Effects of Chitosan on Physiological Responses Relating to Yield Potential of Corn (*Zea mays* L.) under Transient Waterlogged (hypoxia) Condition.

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ผลของไคโตซานต่อการตอบสนองทางสรีรวิทยาซึ่งเกี่ยวข้องกับศักยภาพการ ให้ผลผลิตของข้าวโพดในสภาพน้ำขังชั่วคราว

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ผู้วิจัย: ผู้ช่วยศาสตราจารย์ ดร. สุชาดา   บุญเลิศนิรันดร์
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Abstract:

Experiment # 1 Hypoxic Responses of Corn Seedlings

Hypoxia usually influences plant growth and final yield. The objectives of this study were to evaluate hypoxic responses of waxy corn and field corn and then select to be tolerant and susceptible genotypes. This study was divided into two groups, waxy corn seedlings and field corn seedlings. In each group, pot experiments were conducted using a split plot in randomized complete block design with three replications. Main plots were two treatment conditions, normal irrigation (I-NC) and hypoxic condition (H-NC) and sub plots were a number of corn genotypes. For the waxy corn, it was found that three genotypes, namely Big white 852, Mungkorn Padtaew and Samlee Puifai, were selected as tolerant genotype whereas the susceptible genotype was Neaw Roiet, Nam Wang and Samlee Kaimook Pumpui. In terms of field corn, the tolerant genotype was 30Y87 (Pioneer), NSX 062030 and NSX 062029 but NK48 (Sygenta), 30B80(Pioneer) and Pac224 (Pacific) were selected as susceptible genotype. The main criteria to select to be tolerant and susceptible genotypes were waterlogged index of each characteristic and different value of all recorded data between I-NC and H-NC was also considered.

Key word: hypoxic response, corn seedling, waterlogged index

Experiment # 2 Effects of Chitosan on Physiological and Morphological Responses of Corn Seedlings under Hypoxia.

Chitosan acts as an elicitor in many plant species. It not only activates the immune system of plants, but also increases the yields. The objectives of this study were to investigate the effect of chitosan on physiological and morphological responses of corn seedling genotype, tolerant and susceptible, under hypoxia. This study was divided into two groups, field corn seedlings and waxy corn seedlings. Pot experiments were conducted using a split plot in completely randomized design with four replications. Main plots were tolerant and susceptible genotypes of each corn group; NSX 062030 and 30B80 (Pioneer) were utilized as tolerant and susceptible genotypes respectively in field corn group whereas Big White852 and Neaw Roiet were utilized as tolerant and susceptible genotypes respectively in waxy corn group. Sub plots were three treatment conditions: normal irrigation without chitosan application (I-NC) , chitosan application before hypoxia(C-H) and hypoxia without chitosan application (H-NC).The results were found that slight genotypic effect was observed for aerenchyma development under various treatment conditions. However, a number of new roots, aerenchyma development, root dry weight and leaf chlorophyll content were affected by various treatment conditions. C-H had positive effects on a number of new roots and aerenchyma development and also tended to retain leaf greenness and chlorophyll content but did not affect soluble sugar accumulation and nitrate reductase activity (NR activity) in corn leaves.

Keywords: chitosan, corn seedling, hypoxia, physiological and morphological response

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บทคัดย่อ:

การทดลองที่ # 1 การตอบสนองต่อสภาพน้ำท่วมขังชั่วคราวของ ข้าวโพดในระยะต้นกล้า

สภาพน้ำท่วมขังชั่วคราวมีผลต่อการเจริญเติบโตและจำกัดผลผลิตของพืช การทดลองนี้มีวัตถุประสงค์เพื่อประเมินการตอบสนองของต้นกล้าข้าวโพดข้าวเหนียวและข้าวโพดไร่เมื่ออยู่ในสภาพน้ำขังชั่วคราวและคัดเลือกหาพันธุ์ที่ทนทานและอ่อนแอต่อสภาพน้ำท่วมขังชั่วคราว การทดลองนี้แบ่งเป็น 2 ชุดคือ ในต้นกล้าข้าวโพดข้าวเหนียว และต้นกล้าข้าวโพดไร่ การทดลองนี้กระทำในกระถางโดยวางแผนการทดลองแบบ Split plot in CRD ทำ 3 ซ้ำ ปัจจัยหลักคือ สภาพการให้น้ำปกติ และสภาพน้ำท่วมขังชั่วคราว ปัจจัยรองคือ จำนวนพันธุ์ของข้าวโพดแต่ละชุด (ข้าวโพดข้าวเหนียว 20 พันธุ์และข้าวโพดไร่ 19 พันธุ์) ผลการทดลองในข้าวโพดข้าวเหนียว พบว่า พันธุ์ที่ถูกคัดเลือกว่าเป็นพันธุ์ที่ทนทานต่อสภาพน้ำท่วมขังชั่วคราวคือ บิ๊กไวท์ 852 มังกรแปดแถว และสำลีปุยฝ้าย ในขณะที่พันธุ์เหนียวร้อยเอ็ด น้ำวัง และ สำลีไข่มุกปุ้มปุ้ย ถูกจัดเป็นพันธุ์ที่อ่อนแอต่อสภาพน้ำท่วมขังชั่วคราว ในส่วนของข้าวโพดไร่พบว่า พันธุ์ที่ถูกคัดเลือกว่าเป็นพันธุ์ที่ทนทานต่อสภาพน้ำท่วมขังชั่วคราวคือ 30Y87(Pioneer) NSX 062030 และ NSX 062029 ส่วนพันธุ์ที่ถูกจัดเป็นพันธุ์ที่อ่อนแอต่อสภาพน้ำท่วมขังชั่วคราว คือ NK48 (Syngenta) 30B80(Pioneer) และ Pac224 (Pacific) โดยใช้เกณฑ์ในการคัดเลือกจากค่าดัชนีความทนทานต่อน้ำท่วมขัง (waterlogged index) ของแต่ละลักษณะที่บันทึก ส่วนค่าความแตกต่างของลักษณะข้อมูลที่เก็บระหว่างสภาพให้น้ำปกติกับสภาพน้ำท่วมขังก็อาจนำมาพิจารณาด้วย

การทดลองที่ # 2 ผลของไคโตซานต่อการตอบสนองทางสรีรวิทยาและสัณฐานวิทยาของต้นกล้าข้าวโพดในสภาพน้ำท่วมขังชั่วคราว

ไคโตซาน มีคุณสมบัติเป็นตัวชักนำให้เกิดภูมิคุ้มกันในพืชหลายชนิด นอกจากนี้ยังช่วยเพิ่มผลผลิตพืช วัตถุประสงค์ของการทดลองนี้เพื่อศึกษาผลของไคโตซานต่อ การตอบสนองทางสรีรวิทยาและสัณฐานวิทยาของต้นกล้า ข้าวโพดทั้งพันธุ์ที่ทนทานและอ่อนแอต่อสภาพน้ำท่วมขังชั่วคราว โดยแบ่งการทดลองเป็น 2 กลุ่มคือ ในข้าวโพดไร่และข้าวโพดข้าวเหนียว การทดลองนี้กระทำในกระถางโดยวางแผนการทดลองแบบ Split plot in CRD ทำ 4 ซ้ำ ปัจจัยหลักคือพันธุ์ข้าวโพดที่ถูกคัดเลือกว่าเป็นพันธุ์ที่ทนทานและอ่อนแอต่อสภาพน้ำท่วมขังชั่วคราวของแต่ละกลุ่ม สำหรับข้าวโพดไร่พันธุ์ที่ถูกจัดว่าเป็นพันธุ์ทนทานและอ่อนแอ คือพันธุ์ NSX 062030 และ 30B80 (Pioneer) ตามลำดับส่วนข้าวโพดข้าวเหนียวพันธุ์ที่ทนทานคือ พันธุ์ บิ๊กไวท์ 852 และพันธุ์ที่อ่อนแอ คือ เหนียวร้อยเอ็ด ปัจจัยรองคือ สภาพปลูกที่แตกต่างกัน 3 แบบคือ การปลูกในสภาพให้น้ำปกติและไม่มีการฉีดพ่นไคโตซาน (I-NC) ฉีดพ่นไคโตซานก่อนน้ำท่วมขังชั่วคราว(C-H) และ สภาพน้ำท่วมขังชั่วคราวและไม่มีการฉีดพ่นไคโตซาน (H-NC) ผลการทดลองพบว่า อิทธิพลของพันธุ์ข้าวโพดมีผลเล็กน้อยต่อการการพัฒนา

