	3.37	4.21	3.53	4.93	5.18	4.85
RD23	3.97	4.37	4.21	5.05	4.61	4.61

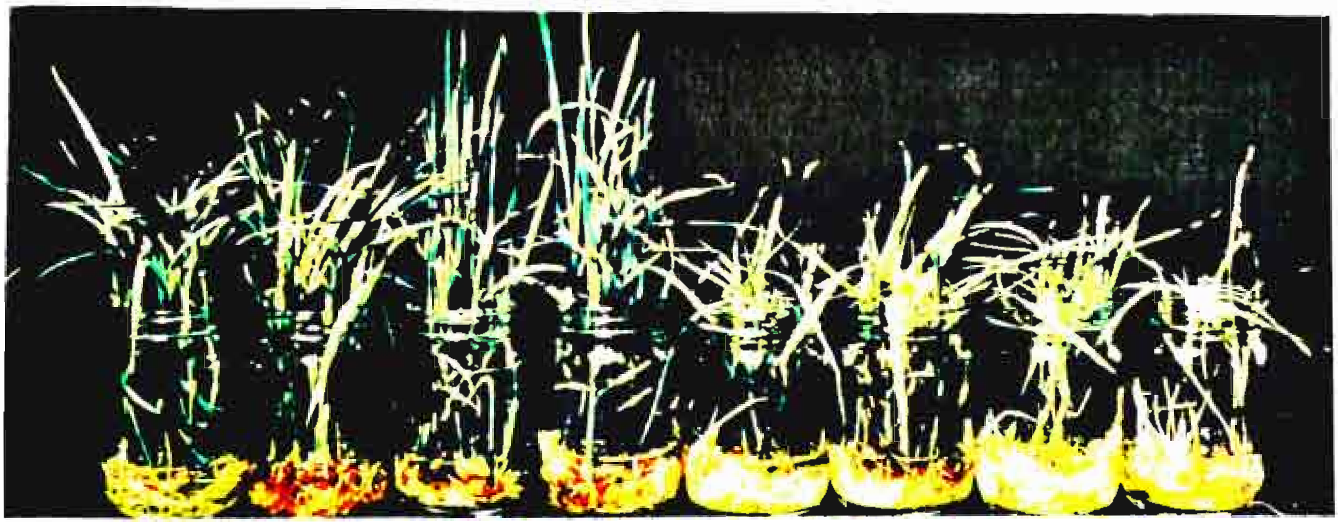

Fig. 1 Morphology of rice seedlings after one month of drought stress condition. From left to right, RD 23A1, RD23A2, RD23A3, RD23B1, RD23B2, RD23B3, RD23C1, and RD23

Table2 Total soluble sugar accumulation level (μmole/g fresh weight) in rice seedlings grown in non stress condition for 6 weeks

Rice Line	Total soluble sugar level (μmole/g fresh weight)					
	Week1	Week2	Week3	Week4	Week5	Week6
RD23A1	85.69	75.23	72.55	72.67	87.61	112.49
RD23B1	80.81	72.84	69.35	67.55	85.63	110.22
RD23B2	69.88	73.54	68.13	71.91	86.21	112.72
RD23C1	70.05	87.78	66.33	75.69	90.98	108.19
RD23	83.48	87.73	64.53	68.95	85.22	114.99

Drought stress induced the increase of solute content in rice leaves

After a week under drought stress, all rice lines accumulated higher proline level than what grown in non-stress condition (Fig. 2). The data showed that the proline level had been increased for 5 weeks and then dropped down in the sixth week (Fig. 2 and Fig.3). The proline accumulation level in original RD23 was significantly different from what in the tolerant lines, when plants were grown in the same condition as shown in Table 3 and Fig.3.

The similar response was also observed when the total soluble sugar level was determined (Table 4, Fig.4 and Fig.5).

