RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2010.36.089

ScienceAsia 36 (2010): **89–93**

Physiological and morphological responses of field corn seedlings to chitosan under hypoxic conditions

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Received 17 Aug 2009
Accepted 7 Apr 2010

ABSTRACT: Chitosan acts as an elicitor in many plant species. It not only activates the immune system of plants, but also increases the crop yields. The objective of this study was to investigate the effects of chitosan on physiological and morphological responses of two field corn genotypes, tolerant and susceptible, under hypoxia. A pot experiment was conducted using a split plot in completely randomized design with two main plots (M) and three subplots (S). Main plots were two genotypes of field corn: NSX 062030 (tolerant) and 30B80 (Pioneer, susceptible), and subplots were three treatment conditions: normal irrigation without chitosan application (I-NC), chitosan application before hypoxia (C-H) and hypoxia without chitosan application (H-NC). Slight genotypic differences were observed for aerenchyma development under hypoxia. However, various treatment conditions influenced the activity of nitrate reductase (NR), leaf greenness, number of prop roots, and aerenchyma development. C-H had positive effects on number of prop roots and aerenchyma development and also tended to retain leaf greenness. The highest leaf NR activity was detected under H-NC and did not differ significantly from that under C-H. However, C-H tended to show positive effects on soluble sugar accumulation, but did not show any significant differences from the others.

KEYWORDS: aerenchyma, leaf greenness, prop root, soluble sugar, nitrate reductase

INTRODUCTION

Chitosan is a natural biopolymer found in exoskeletons of crustaceans and insect cuticles as well as cell walls of fungi and some algae. Chitosan has several agricultural applications. It induces defence mechanisms in several plant species[1,2] and increases the activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), key enzymes of the phenylpropanoid pathways associated with synthesis of secondary plant metabolites under unfavourable conditions[3]. Furthermore, chitosan also promotes plant growth and enhances crop yield of many species such as *Oryza sativa* and *Eustoma grandiflorum*[4,5]. It also accelerates the germination rate and germination index[6]. Hypoxia describes the status of a cell or tissue in a deficient or low O_2 concentration ($< 20\%$) state. This term differs from anoxia, in which the O_2 concentration is zero[7]. Plant survival under hypoxic conditions is a major problem affecting agricultural productivity[8] as it inhibits N uptake and N redistribution within the shoot causing early leaf senescence and retarded shoot growth in flooding plants[9]. Hypoxia also reduces root growth, dry matter accumulation, and final crop yield[10]. It has been estimated that hypoxia reduces crop yields by 20–25% but the loss may exceed 50% depending on the stage of plant development[11]. Water-logging tolerance is probably a complex phenomenon related to the plant morphological and physiological features[12]. It impairs shoot and root growth and decreases chlorophyll content[13]. Adventitious roots, which develop exclusively under flooding conditions, show greater aerenchyma, which may vary among cultivars[14]. Under anoxia, fermentative metabolism is increased after 4 days to supply enough energy to maintain the metabolic activities of the roots for plant survival[15]. Under flooding conditions, tolerant species show a marked increases in nitrate reductase activity in roots and leaves. A greater ability to synthesize amino acid is found in tolerant species than in intolerant species[16].

The objective of this study was to investigate the effects of chitosan on physiological and morphological responses of two field corn genotypes, tolerant and susceptible, under hypoxic conditions.

MATERIALS AND METHODS

Experiments were conducted using a split plot in a completely randomized design with two field corn genotypes: tolerant (NSX 062030) and susceptible (30B80, Pioneer)[17] as main plot (M), and three treatment conditions: normal irrigation without chitosan application(I-NC), chitosan application before hypoxia (C-H) and hypoxia without chitosan application (H-NC) as subplot (S), having four pots per experimental unit and replicated four times. The pot experiments were conducted in an open-ended outdoor greenhouse with daily maximum and minimum temperatures of 33 °C and 23 °C, respectively. Seeds of each genotype were planted in 46 cm-diameter pots containing 7 kg/pot of clay soil with the following chemical properties: pH 5.2, organic matter 0.89% (very low), available phosphorus 5.01 ppm (medium), and exchangeable potassium 66.2 ppm (medium). Chitosan used in this study was a polymeric type with a degree of deacetylation of 96.58%. It was dissolved in 2% acetic acid before spraying it on corn plants. Seven days after coleoptile emergence, plants were thinned leaving 4 healthy plants per pot, and the chitosan application started according to the treatment details. Chitosan at the concentration of 80 ppm was sprayed 7, 14, 21, and 28 days after planting onto the corn leaves. One day after the last chitosan spraying, corn seedlings were subjected to a transient waterlogging (hypoxia) by adding water to the pots until the water level reached 5.0 cm above the soil surface and a level of 2.5–5.0 cm was maintained for 9 days. After the ninth day of waterlogging, two plants per pot were sampled to determine the root features. Only prop root numbers selected from total roots were counted by using a numbering counter. In the case of aerenchyma development, the young nodal roots were prepared by free-hand cross-section at 5 cm from the root tip. An Olympus compound microscope at 10 × was used to magnify the whole root cross-section and the aerenchyma region was photographed. After that, the whole root cross-sectional region was cut and then weighed. The aerenchyma region was also cut and then weighed. The ratio of aerenchyma to the whole root weights was used as a measure of aerenchyma development[18]. For the leaf blade, leaf greenness was measured on one side of the midrib, midway between leaf base and tip by chlorophyll meter (SPAD 502). Leaf nitrate reductase activity was analysed by the method of Jaworski[19] and soluble sugar accumulation in leaves was investigated by the method of Yoshida[20]. This experiment was carried out at Rajamangala University of Technology Suvarnabhumi during September to November 2008. All data were subjected to ANOVA by MSTAT and treatment mean comparison was done by the use of Least Significant Difference (LSD).