ของ อะเรนไคมาภายใต้สภาพการปลูกที่ต่างกัน อย่างไรก็ตาม สภาพการปลูกที่ต่างกันมีผลต่อ จำนวนรากที่เกิดขึ้นใหม่ การพัฒนาของ อะเรนไคมา น้ำหนักแห้งของราก และปริมาณคลอโรฟิลล์ในใบ การฉีดพ่นไคโตซานก่อนเจอสภาพน้ำท่วมขังชั่วคราวมีผลในทางบวกต่อจำนวนรากที่เกิดขึ้นใหม่ และการพัฒนาของ อะเรนไคมา ตลอดจนมีแนวโน้มที่จะรักษาสภาพความเขียวและปริมาณคลอโรฟิลล์ในใบไว้ได้ดีกว่าการไม่ฉีดพ่นไคโตซาน แต่ไม่มีผลต่อปริมาณการสะสม soluble sugar และกิจกรรมของเอนไซม์ในเตรทรีดักเตส

Executive summary:

Field corn is an important economic crop which increases revenue in Thailand about 3,000 -4,000 million baht per year. Field corn production in Thailand is currently about 4 million tons from 6 million rais of total planting areas which is not enough because the demand is 5.5 million tons. Total field corn yields are mostly supplied as raw materials for animal feed factory within the country. Due to the limited planting areas and farmers interest to grow the other crops, i.e. cassava, sugarcane and rubber having more incomes resulted in continuously decreased planting areas. Planting areas were decreased from 5.67 million rais in the years 2005 to 5.14 and 5.08 million rais in the year 2006 and 2007 respectively. In the present time, the prices of field corns are very high all over the world. Because the United States of America, the first producer in the world, supplies field corns to produce ethanol as supplementary energy resulted in decreased field corn exporter. This changing continuously influences the amount of field corns which are not actually enough to supply to the animal feed factory.

With regard to waxy corn, it is popular to grow as green corn due to being gentle and lightly sweet and the harvesting time is quite short (55-70 days). It can be grown through the year in irrigated areas. The total planting areas in Thailand are 80,000 rais, average yield about 1300-1700 kg/rai. The increased population in the world contribute to increased demand.

While the animal feed industry tends to increase. Therefore, to find the ways to increase corn production to supply consumer demand in each year is urgently considered. The decreased planting areas are caused by several factors. Drought stress is one factor which often happens and shows increased seriousness. Moreover, the planting areas in field (rainfed) condition are also limited because of forest invasion. Growing corns in the paddy field after rice harvesting is possible way to increase corn planting areas in Thailand and even increases farmer incomes. Corn planting areas in paddy field after rice harvesting are currently expanded, particularly in the lower central region of Thailand. One characteristic of paddy soil is generally poor drainage resulted occurs hypoxic condition which directly affects root respiration that finally limits growth and yield of corn.

Chitosan is a natural biopolymer found inexoskeletons of crustaceans and insect cuticles as well as cell walls of fungi and some algae. Chitosan can be used in agriculture with many aspects. Nowadays, there are widely applications of chitosan to induce defence mechanism in many plant species. Furthermore, chitosan also promotes plant growth and enhances yield of many crop species. Therefore, the way to solve hypoxic problem in corn growing in paddy field for maintaining corn yield under this condition should be urgently done to increase total corn yield to supply consumer demand and animal feed factory need. This study was divided into two experiments, each experiment was split into two sets, field corn seedling and waxy corn seedling.

Experiment # 1 Hypoxic responses of corn seedlings

This experiment was done two sets, 20 genotypes of field corn and 19 genotypes of waxy corn. The objectives of this experiment were to evaluate hypoxic responses in corn seedlings which was inherited by genetic traits and then selected to be tolerant or susceptible genotypes. For field corn seedling set, the result was found that the tolerant genotype was 30Y87 (Pioneer), NSX 062030 and NSX 062029 whereas the susceptible genotype was NK48 (Syngenta), 30B80(Pioneer) and Pac224 (Pacific). In regard to waxy corn seedling set, it was found that Big White 852, Mungkorn Padteaw and Samlee Puifai were classified as

tolerant genotype but Neaw Roiet, Nam Wang and Samlee Kaimook Pumpui were classified as susceptible genotype**.** The main criteria to select to be tolerant or susceptible genotypes were waterlogged index of each characteristic recorded and differential value of all recorded data between normal irrigation (I-NC) and hypoxic condition (H-NC) was also considered.

Experiment # 2 Effects of Chitosan on Physiological and Morphological Responses of Corn Seedlings under Hypoxia.

This experiment was done two sets, field corn and waxy corn, by using one genotype of tolerant and susceptible genotypes selected from experiment # 1 of each corn set. For field corn seedling set, NSX 062029 and 30B80 (Pioneer) genotypes were represented as tolerant and susceptible genotypes respectively whereas Big white 852 and Neaw Roiet were represented as tolerant and susceptible genotypes of waxy corn seedling set respectively. The objectives of this experiment were to investigate the effect of chitosan on physiological and morphological responses of corn seedlings, tolerant and susceptible, under hypoxia. Various treatment conditions, normal irrigation without chitosan application (I-NC) , chitosan application before hypoxia(C-H) and hypoxia without chitosan application (H-NC), were done to compare corn seedling adaptation in each genotype. The results were found that slight-genotypic effect was observed for aerenchyma development under various treatment conditions. However, a number of new roots, aerenchyma development, root dry weight and leaf chlorophyll content were affected by various treatment conditions. C-H had positive effects on a number of new roots and aerenchyma development and also tended to maintain leaf greenness and chlorophyll content but did not affect soluble sugar accumulation and nitrate reductase activity (NR activity) in corn leaves.

Objectives:

1. To study hypoxic responses of various corn genotypes and then classify as susceptible and tolerant genotypes.

2. To investigate effect of chitosan on physiological and morphological responses of corn seedlings under hypoxic condition.

Materials and method

This study was divided into two experiments as follows:

Experiment # 1**.** Hypoxic responses of corn seedlings, this title was split into waxy corn seedling set (exp.# 1.1) and filed corn seedling set (exp.#1.2) .

Experiment # 2. Effects of Chitosan on Physiological and Morphological Responses of Corn Seedlings under Hypoxia, this title was split into waxy corn seedling set (exp.2.1) and filed corn seedling set (exp.2.2).

Experiment # 1 Hypoxic responses of corn seedlings

This experiment was conducted using a split plot in completely randomized design with three replications. The main plots were two conditions, normal irrigation condition (I-NC) and hypoxic condition (H-NC), and the sub plots were a number of waxy corn genotypes (exp.# 1.1) and a number of filed corn genotypes (exp.# 1.2). The treatment details are as follows:

Main plot was two treatment conditions.

1. Normal irrigation condition (I-NC)
2. Hypoxic condition (H-NC)

Sub plot of exp.1.1 was twenty genotypes of waxy corn.

1. Sri Prae
2. Lam Tong
3. Chat Ngurn
4. Samlee Kaimok Pumpui
5. Samlee Pumpui
6. Koanaew Pumpui
7. Samsri Trasing
8. Khao Samlee
9. Khao Padtaew
10. Teainleung Sainumpeung
11. Naew Roiet
12. Sweet White 25
13. Mungkorn Padtaew
14. Nam Nan
15. Big White 852
16. Ban khoa
17. Nam wang
18. Teaindam Namping
19. Khao Kow-naew
20. Naew Samlee

Sub plot of exp.# 1.2 was nineteen genotypes of field corn.

1. NSX 042005
2. NSX 042007
3. NSX 042010
4. NSX 042001
5. NSX 042022
6. NSX 042029
7. NSX 052014
8. NSX 062029
9. NSX 062030
10. NS 2
11. CP AAA Super (BSI)
12. Pac (Pacific)
13. KSX 4901(KU)
14. SW 4452 (KU)
15. 30B80 (Pioneer)
16. 30Y87 (Pioneer)
17. DK 9905(Monsanto)
18. NK 48 (Syngenta)
19. DK 979 (Monsanto)

Seeds of each genotype were planted in a 46 cm-diameter pot contained 7 kg. pot^{-1} of paddy soil having the following chemical properties; pH= 5.2, % OM = 0.89, aval.P = 5.01 ppm, exch. K = 66.2 ppm. Seven days after emergence, plants were thinned and left four healthy plants per pot. One experimental unit was comprised of four pots. One day before transient waterlogging initiation, dry matter accumulation of all genotypes was separately recorded by weight after drying at 80° C for 48 hours in hot- air oven. When corn seedlings had three to four true leaves (28-30 days after emergence); corn seedlings were subjected to transient waterlogging (hypoxia) by adding water to the pots until the water level reached 5.0 cm above the soil surface and maintained its level at 2.5-5.0 cm for nine days. After the ninth day of waterlogging, dry matter accumulation, plant height, chlorophyll content and leaf greenness, were immediately measured as the following details:

1. Dry matter accumulation: two plants per pot of each genotype were measured by weight after drying at 80° C for 48 hours in hot- air oven and then averaged out to dry matter per plant.