Table 3 Proline accumulation level (μmole/g fresh weight) in rice seedlings grown in stress condition for 6 weeks

Rice Line	Proline level (μmole/g fresh weight)					
	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

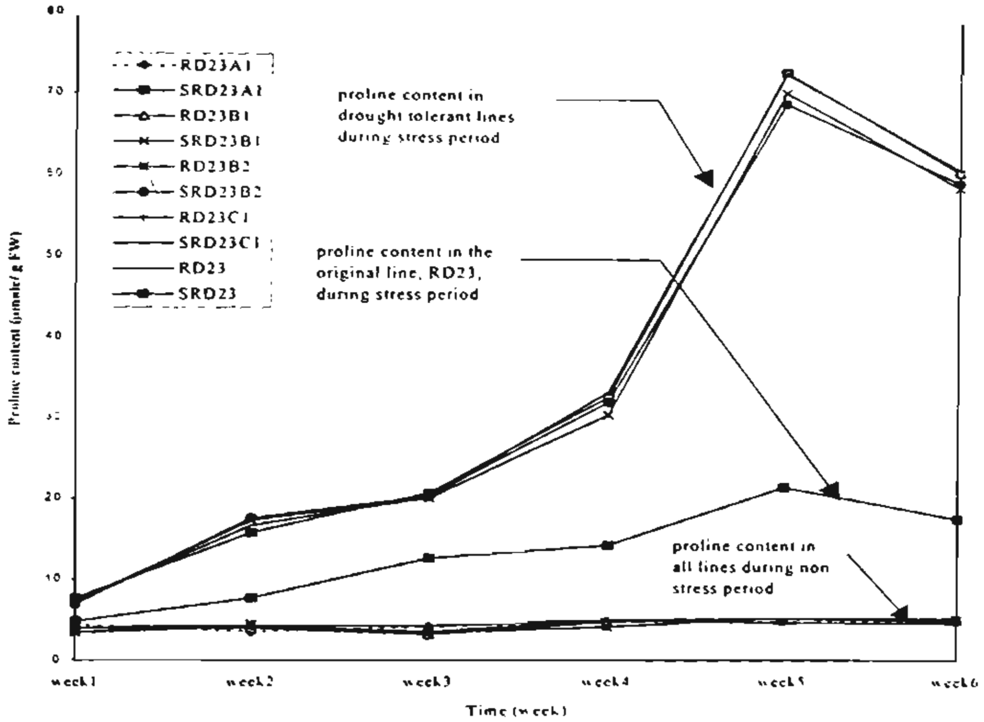

Fig. 2 The comparison of proline content (µmole/g FW) in stressed and non- stressed plants during 6 weeks of the treatments.

