

Drought Recovery and Grain Yield Potential of Rice after Chitosan Application

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ABSTRACT

Chitosan is a biopolymer applied to plants in order to increase the key enzymes related to the closure of the plant's stomata resulting in reduction of water loss. The aim of this experiment was to determine the effect of chitosan on drought recovery and grain yield of rice under drought conditions. The experimental design was arranged in RCBD with four replications of five treatments: sufficient irrigation, drought without chitosan, chitosan applied before drought, chitosan applied during drought and chitosan applied after drought. The experiment was conducted in a greenhouse at the Department of Plant Production Technology, Rajamangala University of Technology Suvarnabhumi, during April to August 2005. The results revealed that the treatment applied with chitosan before drought gave the highest yield and yield components and also showed good recovery. Furthermore, the percentage of damaged leaves was less than those of the other treatments. From this study, it is suggested that the severity of rice plants damaged from drought was reduced by chitosan application.

Key words: drought stress, chitosan application, rice (*Oryza sativa* L.)

INTRODUCTION

Plant stress refers to the condition in which plant cells and tissues are at less than full turgor. This occurs whenever the loss of water by transpiration exceeds the rate of water absorption (Kramer, 1996). Thus, drought stress may occur seasonally as soil moisture reserves are depleted as transpiration exceeds the rate at which water is supplied to the leaves. With the occurrence of drought stress, almost all processes associated with plant growth are affected. The effects may vary with the degree and duration of drought and the growth stage of the plant. Severe drought stress

thus affects the accumulation of biomass, limits plant productivity and yield by reducing photosynthesis, and can also affect partitioning of biomass to harvestable parts of the plant (Boyer, 1982; Bradford and Hsiao, 1982). Khan *et al.* (2003) reported that the various tested chitin and chitosan oligomers produced elevated phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) activities in soybean leaves, which may lead to the induction of other secondary plant metabolites produced by phenylpropanoid pathway that are related to plant stress. Tham *et al.* (2001) revealed that wheat and barley damaged at 2.5 µg/ml of vanadium were recovered by the

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treatment with 10-100 µg/ml chitosan irradiated at 200-700 Kgy of λ-rays in 1% solution. Lee *et al.* (1999) found that chitosan, a component of fungal cell walls, reduced the size of stomatal aperture and inhibited light – induced stomatal opening in tomato epidermis by inducing reactive oxygen species (ROS) including superoxide and hydrogen peroxide which inhibit stomatal opening and promote stomatal closing. Bittelli *et al.* (2001) reported that foliar application of chitosan decreased transpiration in pepper plants, and reduced water use by 26-43% while maintaining biomass production and yield.

MATERIALS AND METHODS

Pot experiment was conducted in a greenhouse. Rice seeds were planted with ten seeds per pot. Eight pots were used as one experimental unit per replication. The experimental design was a RCBD with four replications and five treatments. All treatments were applied with sufficient irrigation until 20 days after planting, drought stress condition were imposed at three growth stages of rice plant: seedling stage, tillering stage and panicle initiation stage. The details of five treatments are as follows:

T₁. Sufficient irrigation throughout cropping season

T₂. Drought period was imposed at each growth stage until rice leaves were tightly rolled (leaf rolling score = 9) and then irrigation was applied (control treatment).

T₃. Chitosan was sprayed one day before drought period imposed at each growth stage and then irrigation was applied when rice leaves in control treatment were tightly rolled.

T₄. Chitosan was sprayed during drought period (rice leaves were fully cupped (leaf rolling score=5)) at each growth stage and then irrigation was applied when rice leaves in control treatment were tightly rolled.

T₅. Chitosan was sprayed after irrigation

was applied one day after drought period at each growth stage.

Chitosan used in this experiment was polymer (degree of deacetylation = 96.62%, molecular weight ~ 100,000 KDa) in the form of flake. It was dissolved in 2% of dilute acetic acid to be liquid chitosan before spraying to rice plants. Drought recovery score and damaged leaf data were collected during recovery period (within seven days after irrigation was applied) after drought period. Leaf rolling score was recorded when the rice leaves in control treatment were tightly rolled. Dry matter and plant height data were collected during recovery period at each growth stage and leaf greenness was also measured by chlorophyll meter (SPAD 502). Yield and yield components were recorded after harvest. This study was conducted during May to August 2005 at Rajamangala University of Technology Suvarnabhumi. All data were analysed by two-way ANOVA (analysis of variance), and LSD (Least Significant Difference) was used to determine the difference between treatment means.