2. Plant height: two plants per plot were measured from the ground to the top of corn leaf and then averaged out to plant height.

3. Chorophyll content was extracted by N,N-dimethyl formamide (DMF) method (Moran and Porath, 1980).

4. Leaf greenness was measured on one side of the midrib, midway between leaf base and tip of leaf blade by chlorophyll meter (SPAD 502).

All data mentioned above were taken to calculate to be waterlogged index and the differential values between I-NC and H-NC whereas dry matter accumulation before and after subjecting waterlogging condition was used to calculate relative growth rate. The equations used to calculate are as follows:

Waterlogged index = (X2/X1)/(Y2/Y1)

X1= characters of each genotype measured in I-NC.
X2= characters of each genotype measured in H-NC
Y1= character average of all genotypes measured in I-NC
Y2= character average of all genotypes measured in H-NC

Differential values = $(X_1-X_2)/X_1$

X_1 = characters measured in I-NC.
X_2 = characters measured in H-NC.

Relative growth rate = $$\frac{\ln W2 - \ln W1}{(T2 - T1)}$$

W1 = dry matter at the beginning (T1)of the sampling period (before subjecting to waterlogging)
W2 = dry matter at the end (T2) of the sampling period (after subjecting to waterlogging)

T1 = timing at the beginning period

T2 = timing at the end period

All data were subjected to analysis of variance (ANOVA) and treatment mean comparision was done by the use of Dancant Multiple Range Test (DMRT).

Results

Twenty genotypes of waxy corn and nineteen genotypes of field corn were evaluated to screen to be tolerant or susceptible genotypes by recording dry matter accumulation , plant height , leaf greenness, chlorophyll content , relative growth rate and the differential values between I-NC and H-NC. Waterlogged index were also recorded by calculating from all recorded data formerly mentioned. Each characteristic of each genotype differently responded to H-NC. However, waterlogged index of each characteristic was the most important parameter to be considered to classify as tolerant or susceptible genotypes. Therefore, in field corn genotype set, the tolerant genotypes were 30Y87 (Pioneer), NSX 062030 and NSX 062029 and the susceptible genotypes were NK48 (Sygenta), 30B80(Pioneer) and Pac224 (Pacific). In regard to waxy corn genotype set, the tolerant genotypes were Big white 852, Mungkorn Padtaew and Samlee Puifai and the susceptible genotypes were Neaw Roiet, Nam Wang and Samlee Kaimook Pumpui. The result of each characteristic was as follows.

1. Dry matter accumulation

Hypoxia (H-NC) caused dry matter decreases in both waxy corn and field corn genotypes. Genotypic differences affected dry matter accumulation under both condition, I-NC and H-NC. Different treatment conditions influenced dry matter accumulation of both waxy corn and field corn genotypes. All genotypes poorly produced dry matter under H-NC when compared with those under I-NC. For waxy corn genotypes, Koanaew Pumpui, showed good adaptation under H-NC over than the other genotype because differential values between I-NC and H-NC was lower than the others. Furthermore, the genotype average between H-NC and I-NC was even higher whereas poorly adaptive genotype was observed in Neaw Roiet. Considering field corn genotypes, NSX 062030 genotype showed good performance under H-NC because the differential values between H-NC and I-NC was less than the other genotypes. Dry matter of DK979 (Monsanto) genotype was more susceptible than the other genotypes due to high differential values between H-NC and I-NC (table 1,2).

2. Plant height

Plant height of waxy corn and field corn genotypes was influenced by hypoxic condition (H-NC). Genotypes of waxy corn which were susceptible and tolerant to hypoxia were the same trends with dry matter accumulation; Mangkorn Padtaew genotype could retained plant height under H-NC greater than the others but plant height of Neaw Roiet genotype was more sensitive to H-NC. In field corn genotypes, the least differential values of plant height between I-NC and H-NC was found in NSX 042029 genotype because it could adapt itself under H-NC. 30Y87(Pioneer) genotype was the most susceptibility to H-NC, its plant height was remarkably decreased under H-NC (table 1,2)

Table 1 Effects of hypoxia on dry matter and plant height of waxy corn genotypes.

Genotype(G)	Dry matter (g)				Plant height (cm)			
	Condition (C)		Differential values	Genotype average	Condition (C)		Differential values	Genotype average
	I-NC	H-NC			I-NC	H-NC		
1 SRI PRAE	5.83	2.50	0.57	4.16 [ab]	102	60	0.41	81 [a]
2 LAM TONG	1.50	1.41	0.06	1.45 [b]	53	35	0.34	44 [b]
3 CHAT NGURN	5.33	3.33	0.38	4.33 [ab]	105	73	0.30	89 [a]
4 SAMLEE KAIMOOK PUMPUI	8.33	3.16	0.62	5.75 [a]	118	74	0.37	96 [a]
5 SAM LEE PUIFAI	5.66	3.83	0.32	4.75 [ab]	110	81	0.26	96 [a]
6 KOA NAEW PUMPUI	4.66	3.50	0.25	4.08 [ab]	103	64	0.38	84 [a]
7 3 SEE TRA SING	7.50	3.16	0.58	5.33 [ab]	103	72	0.30	88 [a]
8 KHAO SAMLEE	6.50	3.00	0.54	4.75 [ab]	112	76	0.32	94 [a]
9 KHAO PAD TAEW	5.16	2.66	0.48	3.91 [ab]	97	67	0.31	82 [a]
10 TEAIN LEUNG SAI NUMPEUNG	7.66	3.00	0.61	5.33 [ab]	101	66	0.35	84 [a]
11 NAEW ROI ET	7.66	1.16	0.85	4.41 [ab]	100	55	0.45	78 [a]
12 SWEET WHITE 25	6.83	4.00	0.41	5.41 [ab]	110	78	0.29	94 [a]
13 MANG KORN PAD TAEW	8.00	4.33	0.46	6.16 [a]	106	79	0.25	92 [a]
14 NAM NAN	6.16	3.16	0.49	4.66 [ab]	104	66	0.37	85 [a]
15 BIG WHITE 852	8.16	4.16	0.49	6.16 [a]	112	79	0.29	95 [a]
16 BAN KHOA	7.83	2.83	0.64	5.33 [ab]	115	74	0.36	94 [a]
17 NAM WANG	6.33	1.83	0.71	4.08 [ab]	106	66	0.38	86 [a]
18 TEAIN DAM NAM PING	6.16	2.16	0.65	4.16 [ab]	103	63	0.39	83 [a]
19 KHAO KHOA NAEW	8.00	2.83	0.65	5.14 [ab]	113	70	0.38	91 [a]
20 NAEW SAMLEE	8.16	3.76	0.54	5.96 [a]	114	71	0.38	92 [a]
Condition average	6.57	2.99			104	68		
F-test								
Condition (C)	*				*			
Genotype (G)	*				*			
Condition (C) x Genotype (G)	ns				ns			
CV(%)	32.79				9.71			

Table 2 Effects of hypoxia on dry matter and plant height of field corn genotypes.

Genotype (G)	Dry matter (g)		Differential values	Genotype average	Plant height(cm)		Differential values	Genotype average
	Condition (C)				Condition (C)			
	I-NC	H-NC			I-NC	H-NC		
NSX 042005	12.27	6.32	0.48	9.29^{ab}	24.19	14.82	0.39	19.50^{abc}
NSX 042007	14.51	7.86	0.46	11.19^{ab}	25.17	15.53	0.38	20.35^{abc}
NSX 042010	14.69	9.25	0.37	11.97^{ab}	27.42	18.11	0.34	22.76^{a}
NSX 042011	12.26	6.30	0.49	9.28^{ab}	23.99	15.95	0.34	19.97^{abc}
NSX 042022	10.54	6.93	0.34	8.73^{ab}	23.71	15.66	0.34	19.69^{abc}
NSX 042029	10.90	10.05	0.08	10.48^{ab}	24.50	18.27	0.25	21.39^{abc}
NSX 052014	11.51	9.26	0.20	10.38^{ab}	24.92	17.85	0.28	21.39^{abc}
NSX 062029	11.01	10.21	0.07	10.61^{ab}	25.01	17.78	0.29	21.39^{abc}
NSX 062030	10.85	9.09	0.16	9.97^{ab}	24.14	16.38	0.32	20.26^{abc}
NS 2	8.68	7.13	0.18	7.91^{b}	21.30	14.72	0.31	18.01^{c}
CP AAA Super (BSI)	10.31	5.91	0.43	8.11^{b}	22.63	16.51	0.27	19.57^{abc}
Pac224 (Pacific)	15.15	7.41	0.51	11.28^{ab}	25.93	16.66	0.36	21.29^{abc}
KSX 4901 (KU)	11.36	8.03	0.29	9.7^{ab}	23.99	16.72	0.30	20.35^{abc}
SW 4452 (KU)	16.26	8.17	0.50	12.21^{ab}	26.60	16.35	0.39	21.48^{abc}
30B80 (Pioneer)	17.12	7.69	0.55	12.40^{ab}	24.64	15.03	0.39	19.83^{abc}
30Y87 (Pioneer)	11.96	6.18	0.48	9.07^{ab}	25.38	15.28	0.40	20.33^{abc}
DK 9905 (Monsanto)	15.08	9.66	0.36	12.37^{ab}	27.79	17.12	0.38	22.45^{ab}
NK48 (Syngenta)	18.94	8.88	0.53	13.91^{a}	26.30	16.07	0.39	21.18^{abc}
DK979 (Monsanto)	19.76	7.11	0.64	13.44^{ab}	21.69	15.04	0.31	18.37^{bc}
Condition average	3.32	7.97			24.70	16.31		
F-test								
Condition (C)	*				*			
Genotype (G)	*				*			
Condition (C) x Genotype(G)	ns				ns			
CV.(%)	26.38				10.65			