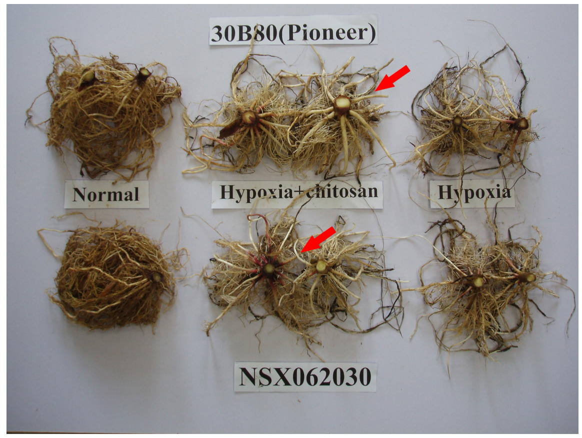

Fig. 1 Prop roots (arrow) of tolerant genotype (NSX 062030) and susceptible genotype (30B80 (Pioneer)) under various treatment conditions.

RESULTS AND DISCUSSION

Number of prop roots

The number of prop roots (NPR) of both NSX 062030 and 30B80 (Pioneer) observed in C-H conditions was significantly greater than in I-NC and H-NC. All newly generated roots were thicker than the existing roots (Table 1, Fig. 1). Different field corn genotypes did not affect NPR in this study. These findings may suggest that chitosan may stimulate NPR under hypoxia resulting in increased root surface exposed to air and consequently increased aerobic respiration. This is consistent with studies on *Raphanus sativus*[21] and *Brassica campestris*[22].

Aerenchyma development

The aerenchyma tissue in root cortex of NSX 062030 genotype dramatically developed under C-H; the percentage of aerenchyma development was higher than that of 30B80 (Pioneer) genotype under the same conditions. It may be conceivable that 30B80 (Pioneer) genotype was susceptible to hypoxia, thus it was poorly adapted to generate aerenchyma under hypoxia even though it was exposed to chitosan (Table 1). Development of aerenchyma and adventitious roots is a more recessive factor that increases hypoxic tolerance

Table 1 Effects of chitosan on number of prop root and aerenchyma development of two field corn genotypes under various treatment conditions.

Field corn genotype (M)	Number of prop root (root)				Aerenchyma development (%)			
	Treatment condition (S)			M average	Treatment condition (S)			M average
	I-NC	C-H	H-NC		I-NC	C-H	H-NC	
Tolerant (NSX 062030)	2.75	11.50	5.25	6.50	4.18^{p}	19.57^{m}	9.19^{o}	10.96^{a}
Susceptible (30B80 (Pioneer))	3.00	13.50	7.00	7.83	3.93^{p}	7.79^{o}	12.90^{n}	8.21^{b}
S average	2.87^{c}	12.50^{a}	6.12^{b}		4.05^{b}	13.65^{a}	11.04^{a}	
LSD.05 (M)	ns				1.360			
LSD.05 (S)	1.418				3.196			
LSD.05 (M) × (S)	ns				2.967			
CV(%)	12.84				13.42			

In this table and the next two, the mean followed by the same superscript within the same row and the same column of each characteristic indicates no significant difference among treatment conditions (S) and field corn genotype (M) respectively ($p < 0.05$) and ns indicates no significant differences.

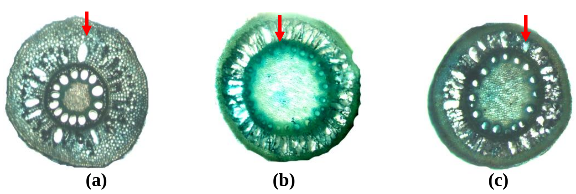

Fig. 2 Aerenchyma development (arrows) of tolerant genotype (NSX 062030) under (a) I-NC (b) C-H (c) H-NC conditions.