RESULTS

1. Leaf rolling

Table 1 shows leaf rolling score after chitosan application under drought stress conditions. There were highly significant differences ($P < 0.01$) among treatments with and without chitosan. The average leaf rolling score throughout the cropping season indicated that while rice leaves under drought without chitosan application were tightly rolled, rice leaves in treatment applied with chitosan before drought were only fully cupped (U-shape).

2. Damaged leaves

Effect of chitosan application on damaged leaves of rice plants under drought is shown in Table 1. The percentage of damaged leaves in treatment without chitosan application was highest and significantly different ($P < 0.01$)

from other treatments. The average percentage of damaged leaves obtained from the treatment with chitosan application before drought was 22.8% and less than those of the other treatments. The average percentage of damaged leaves of the treatment without chitosan was 50.4%.

3. Drought recovery

Drought recovery of rice plants is shown in Table 2. Rice plant recovery score from treatment applied with chitosan before drought was significantly different from the treatment without chitosan application, but there were no significant differences between treatments applied chitosan during and after drought stress. Considering throughout the cropping season, it was found that there were about 68-83% of rice plants from treatment without chitosan application were recovered after applying irrigation for 4-5 days, while 81% of rice plants in the treatment applied with chitosan before drought were recovered after irrigation applied for 1-2 days.

4. Dry matter accumulation

The accumulation of dry matter of rice plants at various growth stages under drought is shown in Table 2. There were no significant

differences between treatments with and without chitosan under drought in all growth stages. However, the treatment applied chitosan before drought tended to accumulate more dry matter than those of the other treatments.

5. Yield and yield components

Yield and yield components of rice applied with chitosan under drought conditions are shown in Table 3. Yield and yield components (tiller/plant, panicle/plant and seed/panicle) obtained from the treatment applied with chitosan before drought showed significant differences ($P < 0.01$) from those of treatment without chitosan. However, panicle per plant, seed per panicle and thousand-grain weight were not significantly different among treatments applied with chitosan before, during and after drought stress.

6. Plant height and leaf greenness

As shown in Table 4, the height of rice plants was not affected by chitosan application under drought conditions. The height of rice plants in treatment without chitosan tended to be lower than those of others in all growth stages. For the leaf greenness, chitosan application had no effects in all growth stages.

Table 1 Effects of chitosan on leaf rolling and damaged leaves of rice under drought condition.

Treatment	Leaf rolling score ^{a/} (score)				Damaged leaves (%)			
	Seedling	Tillering	Panicle	Average	Seedling	Tillering	Panicle	Average
	stage	stage	initiation		stage	stage	initiation	stage
Sufficient irrigation	0	0	0	0	0	0	0	0
Drought stress without chitosan	9	9	9	9	25	55	71.3	50.4
Chitosan applied before drought stress	6.8	6	5.5	6.1	12.5	32	23.8	22.8
Chitosan applied during drought stress	9	8	7.5	8.2	15	52	62.5	43.2
Chitosan applied after drought stress	9	7.5	7	7.8	17.5	42	54.3	37.2
LSD(0.01)	1.14	1.67	1.42		10.25	19.62	16.70	
(%) C.V.	11.17	12.70	11.35		23.88	23.59	18.52	

a/ = Leaf rolling score by SES (IRRI,1980)

0 = Leaves healthy

1 = Leaves start to fold (shallow V-shape)

3 = Leaves folding (deep V- shape)

5 = Leaves fully cupped (U-shape)

7 = Leaf margins touching (O-shape)

9 = Leaves tightly rolled

Table 2 Effects of chitosan on drought recovery and dry matter accumulation of rice under drought condition.

Treatment	Drought recovery score ^{a/}				Dry matter accumulation		
	(score)				(g/trt)		
	Seedling stage	Tillering stage	Panicle initiation	Average	Seedling stage	Tillering stage	Panicle initiation
Sufficient irrigation	0	0	0	0	53.5	90.5	216.1
Drought stress without chitosan	3	7	8	6	41.9	54.0	75.9
Chitosan applied before drought stress	1	2	5.5	2.8	45.1	69.0	118.2
Chitosan applied during drought stress	1.5	5.5	7	4.6	48.0	56.4	99.7
Chitosan applied after drought stress	2.5	6	6.5	5	49.7	56.3	104.0
LSD(0.01)	1.89	1.47	1.42		ns	20.39	43.15
(%) C.V.	24.72	16.66	10.62		15.98	14.80	16.27

a/= Drought recovery score by SES (IRRI,1980)