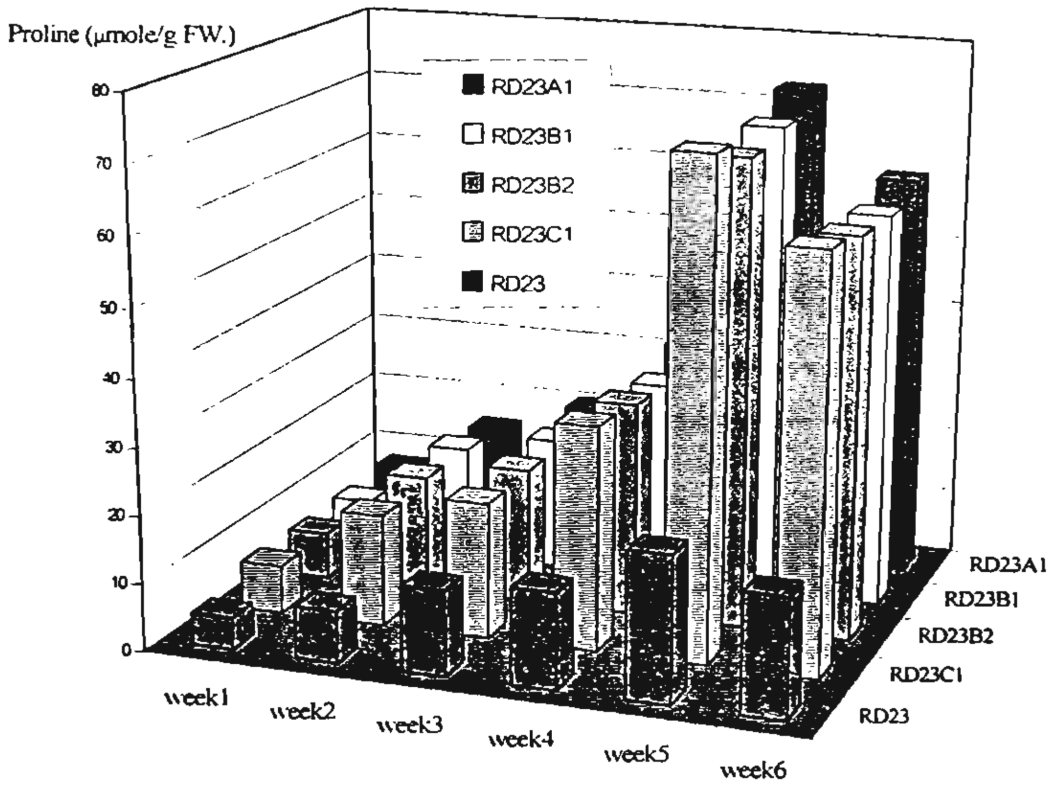

Fig. 3 Proline content (µmole/g FW) in drought stressed seedlings during 6 weeks of the drought treatment.

Table 4 Total soluble sugar content (µmole/g fresh weight) in rice seedlings grown in stress condition for 6 weeks

Rice Line	Total soluble sugar level (µmole/g fresh weight)					
	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]

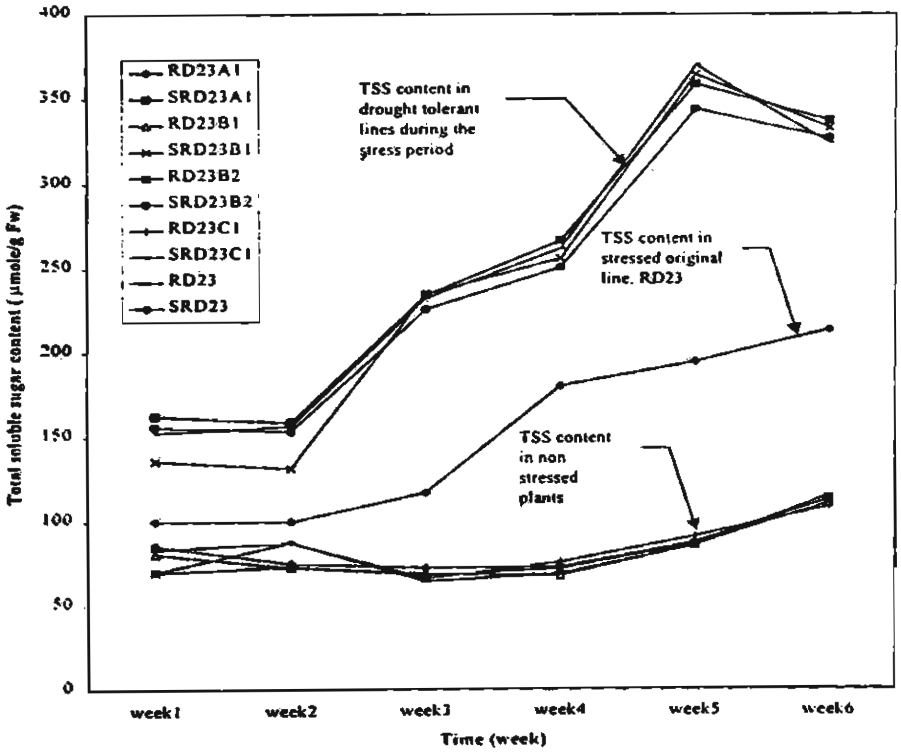

Fig. 4 The comparison of total soluble sugar (TSS) content (µmole/g FW) in stressed and non- stressed plants during 6 weeks of the treatments.