3. Leaf greenness

Hypoxia influenced leaf greenness of both field corn and waxy corn genotypes. Under hypoxia (H-NC), leaf greenness value of all genotypes were remarkably decreased. For waxy corn genotype, the most adaptive genotype under H-NC in terms of leaf greenness retention was Sumlee Pufai because the differential value of leaf greenness between I-NC and H-NC was not high like the other genotypes whereas Khao Samlee was the most sensitive genotype to H-NC. With regard to field corn genotypes, three genotypes showed good adaptation to retain their leaf greenness, namely NSX062029, 30B80 (Pioneer) and 30Y87 (Pioneer) but Pac 224 (Pacific) genotype poorly retained its leaf greenness under H-NC (table 3,4).

4. Chlorophyll content

Chlorophyll content in leaves of waxy corn and field corn genotypes was dramatically decreased under H-NC. Leaf chlorophyll content responses to H-NC showed the same trend with leaf greenness. In waxy corn genotypes, Big white 852 (commercial hybrid) showed an ability to retain chlorophyll content under H-NC greater than the other genotypes. On the other hand, the most sensitive genotype to H-NC in terms of chlorophyll content was Khao Padteaw and See Tra Sing. For the field corn genotypes, 30B80 (Pioneer) was the most adaptive genotype to retain chlorophyll content under H-NC. The poorly adaptive genotype was Pac 224(Pacific) (table 3,4).

Table 3 Effects of hypoxia on chlorophyll content and leaf greenness of waxy corn genotypes.

Genotype(G)	Chlorophyll content (mg/g fresh wt.) Condition (C)		Differential values	Genotype average	Leaf greenness (spad unit) Condition (C)		Differential values	Genotype average
	I-NC	H-NC			I-NC	H-NC		
1 SRI PRAE	2.22	1.29	0.42	1.76 [ab]	33.96	22.96	0.32	28.47 [ab]
2 LAM TONG	1.92	1.20	0.38	1.56 [b]	27.43	19.46	0.29	23.45 [b]
3 CHAT NGURN	2.18	1.47	0.33	1.83 [ab]	31.03	22.10	0.29	26.57 [ab]
4 SAMLEE KAIMOOK PUMPUI	2.25	1.22	0.46	1.73 [ab]	30.76	21.36	0.31	26.07 [ab]
5 SAM LEE PUIFAI	2.06	1.53	0.26	1.79 [ab]	30.36	22.80	0.25	26.78 [ab]
6 KOA NAEW PUMPUI	2.38	1.84	0.23	2.11 [ab]	41.46	25.66	0.38	33.57 [a]
7 3 SEE TRA SING	2.57	1.16	0.55	1.84 [ab]	38.80	23.23	0.40	31.02 [ab]
8 KHAO SAMLEE	2.14	1.28	0.40	1.71 [ab]	39.20	21.00	0.46	30.10 [ab]
9 KHAO PAD TAEW	2.80	1.26	0.55	2.03 [ab]	36.26	20.10	0.45	28.18 [ab]
10 TEAIN LEUNG SAI NUMPEUNG	2.07	1.45	0.30	1.76 [ab]	36.63	25.00	0.32	30.82 [ab]
11 NAEW ROI ET	2.33	1.28	0.45	1.81 [ab]	35.96	22.73	0.37	29.35 [ab]
12 SWEET WHITE 25	2.15	1.58	0.27	1.87 [ab]	40.66	26.40	0.35	33.53 [a]
13 MANG KORN PAD TAEW	2.22	1.39	0.37	1.81 [ab]	32.56	24.00	0.26	28.28 [ab]
14 NAM NAN	2.75	1.70	0.38	2.23 [a]	36.60	24.43	0.33	30.52 [ab]
15 BIG WHITE 852	2.37	2.02	0.15	2.19 [a]	40.66	29.76	0.27	35.22 [a]
16 BAN KHOA	2.35	1.40	0.40	1.87 [ab]	31.50	21.06	0.33	26.28 [ab]
17 NAM WANG	2.74	1.83	0.33	2.29 [ab]	42.46	24.13	0.43	33.30 [a]
18 TEAIN DAM NAM PING	2.15	1.78	0.17	1.96 [ab]	41.20	25.16	0.39	33.18 [a]
19 KHAO KHOA NAEW	2.25	1.64	0.27	1.94 [ab]	33.06	24.53	0.26	28.80 [ab]
20 NAEW SAMLEE	2.40	1.20	0.50	1.80 [ab]	34.26	20.66	0.40	27.47 [ab]
Condition average	2.31	1.47			35.76	23.33		
F-test								
Condition (C)	*				*			
Genotype(G)	*				*			
Condition (C) x Genotype (G)	ns				ns			
CV.(%)	16.65				12.13			

Table 4 Effects of hypoxia on chlorophyll content and leaf greenness of field corn genotypes.

Genotype (G)	chlorophyll content (mg/g fresh wt.) Condition (C)		Differential values	Genotype average	Leaf greenness (spad unit) Condition (C)		Differential values	Genotype average
	I-NC	H-NC			I-NC	H-NC		
NSX 042005	8.50	5.02	0.41	6.76	46.86	30.53	0.35	38.70
NSX 042007	10.43	5.37	0.49	7.90	41.33	25.20	0.39	33.26
NSX 042010	9.90	4.27	0.57	7.08	48.06	25.40	0.47	36.73
NSX 042011	9.97	4.73	0.53	7.35	48.50	24.03	0.50	36.26
NSX 042022	10.03	4.00	0.60	7.01	46.53	25.63	0.45	36.08
NSX 042029	10.43	3.77	0.64	7.10	46.33	26.66	0.42	36.50
NSX 052014	9.73	4.43	0.54	7.08	47.53	29.63	0.38	38.58
NSX 062029	10.63	5.30	0.50	7.96	48.70	33.46	0.31	41.08
NSX 062030	9.93	4.50	0.55	7.21	49.00	31.76	0.35	40.38
NS 2	11.17	4.90	0.56	8.03	49.80	29.93	0.40	39.86
CP AAA Super (BSI)	10.27	4.73	0.54	7.50	49.76	30.73	0.38	40.23
Pac 224(Pacific)	9.20	3.20	0.65	6.20	49.40	22.03	0.55	35.71
KSX 4901 (KU)	9.10	5.27	0.42	7.18	47.76	31.70	0.34	39.73
SW 4452 (KU)	9.03	4.65	0.49	6.84	47.40	29.73	0.37	38.56
30B80 (Pioneer)	9.20	5.47	0.41	7.33	47.10	32.70	0.31	39.90
30Y87 (Pioneer)	10.43	5.80	0.44	8.11	47.60	32.96	0.31	40.28
DK 9905 (Monsanto)	10.03	5.47	0.45	7.75	48.66	31.63	0.35	40.15
NK48 (Syngenta)	8.63	4.10	0.52	6.36	47.43	26.36	0.44	36.90
DK979 (Monsanto)	8.83	3.53	0.60	6.18	46.53	25.50	0.45	36.01
Condition average	9.76	4.65			47.59	28.71		
F-test								
Condition (C)			**				**	
Genotype(G)			ns				ns	
Condition (C) x Genotype (G)			ns				ns	
CV.(%)			20.63				10.67	

4. Relative growth rate

Relative growth rate of both waxy corn and field corn genotypes was not significantly different within their groups under the same condition. In regard to waxy corn genotypes, Khao Padtaew could maintain relative growth rate under H-NC greater than the other genotypes but relative growth rate of Neaw Roiet under H-NC was poorly; it meant that it was very susceptible to H-NC. Considering field corn genotypes, relative growth rate was not differently between I-NC and H-NC. Because most of field corn genotypes used in this study were precommercial hybrid which showed good adaptation in wide environment. However, NSX062030 tended to accumulate dry matter under H-NC which associated to relative growth rate better than the other genotypes. NK48 (Syngenta) was the most sensitive genotype to H-NC because relative growth rate under H-NC was lower than the other genotypes (table5,6).