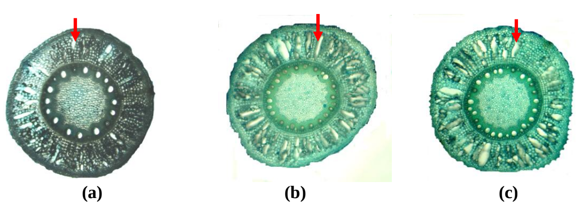

Fig. 3 Aerenchyma development (arrows) of susceptible genotype (30B80, Pioneer) under (a) I-NC (b) C-H (c) H-NC conditions.

in *Zea mays*[23]. The aerenchyma formation and adventitious roots in the vicinity of cotyledonary nodes is an indicator of adaptive mechanisms presented in flood-tolerant plants[24]. Within a genotype, it was found that C-H increased aerenchyma development of NSX 062030 genotype, the tolerant hybrid, but did not affect that of 30B80 (Pioneer) genotype. However, aerenchyma development under C-H and H-NC of both genotypes was higher than that under I-NC (Table 1, Figs. 2 and 3).

Leaf greenness

Under normal irrigation without chitosan (I-NC), leaf greenness values of both genotypes were higher than those under hypoxic condition (C-H and H-NC, Table 2). Leaf greenness values of both genotypes under C-H and H-NC conditions were not significantly different. However, C-H tended to retain leaf greenness of the two field corn genotypes. Tomato and lettuce leaves turn darker green with increasing chitosan concentrations[25].

Soluble sugar accumulation

The soluble sugar content in corn leaves of two genotypes was not significantly different under the various treatment conditions (Table 3). Under C-H condition, corn tended to accumulate a high level of soluble sugar in both NSX 062030 and 30B80 (Pioneer) genotypes. This can be explained by the fact that chitosan is poly-β-(1,4)-2-amino-2-deoxy-D-glucose which degrades to ammonia, an oligosaccharide, and a monosaccharide, hence increasing the soluble sugar under C-H. The content of soluble sucrose in non-heading Chinese cabbage leaves increases after dressing the seed or spraying the leaves with chitosan[22]. However, NSX 062030 and 30B80 (Pioneer) genotypes showed comparable soluble sugar accumulation under the same condition. In maize, flooding increases the amount of soluble sugar increased in both the roots and shoots[23]. In soybean and rice, the soluble sugar consumption under fermentative metabolism also increases[15].

Nitrate reductase activity

The NR activities in both genotypes under H-NC and C-H were higher than those under I-NC (Table 3).

Table 2 Effects of chitosan on leaf greenness (spad unit) of two field corn genotypes under various treatment conditions.

Field corn genotype (M)	Treatment condition (S)			M Average
	I-NC	C-H	H-NC	
Tolerant (NSX 062030)	43.85	33.75	32.12	36.57
Susceptible (30B80 (Pioneer))	41.45	30.85	29.52	33.94
S average	42.65[a]	32.30[b]	30.82[b]	
LSD.05 (M)		ns		
LSD.05 (S)		3.584		
LSD.05 (M) × (S)		ns		
CV(%)		6.6		

Table 3 Effects of chitosan on soluble sugar accumulation and nitrate reductase activity of two field corn genotypes under various treatment conditions.

Field corn genotype (M)	Soluble sugar accumulation (mg glucose/g leaf dry wt.)				Nitrate reductase activity (µg nitrate/g leaf fresh wt.)			
	Treatment condition (S)			M average	Treatment condition (S)			M average
	I-NC	C-H	H-NC		I-NC	C-H	H-NC	
Tolerant (NSX 062030)	0.067	0.080	0.068	0.071	0.098	0.136	0.138	0.124
Susceptible (30B80 (Pioneer))	0.073	0.083	0.064	0.073	0.099	0.116	0.125	0.113
S average	0.070	0.081	0.066		0.098[b]	0.126[a]	0.131[a]	
LSD.05 (M)		ns				ns		
LSD.05 (S)		ns				0.015		
LSD.05 (M) × (S)		ns				ns		
CV(%)		17.80				11.63		

This could be explained by the fact that under waterlogging, the most important ion used by soil microorganisms as an alternative electron acceptor for respiration is nitrate (NO_3^-), which is rapidly reduced to nitrite (NO_2^-), resulting in increased NR activity. During flooding, the activity of NR in roots of flooded-tolerant plants rapidly increases[16]. For crop plants, some species are root-reducers for NO_3^- and some species are leaf-reducers. No significant difference in terms of NR activity between two field corn genotypes was detected. NR activity under C-H and H-NC was not significantly different. C-H did not contribute to NR activity increases in NSX 062030 and 30B80 (Pioneer) genotypes under hypoxia.

The present results suggest that chitosan is able to induce physiological and morphological responses that allow corn seedlings to survive under hypoxic condition.

Acknowledgements: The authors would like to thank Thailand Research Fund and the Commission of Higher Education for the research budget. Special thanks were extended to Dr Sansern Jampathong from National Corn and Sorghum Research Centre and Mr. Pichet Krudloyma from Nakhon Sawan Field Crop Research Centre for providing field corn seeds for the experiments.

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