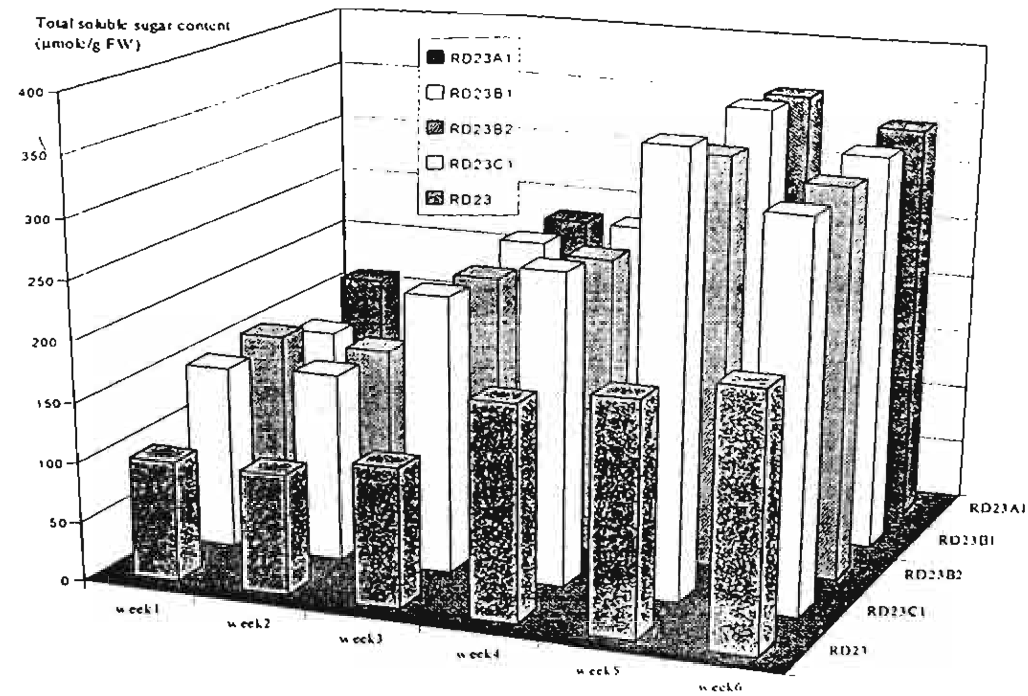

Fig. 5 Total soluble sugar content (µmole/g FW) in drought stressed seedlings during 6 weeks of the drought treatment.

The solute concentration levels were decreased, when stressed plants were rewatered.

After six weeks under drought stress, all rice seedlings were moved to grow in the nutrient solution containing no PEG. A week later, the proline and TSS content in leaves were measured. It was found that the proline and TSS accumulation levels were similar to what found in the non-stress plants (Fig.6 and Fig. 7).

Discussion

The proline and total soluble sugar accumulation, occurring during drought stress in rice seedlings is similar to the response in other species; for example wheat (*Triticum durum*) (Mattioni et al, 1997), sorghum (*Sorghum bicolor*) (Premanchandra et al, 1995) and alfalfa (*Medicago sativa*) (Irigoyen et al, 1992).

The drought tolerant lines accumulates up to fifteen fold of proline content after five week of dehydration stress while the original line, RD23 showed about five fold increasing (Table5).