Table 5 Effects of hypoxia on relative growth rate of waxy corn genotypes.

Genotype (G)	Condition (C)		Differential values	Genotype average
	I-NC	H-NC		
1 SRI PRAE	0.19	0.13	0.32	0.16
2 LAM TONG	0.13	0.12	0.08	0.12
3 CHAT NGURN	0.14	0.08	0.43	0.11
4 SAMLEE KAIMOOK PUMPUI	0.20	0.06	0.70	0.13
5 SAM LEE PUIFAI	0.11	0.06	0.45	0.08
6 KOA NAEW PUMPUI	0.14	0.10	0.29	0.12
7 3 SEE TRA SING	0.18	0.10	0.44	0.14
8 KHAO SAMLEE	0.18	0.09	0.50	0.13
9 KHAO PAD TAEW	0.16	0.12	0.25	0.14
10 TEAIN LEUNG SAI NUMPEUNG	0.22	0.10	0.55	0.16
11 NAEW ROI ET	0.25	0.01	0.96	0.13
12 SWEET WHITE 25	0.17	0.08	0.53	0.12
13 MANG KORN PAD TAEW	0.19	0.14	0.26	0.16
14 NAM NAN	0.16	0.08	0.50	0.12
15 BIG WHITE 852	0.17	0.12	0.29	0.14
16 BAN KHOA	0.17	0.08	0.53	0.12
17 NAM WANG	0.19	0.04	0.79	0.11
18 TEAIN DAM NAM PING	0.18	0.09	0.50	0.13
19 KHAO KHAO NAEW	0.17	0.10	0.41	0.13
20 NAEW SAMLEE	0.16	0.10	0.38	0.13
Condition average	0.17	0.09		
F-test				
Condition (C)	*			
Genotype (G)	ns			
Condition (C) x Genotype(G)	ns			
CV.(%)	**32.79**			

Table 6 Effects of hypoxia on relative growth rate of field corn genotypes.

Genotype (G)	Condition (C)		Differential values	Genotype average
	I-NC	H-NC		
NSX 042005	0.179	0.160	0.11	0.169
NSX 042007	0.180	0.138	0.23	0.159
NSX 042010	0.203	0.152	0.25	0.177
NSX 042011	0.209	0.142	0.32	0.175
NSX 042022	0.205	0.137	0.33	0.171
NSX 042029	0.224	0.155	0.31	0.189
NSX 052014	0.195	0.126	0.35	0.160
NSX 062029	0.190	0.155	0.18	0.172
NSX 062030	0.170	0.164	0.04	0.167
NS 2	0.205	0.181	0.12	0.193
CP AAA Super (BSI)	0.217	0.143	0.34	0.180
Pac224 (Pacific)	0.210	0.148	0.30	0.179
KSX 4901 (KU)	0.207	0.183	0.12	0.195
SW 4452 (KU)	0.205	0.161	0.21	0.183
30B80 (Pioneer)	0.232	0.185	0.20	0.208
30Y87 (Pioneer)	0.209	0.196	0.06	0.202
DK 9905 (Monsanto)	0.222	0.186	0.16	0.204
NK48 (Syngenta)	0.232	0.145	0.38	0.188
DK979 (Monsanto)	0.233	0.205	0.12	0.219
Condition average	0.206	0.161		
F-test				
Condition (C)	ns			
Genotype(G)	ns			
Condition (C) x Genotype(G)	ns			
CV.(%)	25.16			

5. Waterlogged index

Waterlogged index was the most important parameter to classify corn genotype to be tolerant or susceptible genotype. The value of waterlogged index of each characteristic of both waxy corn and field corn genotypes was so varied. However, if some genotypes showed high waterlogged index values in many characteristics; it would be classified to be tolerant genotype. On the other hand, some genotypes showed low waterlogged index values; it would be classified to be susceptible genotype. Three waxy corn genotypes named Big white 852, Mungkorn Padtaew and Samlee Puifai were classified as tolerant genotypes but Neaw Roiet, Nam Wang and Samlee Kaimook Pumpui were classified as susceptible genotypes. For field corn genotypes, 30Y87 (Pioneer), NSX 062030 and NSX 062029 genotypes were classified as tolerant genotype but NK48 (Sygenta), 30B80(Pioneer) and Pac224 (Pacific) were classified as susceptible genotype (table 7-8).

Table 7 Waterlogged index value of each characteristic of waxy corn genotypes .

Genotype(G)	waterlogged index				
	Relative growth rate	Plant height	Dry matter accumulation	Chlorophyll content	Leaf greenness
1 SRI PRAE	1.16	0.90	0.94	0.91	1.03
2 LAM TONG	1.85	1.01	2.06	0.98	1.08
3 CHAT NGURN	0.97	1.05	1.37	1.06	1.09
4 SAMLEE KAIMOOK PUMPUI	0.51	0.95	0.83	0.85	1.06
5 SAM LEE PUIFAI	0.92	1.12	1.48	1.16	1.13
6 KOA NAEW PUMPUI	1.21	0.94	1.65	1.21	0.94
7 3 SEE TRA SING	0.94	1.07	0.92	0.67	0.91
8 KHAO SAMLEE	0.85	1.03	1.01	0.94	0.82
9 KHAO PAD TAEW	1.27	1.05	1.13	0.70	0.85
10 TEAIN LEUNG SAI NUMPEUNG	0.77	0.99	0.86	1.10	1.04
11 NAEW ROI ET	0.06	0.83	0.33	0.86	0.96
12 SWEET WHITE 25	0.80	1.07	1.28	1.15	0.99
13 MANG KORN PAD TAEW	1.25	1.14	1.19	0.98	1.13
14 NAM NAN	0.85	0.96	1.12	0.96	1.02
15 BIG WHITE 852	1.20	1.07	1.12	1.33	1.12
16 BAN KHOA	0.80	0.97	0.79	0.93	1.02
17 NAM WANG	0.35	0.95	0.63	1.04	0.87
18 TEAIN DAM NAM PING	0.85	0.93	0.77	1.29	0.93
19 KHAO KHOA NAEW	1.0	0.94	0.77	1.14	1.13
20 NAEW SAMLEE	0.10	0.94	1.01	0.78	0.92

Table 8 Waterlogged index value of each characteristic of field corn genotypes.

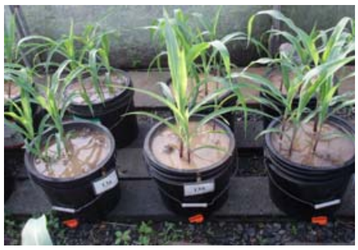
Genotype (C)	Waterlogged index				
	Plant height	Leaf greenness	Relative growth rate	Dry matter	Chlorophyll content
NSX 042005	0.10	1.08	1.14	0.86	1.24
NSX 042007	0.93	1.01	0.98	0.90	1.07
NSX 042010	1.00	0.88	0.95	1.05	0.89
NSX 042011	1.00	0.82	0.86	0.85	1.00
NSX 042022	1.00	0.91	0.85	1.09	0.84
NSX 042029	1.12	0.95	0.88	1.54	0.75
NSX 052014	1.08	1.03	0.82	1.34	0.95
NSX 062029	1.07	1.14	1.04	1.55	1.05
NSX 062030	1.02	1.08	1.23	1.40	0.95
NS 2	1.04	1.00	1.13	1.37	0.93
CP AAA Super (BSI)	1.10	1.02	0.84	0.95	0.97
Pac224 (Pacific)	0.97	0.74	0.90	0.81	0.73
KSX 4901 (KU)	1.05	1.10	1.13	1.18	1.20
SW 4452 (KU)	0.93	1.04	1.00	0.83	1.07
30B80 (Pioneer)	0.92	1.15	1.02	0.75	1.23
30Y87 (Pioneer)	0.91	1.15	1.20	0.86	1.17
DK 9905 (Monsanto)	0.93	1.08	1.07	1.07	1.13
NK48 (Syngenta)	0.92	0.92	0.80	0.78	1.00
DK979 (Monsanto)	1.05	0.91	1.12	0.60	0.83

Experiment # 2. Effects of Chitosan on Physiological and Morphological Responses of Corn Seedlings under Hypoxia.

