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Effect of Calcium on the Response of A Field-grown Rice Plant to Water Stress

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There was a gradual decrease in relative water content and leaf water potential in water-stressed rice plant (cv. Ratna) during transition from vegetative to reproductive stage. Seed treatment with calcium (as $CaCl_2$) improved the water status of stressed plants. As compared to control, water-stress induced a higher decline in the contents of chlorophyll, protein and RNA and an increase in free amino acids and proline in plants; however, treatment with higher concentration of calcium (10^{-2}M) significantly checked the trend in the stressed plants. Plants grew less responsive to calcium treatment with the advancement of age. A significant reduction in yield-parameters was observed in water-stressed plant but calcium treatment of seeds considerably compensated such loss in yield brought about by water-stress.

Key Words: Rice plant, Developmental stage, Calcium chloride, Seed treatment, Water-stress, RWC, LWP, Chlorophyll, Protein, Free amino acids, Proline, Yield

Introduction

Water-stress adversely affects the growth and metabolism of plants (Levitt 1972 and Hsiao 1973) and thus, lowers the yield of crop plants (Boyer & Mcpherson 1975 and Eck & Musick 1979). Pre-sowing seed-treatment with some inorganic salts increases the stress-tolerance in some crop plants as is evident from improved growth and yield of the plants (Bozhenko 1968 and Levitt 1972). But the mode of action of calcium on yield improvement under water-stress still remains unclear.

The present work attempts at analysing the changes in water status, some biochemical variables, free proline content and yield components of rice plants grown in the field under water-stress and to evaluate the effect of calcium on such changes.

Material and Methods

Certified seeds of rice *(Oryza sativa* L. cv. Ratna) obtained from Crop Research Farm, Burdwan University, Burdwan, were grown in well-watered seed beds and 40 day old seedlings were then transplanted at a spacing of 15 × 15 cm in three plots (5 × 10m) for each treatment including control under stressed and non-stressed conditions. The experiments were carried out during April to June (1980 and 81).

Water-stress was imposed by withholding water supply. Control field was maintained at saturated soil water level (about 4 cm above the ground surface). The content of soil-water in water-stressed field was restricted to 25% by careful manipulation of watering in the field. The clayee-loam field soil was not fertilized with either organic or inorganic manure throughout the experimental period.

Two lots of seeds were separately treated with 10^{-2} and 10^{-4}M concentrations of $CaCl_2$ for 8 hr. For control seeds were soaked in distilled water for the same period. After treatments, the seeds were sun-dried for 12 hr. and then allowed to germinate in a germinator at 37°C. Two-day old seedlings were carefully transferred to seed beds. Forty-day old seedlings were then transplanted in the same way as mentioned earlier. The data were taken at different developmental stages of the plant, viz., (i) vegetative (V, 65 days old); (ii) boot (B, 75±3 days old); (iii) milk-ripe (MR, 82±3 days old); and (iv) mature fruiting (MF, 101±3 days old).

At each developmental stage, randomized samples were collected from the treated and untreated plants (ten plants in each plot) under both stressed and non-stressed conditions at a fixed time in the morning (11 AM). After mixing the leaf samples, three replicates were made for each treatment and control for the estimation of the various parameters. The relative water content (RWC), defined as water content of fresh tissue expressed as a percentage of water content of the fully turgid tissue, was determined following the method of Weatherley (1950). The leaf water potential (LWP) was measured by liquid immersion method described by Knipling (1967). To measure the water content of the soil at the time of collection of data, 100g of soil samples, collected from the depth of 2.5 cm from the field surface from different regions of experimental plots, was weighed. The average of the percentage of water content of soil samples, thus collected, was determined.

Chlorophyll was estimated following Arnon (1949). Protein was extracted from the residue of methanolic extract by digesting with 1 N NaOH at 80°C for an hour and were estimated by the method of Lowry et al. (1951). Total free amino acid content was measured by the modified colorimetric method of Rosen (1957). Nucleic acids were extracted according to Cherry (1962) and estimated by orcinol reagent (Choudhuri & Chatterjee 1970). Proline was extracted with 3% aqueous solution of sulphosalcyclic acid and was estimated following Bates et al. (1973).