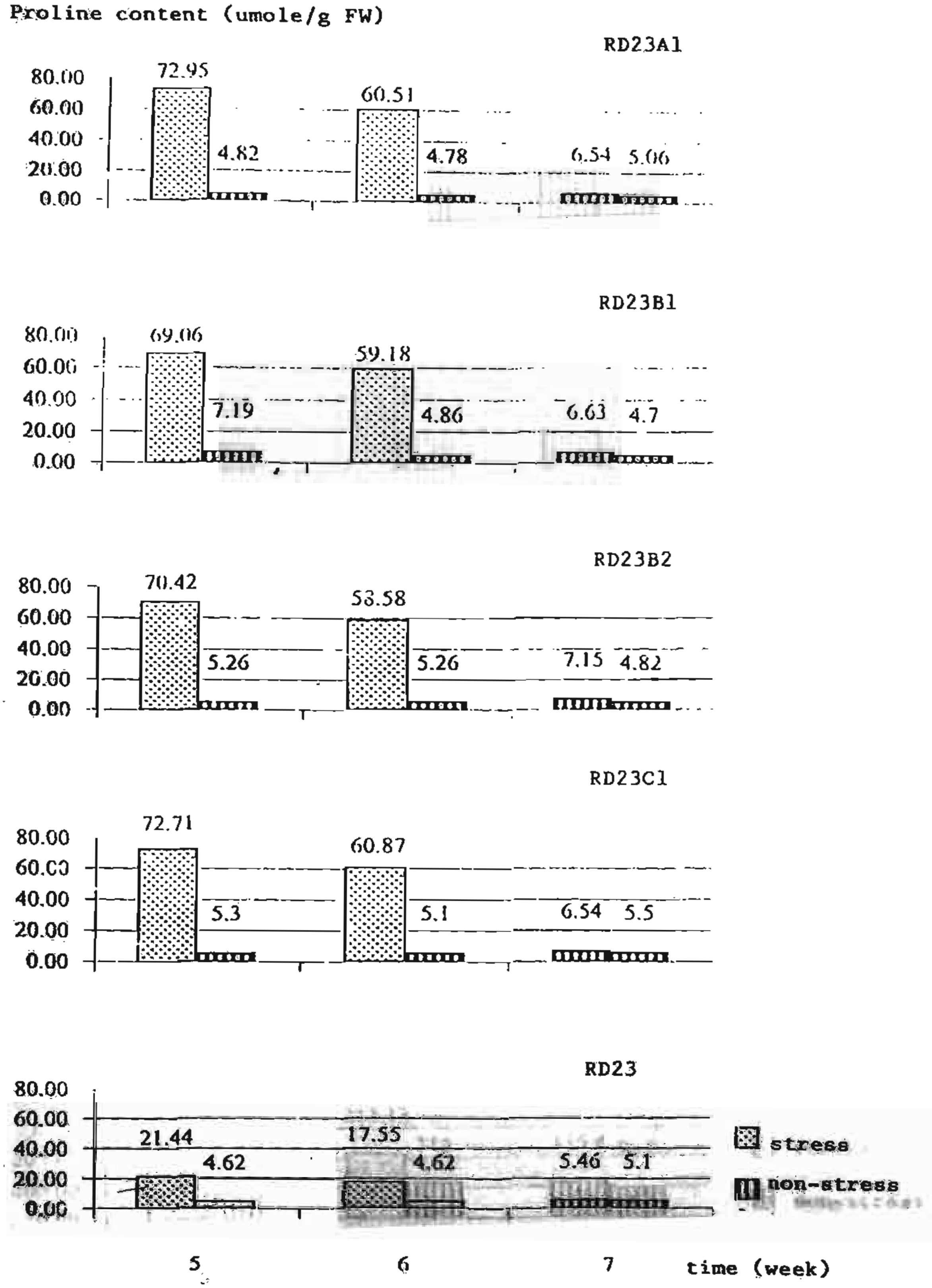

Fig. 6 Proline content in stressed and non stressed plants during week5-week7. After the sixth week, the drought stressed plants were moved out of the stress condition.