This experiment was done two sets, in field corn genotype and waxy corn genotype. Each set was conducted using a split plot in completely randomized design with two main plots ,tolerant and suceptible genotype, of each corn set. Three sub plots, normal irrigation without chitosan application(I-NC) , chitosan application before hypoxia(C-H) and hypoxia without chitosan application (H-NC), were utilized having four pots per an experimental unit and replicated four times. This study was the pot experiment conducted in an open-ended outdoor greenhouse with daily maximum and minimum termperature of 33 °C and 23 °C . Seeds of each genotype were planted in a 46 cm-diameter pot contained 7 kg pot^{-1} of paddy soil having the following chemical properties; pH= 5.2, % OM = 0.89, aval.P = 5.01 ppm, exch. K = 66.2 ppm. Seven days after emergence, plant were thinned and left four healthy plants per pot. Chitosan at the concentration of 80 ppm was sprayed four times (7, 14, 21 and 28 days after planting) on corn leaves before initiation of hypoxia. After one day of the last chitosan spraying; corn seedlings were subjected to transient waterlogging (hypoxia) by adding water to the pots until the water level reached 5.0 cm above the soil surface and maintained its level at 2.5-5.0 cm for nine days. After the ninth day of waterlogging, irrigation was withdrawn from the pot and then two plants per plot were sampled. The newly generated roots (young white root) were counted and the total roots were dried at 80 °C for 24 hours until the root weight was constant, and then root dry weight was recorded. Percentage of aerenchyma development was evaluated using the method described by IRRI 1995. Free-hand cross-section of young nodal roots were done at 5 cm from root tips and photographed with the Olympus compound microscope at 10X. For each photograph the whole root cross-sectional region was cut and weighed (A) and, the aerenchyma region was cut and weighed (B) separately. Then B/A ratio was calculated and finally converted to percentage of aerenchyma development. For the leaf blade, leaf greenness was measured on one side of the midrib, midway between leaf base and tip by chlorophyll meter. Corn leaves in each treatment were immediately removed from the plants for chlorophyll content extraction by N,N-dimethyl formamide (DMF) method (Moran and Porath, 1980). Leaf nitrate reductase activity was analysed by the method of Jaworski (1971) and soluble sugar accumulation in leaves was investigated by the method of Yoshida (Yoshida *et al.*,1976).This experiment was carried out at Rajamangala University of Technology Suvarnabhumi during September to November 2008. All data obtained were subjectd to Analysis of Variance (ANOVA) and treatment mean comparision was done by the use of Least Significant Difference (LSD).

Note: I-NC = Irrigation was sufficiently applied in each pot to prevent drought stress.
H-NC = Pots were flooded with irrigation and maintained at 2.5-5.0 cm above the soil surface for nine days.

I-NC



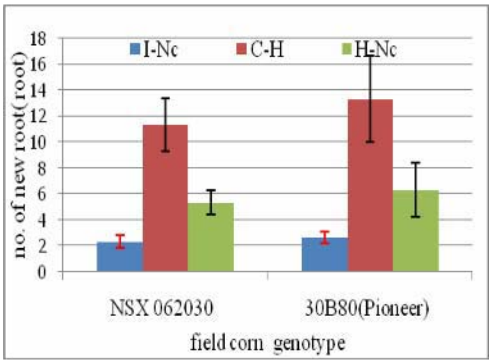
H-NC

Results and discussion

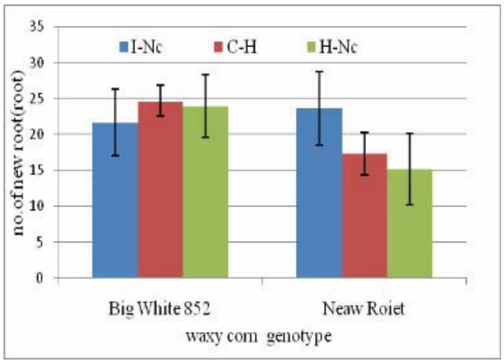
1. Morphological responses

1.1 New root development

With regard to field corn genotypes, the number of new root development(NNR) of both NSX 062030) and 30B80(Pioneer) observed in C-H was significantly grether than in I-NC and H-NC (figure 1a). All new roots generated were thicker than the existing roots. Effects of genotype did not influence NNR in this study. This finding suggested that chitosan may cause to increase NNR under hypoxia resulting in increased root surface exposed to air and consequently increased aerobic respiration. This result was supported by the work of Chibu *et al.,*(1999), who revealed that the numbers of first order, thick lateral roots and second order, lateral roots of radish (*Raphenus sativus*, radicula group) were apparently increased with chitosan application at the concentration of 0.1 %. Similar results were reported by Shou-Qiang and Lang-Lai (2003)who indicated that root length of chinese cabbage (*Brassica campestris*) cv. Dwarf hybrid No. 1 was increased after chitosan was applied by seed dressing with 0.4-0.6 mg chitosan g^{-1}seed and leaf spraying with 20-40 µg chitosan ml^{-1}. In regard to waxy corn genotypes, no statistic difference was found in terms of NNR even though different genotypes and treatment conditions were applied. Big white 852 showed good adaptation greater than Neaw Roiet under C-H and H-NC due to commercial variety.C-H did not influence NNR in both waxy corn genotypes (figure 1b). .



(a)

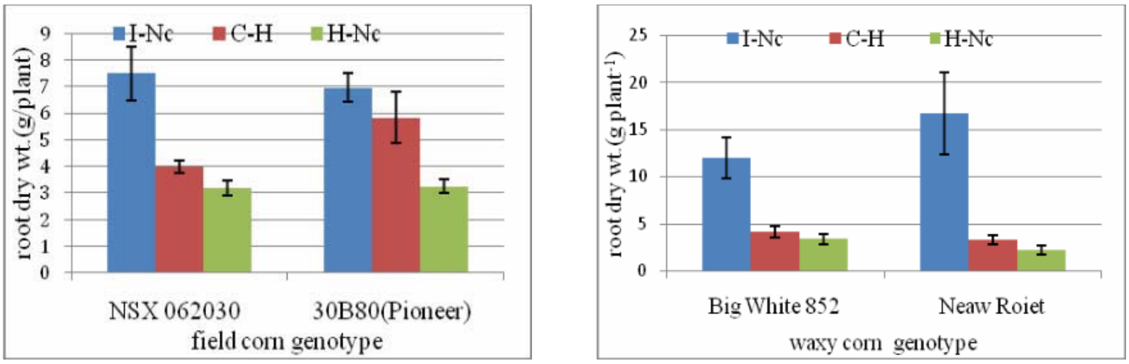


(b)

Figure 1. Effects of chitosan on number of new root development (NNR) of field corn (a) and waxy corn (b) genotypes under different conditions.

1.2 Root dry weight

Under I-NC, root dry weight of both field corn genotypes was greater than that under C-H and H-NC. NSX 062030 genotype, the tolerant one, significantly produced greater root dry weight whereas C-H did not affect root dry weight of this genotype. Root dry weight of 30B80 genotype, the suscepible one, did not significantly differ between under I-NC and H-NC. It can be explained that chitosan clearly contributed to increased NNR being thicker than the existing roots under hypoxia which was also resulted in increase of total root dry weight (figure 2a). This is one of morphological responses that sometimes help plants to survive under hypoxia. Xue *et al*. (2002) found that rapeseed (*Brassica chinensis*) *c.v.* Aikangqin coated with chitosan showed positive effects on root length . For the waxy corn genotypes, root dry weight of both genotypes, Big white 852 and Neaw Roiet, under I-NC was certainly higher than that under C-H and H-Nc and showed significant differences. Adaptation under C-H and H-NC of both waxy corn genotypes was quite low and C-H did not contribute to increased root dry weight (figure 2b).

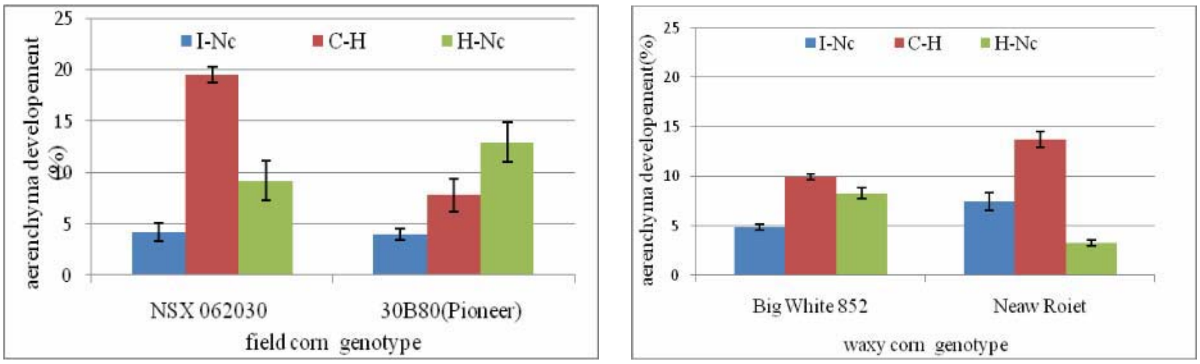


(a) (b)

Figure 2 . Effects of chitosan on root dry weight of field corn (a) and waxy corn (b) genotypes under different conditions.