0 = No symptoms

1 = 90 % of all plants produce new leaves and tillers after irrigation applied 1-2 days

3 = 75 % of all plants produce new leaves and tillers after irrigation applied 1-2 days

5 = 75-90% of all plants produce new leaves and tillers after irrigation applied 4-5 days

7 = 50-75% of all plants produce new leaves and tillers after irrigation applied 4-5 days

9 = Less than 50% of all plants produce new leaves and tillers after irrigation applied 7days

Table 3 Effects of chitosan on yield and yield components of rice under drought condition.

Treatment	Yield (g/trt)	Tiller/plant	Panicle/plant	Seed/panicle	1,000-grain weight (g)
Sufficient irrigation	196.5	4.6	3.5	111.5	26.6
Drought stress without chitosan	74.8	2.5	1.6	65.8	21.4
Chitosan applied before drought stress	133.0	4.4	3.1	88.8	23.2
Chitosan applied during drought stress	96.0	3.9	2.0	78.5	22.3
Chitosan applied after drought stress	96.8	3.6	2.0	72.5	22.5
LSD.01	24.39	0.89	1.18	17.27	2.65
(%) C.V.	9.46	10.94	22.61	9.59	5.35

Table 4 Effects of chitosan on plant height and leaf greenness of rice under drought condition.

Treatment	Plant height (cm)			Leaf greenness (spad unit)		
	Seedling stage	Tillering stage	Panicle initiation	Seedling stage	Tillering stage	Panicle initiation
			stage			stage
Sufficient irrigation	86.3	99.3	118.5	39.9	39.7	32.7
Drought stress without chitosan	78.3	89.3	112.3	37.5	37.8	31.1
Chitosan applied before drought stress	82.0	91.0	121.8	38.6	39.0	31.8
Chitosan applied during drought stress	82.8	90.0	114.0	37.9	38.8	30.7
Chitosan applied after drought stress	78.3	89.8	119.0	37.2	37.7	31.8
LSD.05	4.71	ns	ns	ns	1.46	ns
(%) C.V.	3.76	13.16	7.87	4.45	8.67	5.5

DISCUSSION

Chitosan has been shown to trigger defense mechanisms in plants (Ryan, 1982). Khan *et al.* (2003) reported that plants treated with chitin and chitosan produce chitinase that breaks down the chain of chitin and chitosan into more soluble form. They also found that application of various chitin and chitosan oligomers to soybean leaf tissues caused increased activity of phenylalanine ammonialyase (PAL) and tyrosine ammonialyase (TAL) enzymes. The elevation of enzyme activity was dependent on the chain length of the oligomers and time after treatment. Nicholson and Hammerschmidt (1992) reported that increases in PAL activity have been demonstrated to be one of the earliest responses of plants to the onset of stress by pathogen infection and are considered as an indication of resistance. Since PAL is the key enzyme in the phenylpropanoid pathway, its activity leads to synthesis of phenols, which are compounds associated with expression of resistance. Loschke *et al.* (1983) reported that chitosan induces the expression of a variety of genes involved in plant defense response that, in some cases, results to increased synthesis of secondary plant metabolites. Chitosan influenced pathways involving jasmonic acid (Walker-Simmons *et al.*, 1983; Famer and Ryan, 1990; Doares *et al.*, 1995). Jasmonate exhibits some activities similar to the plant hormone abscisic acid (ABA), which plays a key role in the regulation of water use by plants (Sembdner and Parthier, 1983). Increased levels of ABA result in closure of the plant's stomata and reduced transpiration (Willmer and Pricker, 1996; Leung and Giraudat, 1998). Thus, manipulating the ABA signaling pathway offers the possibility to reduce water consumption by plants (Grill and Ziegler, 1998). In this study, it was demonstrated that the treatment applied with chitosan before drought stress showed the best results over those of the other treatments in all characters. It may be

explained that rice plants treated with chitosan can produce some metabolites which causes closure of the plant's stomata resulting in reduction of transpiration. This may be the reason that the rice plants of the treatment with chitosan application before drought tended to resist drought stress because of stomata closing resulting in less water use. Consequently, the damage of rice plants treated with chitosan was less than that of untreated plants under drought condition. Therefore, it is suggested that the severity of rice plants damaged from drought stress may be reduced by chitosan application.

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