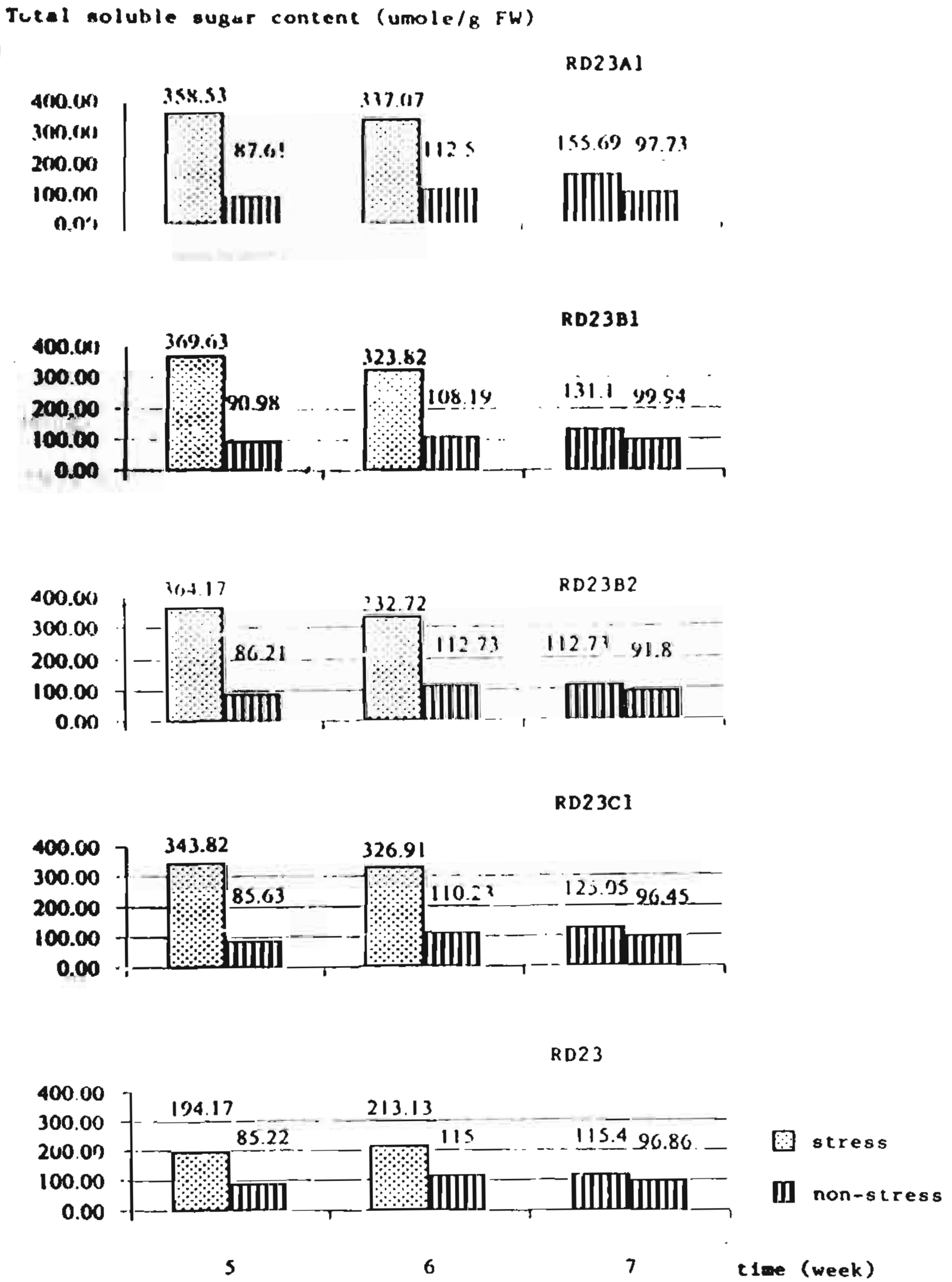

Fig. 7 Total soluble sugar content in stressed and non stressed plants during week5-week7. After the sixth week, the drought stressed plants were moved out of the stressed condition.

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