1.3 <u>Aerenchyma developement</u>

For field corn genotypes, aerenchyma tissue in root cortex of NSX062030 genotype dramatically developed under C-H; the percentage of aerenchyma development was higher than that of 30B80 (Pioneer) genotype under the same condition. It may be conceivable that 30B80 (Pioneer) genotype was susceptible to hypoxia, thus it poorly adapted to generate aerenchyma under C-H and H-NC. Considering within genotype under hypoxic condition, it was found that C-H affectd aerenchyma development of NSX062030 genotype, the tolerant genotype, but did not affect that of 30B80 (Pioneer) (figure 3a,4). In terms of waxy corn genotypes, aerenchyma development of Big white 852, the tolerant genotype, under C-H was not higher. Neaw Roiet formed aerenchyma better than Big white 852 under C-H and showed significant differences from I-NC and H-NC. Different genotypes did not dramatically show aerenchyma development differences (figure 3b,5). This results were suported by the work of Pourabdal *et al.,* (2008) who indicated that development of aerenchyma and adventious roots are more recessive factors that increases hypoxic tolerance in *Zea mays.* The aerenchyma formation and adventitious roots in the vicinity of cotyledonary nodes is an indicator of adaptive mechanisms present in flood-tolerant plants (Kawase, 1981). The most conspicuous anatomical response of crop roots to soil waterlogging or anoxia is the development of an extensive aerenchyma system in their cortex (Konings and Lambers ,1991).

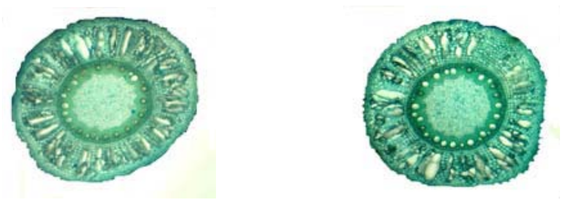


(a) **(b)**

Figure 3 Effects of chitosan on aerenchyma development of field corn (a) and waxy corn (b) genotypes under different conditions.

(a) (b) (c)

NSX 062030 (tolerant genotype)



(d) (e) (f)

30B80 (Pioneer) (susceptible genotype)

Figure 4 Aerenchyma development of field corn genotypes, namely NSX 062030 and 30B80(Pioneer) under different conditions: I-NC (a and d), C-H (b and e) and H-NC (c and f).

(a) (b) (c)

Big white 852(tolerant genotype)

(d)

(e)

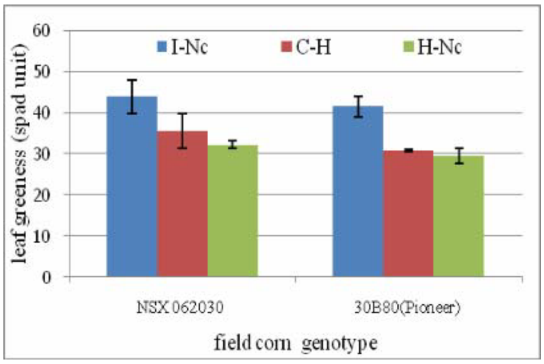
(f)

Neaw Roiet (susceptible genotype)

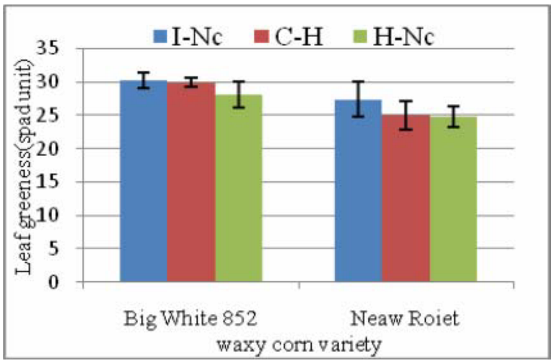
Figure 5 Aerenchyma developement of waxy corn genotypes, namely Big white 852 and Neaw Roiet under different conditions: I-NC (a and d), C-H (b and e) and H-NC (c and f).

1.4 <u>leaf greenness</u>

Under I-NC, leaf greenness values of both field corrn genotypes were dramatically higher than those of under C-H and H-NC. C-H did not significantly affect leaf greenness, however, tended to maintain leaf greenness (figure 6 a). In regard to waxy corn genotypes, it was found that leaf greenness values of the tolerant genotype, Big white 852, were higher than those of susceptible genotype ,Neaw Roiet, in all conditions. C-H did not contribute to leaf greeness retention in Neaw Roiet genotype but slightly retained leaf greeness in Big White 852 genotype (figure 6 b). The previous study of Chibu and Shibayama (1999) reported that tomato and lettuce leaves turned darker green with increasing chitosan concentrations.



(a)



(b)

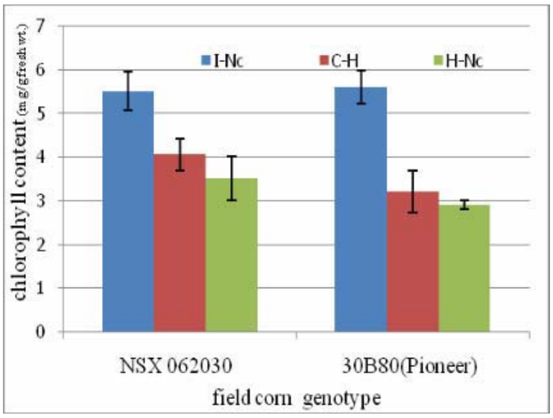
Figure 6 Effects of chitosan on leaf greenness of field corn (a) and waxy corn(b) genotypes under different conditions**.**

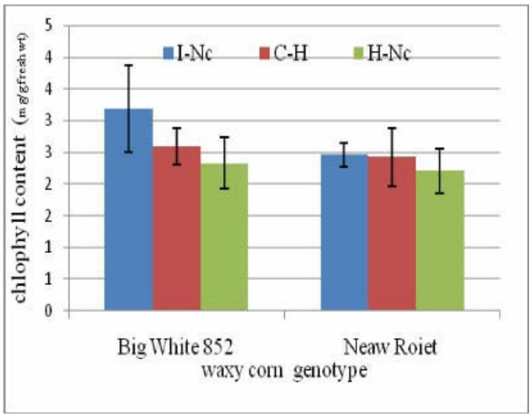
2. Physiological responses

2.1 <u>chlorophyll content</u>

Total chlorophyll content of both field corn genotypes significantly decreased under C-H and H-NC. It was high in I-NC and gradually decreased in C-H and H-NC respectively (figure 7a). NSX 062030 genotype adapted itself to retain chlorophyll content greater than 30B80 (Pioneer) genotype under C-H. With regard to waxy corn genotypes, it showed the same responses with field corn genotypes. However, total chlorophyll content of both waxy corn genotypes did not differ under C-H and H-NC. C-H did not conspicuously affect chlorophyll retention (figure

7b). This study was contrary to the report of XianLing *et al.* (2002) who found that chlorophyll content of mulberry cultivar Sha 2 was increased with chitosan application at the concentration of 0.5-2.0%.Furthermore, application of chitosan at 0.5 to 2.0 g kg^{-1}seed of soybean cv. Suinong as seed coating positively affected chlorophyll content (Hong Yan and ShuYu, 2001) .



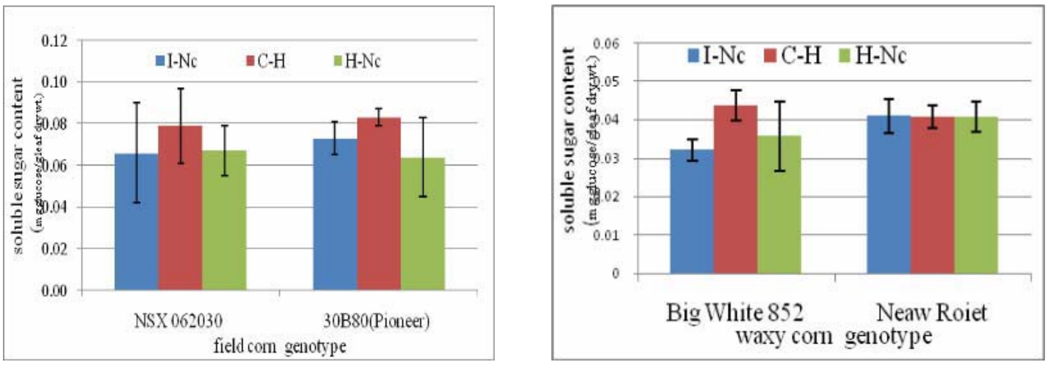


(a) (b)

Figure7. Effects of chitosan on total chlorophyll content of field corn (a) and waxy corn (b) genotypes under different conditions.