The yield components taken into consideration was fertility percentage, 1000-grain weight, grain weight per plant and yield/m^2.

The data included in the tables were statistically analysed for C.D. (critical difference) values at 95% confidence limits at the treatment and replication levels (Panse & Sukhatme 1967). The data which were represented in percentage (RWC and fertility) were transformed into angular variances (Sin^{-1} transformation of Fisher & Yates 1974) and statistical representations were made according to the above method.

Results

The RWC and LWP in both stressed and non-stressed plants gradually decreased with increase in plant age (table 1). Seed treatment with $CaCl_2$ at higher concentration (10^{-2}M) significantly improved the RWC and LWP, particularly at the early stage of development, but the effects of calcium gradually faded away with plant age. However the lower concentration of $CaCl_2$ (10^{-4}M) had little effect on RWC and LWP.

The contents of chlorophyll, protein and RNA decreased with increase in the plant age (table 2), but the magnitude of decline of these macromolecules was higher in stressed plants than that in non-stressed plants. The treatment of seed with calcium chloride (10^{-2} and 10^{-4}M concentrations) significantly increased the chlorophyll, protein and RNA contents and checked the fast rate of their decline in stressed plants up to milk-ripe stage in comparison to non-stressed plants. But the effects of calcium gradually faded away with time.

The contents of total amino acids and proline rose steadily in both stressed and non-stressed plants up to milk-ripe stage (table 3). But there was a decline only in the case of total free amino acid content thereafter (up to mature fruiting stage). The contents of free amino acids and proline were always higher in stressed plants as compared to non-stressed plants and calcium treatment reduced both free amino acid and proline contents in the stressed plants.

The yield components like fertility percentage, 1000-grain weight, grain weight/plant, and yield/m^2 were significantly reduced in stressed plants (table 4). There was, in general, a significant improvement in yield parameters in stressed plants raised from calcium treated seeds as compared with untreated stressed plants (table 4).

Discussion

Hsiao (1973) suggested that changes in metabolism brought about by mild water stress represent plant regulatory responses. Thus, the direct effect of water stress on water status of plants might, in turn, lead to a number of secondary changes in plants. The present results show that water-stress induced significant deteriorative changes in water status (RWC and LWP) and concentration of metabolites in field-grown rice plants. The changes in water status, probably decreased the concentration of cellular metabolites like chlorophyll, protein and RNA and increased the concentration of total free amino acids and proline. There was a marked decrease in free amino acids after milk-ripe stage indicating the utilization of the pool of amino acids, probably in grain-filling at the mature fruiting stage but the proline content remained high. Presowing treatment with calcium significantly improved the water status of both stressed and non-stressed plants and the effects were reflected in limited decline of cellular metabolites and slight rise in free amino acids and proline. However, the beneficial effect of calcium on cellular components became less apparent with plant age suggesting that the effect of seed treatment with calcium is most pronounced at the early stages of development.

Water-stress significantly decreased yield components and thus yield of rice. Seed treatment with calcium however improved yield parameters and yield of stressed plants. The results suggest that calcium treatment of seeds is advantageous to rice plant grown under water-stressed condition.

Table 1 *Effect of calcium treatment of seeds on relative water content (RWC) and leaf water potential (LWP) in* Oryza sativa *at different developmental stages*

Parameters/Treatment		Developmental stages			
		Vegetative stage	Boot stage	Milk-ripe stage	Maturing fruiting stage
RWC (%)	NS*	95.4	93.9	92.0	88.3
	NS + Ca 10^{-2}M	97.3	95.4	94.2	91.9
	NS + Ca 10^{-4}M	94.2	94.0	93.9	89.3
	WS*	85.6	83.8	83.6	80.2
	WS + Ca 10^{-2}M	88.6	86.7	86.3	83.4
	WS + Ca 10^{-4}M	87.3	86.7	85.5	82.3
	CD at $P_{0.05}$	2.5	2.2	2.6	1.02
LWP (bars)	NS	−5.6	−6.0	−6.5	−6.6
	NS + Ca 10^{-2}M	−5.5	−5.9	−6.3	−6.4
	NS + Ca 10^{-4}M	−5.6	−5.9	−6.4	−6.4
	WS	−9.0	−9.8	−10.7	−12.9
	WS + Ca 10^{-2}M	−6.8	−7.8	−8.4	−8.7
	WS + Ca 10^{-4}M	−7.7	−8.2	−9.6	−10.8
	CD at $P_{0.05}$	0.4	0.2	0.3	0.2

**NS*, non-stressed; *WS*, water-stressed

Table 2 *Effect of calcium treatment of seeds on the quantitative changes in chlorophyll, protein and RNA in* Oryza sativa *at different developmental stages*

Parameters/Treatment		Developmental stages			
		Vegetative stage	Boot stage	Milk-ripe stage	Mature-fruiting stage
Chlorophyll (mg/g dry wt)	NS*	7.8	7.6	7.1	5.3
	NS + Ca 10^{-2}M	9.6	9.2	7.6	4.8
	WS*	5.2	5.8	4.0	3.8
	WS + Ca 10^{-2}M	7.4	6.6	5.2	4.4
	CD at $P_{0.05}$	0.3	0.4	0.4	0.2
Protein (mg/g dry wt)	NS	172.2	160.3	120.3	80.3
	NS + Ca 10^{-2}M	192.6	176.6	124.1	88.6
	WS	136.3	132.3	76.2	64.3
	WS + Ca 10^{-2}M	152.8	148.1	92.3	72.8
	CD at $P_{0.05}$	12.2	13.6	8.2	8.7
RNA (mg/g dry wt)	NS	26.5	24.8	18.6	16.1
	NS + Ca 10^{-2}M	30.3	27.3	20.3	17.3
	WS	21.4	19.6	16.5	14.6
	WS + Ca 10^{-2}M	24.5	21.5	17.2	15.7
	CD at $P_{0.05}$	2.2	1.7	1.8	0.7

**NS*, non-stressed; *WS*, water-stressed

Table 3 *Effect of calcium treatment of seeds on the quantitative changes in total free amino acid and proline of water in* Oryza sativa *at different developmental stages*

Parameters/Treatment		Developmental stages			
		Vegetative stage	Boot stage	Milk-ripe stage	Mature-fruiting stage
Free amino acid (mg/g dry wt)	NS	16.4	21.5	26.5	19.2
	NS + Ca 10^{-2}M	14.5	18.7	21.6	17.5
	WS	21.5	26.4	30.5	19.7
	WS + Ca 10^{-2}M	19.3	23.8	28.3	18.4
	CD at $P_{0.05}$	2.3	2.4	2.1	1.9
Proline (μ moles/g dry wt)	NS	40.75	60.25	71.31	211.61
	NS + Ca 10^{-2}M	40.40	58.52	71.18	207.75
	WS	88.74	153.09	261.20	376.79
	WS + Ca 10^{-2}M	73.88	91.16	240.1	262.75
	CD at $P_{0.05}$	6.2	5.7	11.3	13.8

Table 4 *Effect of calcium treatment of seed on yield parameters in* Oryza sativa

Treatments	Percentage fertility	105-grain wt (g)	Grain wt (g/plant)	Yield/m² (kg)
NS*	81.2	21.671	30.60	1.377
NS + Ca 10^{-2}M	84.4	22.773	32.78	1.582
NS + Ca 10^{-4}M	81.8	21.685	31.52	1.413
WS*	60.8	18.625	12.10	0.550
WS + Ca 10^{-2}M	66.2	20.485	15.80	0.762
WS + Ca 10^{-4}M	62.7	19.565	13.62	0.648
CD at $P_{0.05}$	3.2	1.19	3.7	0.155

**NS*, non-stressed; *WS*, water-stressed

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