2.2 soluble sugar accumulation

For field corn genotypes, soluble sugar content in leaves of two genotypes was not significantly different in all treatment conditions. C-H tended to accumulate high soluble sugar in both NSX 062030 and 30B80 (Pioneer) genotypes (figure 8a). As regards waxy corn genotypes, C-H contributed to increased soluble sugar in Big White 852 but did not affect it in Neaw Roiet. It might be explained that Neaw Roiet is susceptible to hypoxia, therefore it poorly adapted to accumulate soluble sugar when it was subjected to hypoxia (figure 8b). Chitosan may induce plant cells to synthesize soluble sugar by increasing the activities of some enzymes which associated to produce secondary metabolites (Khan *et al*., 2003). The results were in line with Shou-Qiang and Langlai (2003) who showed that the content of soluble sucrose in non-heading Chinese cabbage leaves was increased after seed dressing and leaf spraying with chitosan. The amount of soluble sugar had been increased in both the roots and shoots of maize seedlings under flooding (Pourabdal, 2008). The soluble sugar consumption under fermentative metabolism during flooding condition had been increased (Mohanty *et al.,*1993).

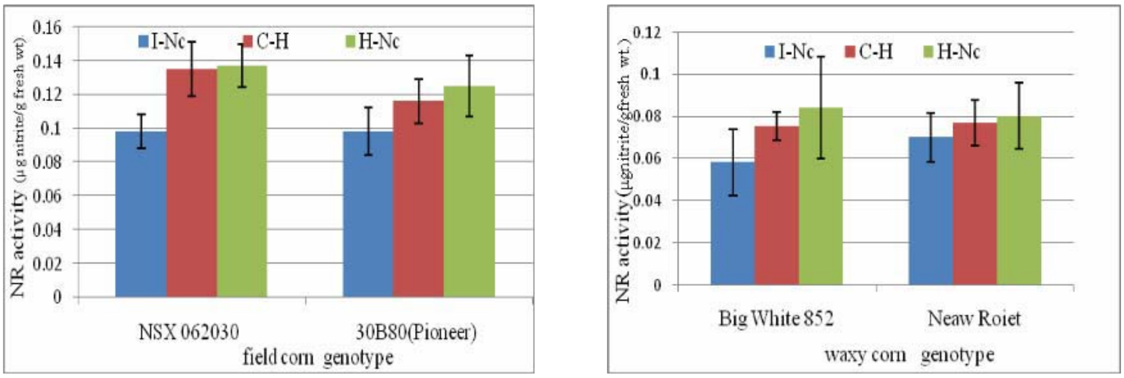


(a) (b)

Figure 8. Effects of chitosan on soluble sugar content in leaves of field corn (a) and waxy corn(b) genotypes under different conditions.

2.3 Nitrate reductase activity

The NR activities in both field corn genotypes under C-H and H-NC were higher than those under I-NC. No significant difference in terms of NR activity between two field corn genotypes was detcted. NR activitiy of both genotypes did not differ between C-H and H-NC(figure 9a). It could be explained that under waterlgging, the most important compound utilized by soil microorganisms as alternative electron acceptors for respiration is NO_3^-, which is rapidly reduced to be NO_2^- resulted in increased NR activity. In terms of waxy corn genotypes, the NR activity trend was similar to that in field corn genotypes. However, C-H did not increase NR activity in both Big White 852 and Neaw Roiet genotyppes (figure 9b). For crop plants, some species are root-reducers for NO^-_3 and some species are leaf-reducers. Garcia-Novo and Crawford (1973) and Lambers(1976) reported that the activity of NR in roots of flooded-tolerant plants increased rapidly during flooding, as did the amino acid synthesis capability. In contrast, Xian-Ling *et al*. (2002) revealed that seeds of mulberry cv. Sha2 coated with chitosan solution at 0.5-2.0 % could increase NR activities. On the other hand, the activity of NR in roots of wax- apple tree was significantly decreased with flooding (Hsu *et al.,*1999). The contradictory results may be because nitrate reductase (NR) is well known as a substrate-inducible enzyme in cereals (Beevers.,1965). However, the use of NR activity as marker of NO^-_3 utilization in individual plant species is impossible without the precise method of optimization (Munzarovo *et al.,* 2006).



(a) (b)

Figure 9 Effects of chitosan on nitrate reductase activity in leaves of field corn (a)and waxy corn(b) genotypes under different conditions .

Conclusions

In this study, it was divided into two experiments. For experiment #1 we screened tolerant and susceptible genotypes in field corn and waxy corn genotypes by focusing on waterlogged index. For field corn genotype, the result was found that the tolerant genotype was 30Y87 (Pioneer), NSX 062030 and NSX 062029 whereas the susceptible genotype was NK48 (Syngenta), 30B80(Pioneer) and Pac224 (Pacific). In regard to waxy corn genotype, it was found that Big White 852, Mungkorn Padteaw and Samlee Puifai were classified as tolerant genotype but Neaw Roiet, Nam Wang and Samlee Kaimook Pumpui were classified as susceptible genotype**.** For the experiment #2 we studied on effects of chitosan on physiological and morphological responses of corn seedlings under hypoxia.The results found that chitosan application showed positive effects on the number of new root development and aerenchyma formation of corn seedlings under hypoxia. Moreover, chitosan application tended to keep leaf greenness and chlorophyll content in corn leaves under hypoxia but did not affect NR activity and soluble sugar content in corn leaves. According to this result, it might be suggested that chitosan had an ability to stimulate some responses in corn seedlings relating to survival under hypoxic condition.

Acknowledgements

The authors would like to thank Thailand Research Fund (TRF) and the Commission of Higher Education (CHE) for the research budget. Special thanks are extended to Dr. sansern Jompathong from National Corn and Sorghum Research Center and Mr.Pichet Krudloyma from Nakhon Sawan Field Crop Research Center for providing field corn seeds in this experiment.

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APPENDIXS

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. การนำเสนอผลงานวิจัยที่ได้รับทุนจาก สกว. ในการประชุม 9th International Conference of the European Chitin Society. ที่เกาะ San Servolo เมือง Venice ประเทศ ITALY. **(เอกสารแนบ)**

2. Manuscript เรื่อง Physiological and Morphological Responses of Corn Seedlings Applied with Chitosan under Hypoxic Condition กำลังอยู่ระหว่างการตรวจทานของนักวิจัยที่ปรึกษา และคาดว่าจะส่งตีพิมพ์ในวารสาร ScienceAsia ภายในเดือน สิงหาคม 2552 นี้ (เอกสารแนบ)

EUCHI 2009

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EFFECTS OF CHITOSAN ON PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF FIELD CORN SEEDLINGS UNDER HYPOXIA

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INTRODUCTION

Chitosan is a natural biopolymer found inexoskeletons of crustaceans and insect cuticles as well as cell walls of fungi and some algae. Chitosan has been shown to induce defence mechanism in many plant species (Ben-Shaloam *et al.* 2003; Photchanachai *et al.* 2006) and increase the activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) ,the key enzymes of phenylpropanoid pathways associated with synthesis of secondary plant metabolites under unfavorable condition

(Khan *et al,* 2003). Furthermore, chitosan also promotes plant growth and enhances yield of many crop species such as *Oryza sativa* L.and *Eustoma grandiflorum* (Boonlertnirun *et al*., 2008; Ohta *et al*., 1999) as well as accelerates germination rate and germination index (Xue-Yan *et al.,* 2002). Hypoxia describes the status of cell or tissue which is deficient or low in O_2 concentration (<20%). This term is different from anoxia, in which the O_2 concentration is zero (Pradet and Bomsel, 1978). Hypoxia also reduces shoot and root growth, dry matter accumulation and final yield (Malik *et al*., 2002). It is estimated to reduce yields by 20 to 25% but the loss may exceed 50% depending on the stage of plant development (Setter *et al.,* 1999). The most conspicuous anatomical response of crop roots to soil waterlogging is the development of an extensive aerenchyma system in their cortex (Konings and Lamber, 1991). 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 condition.

MATERIALS and METHODS

This experiment was conducted using a split plot in completely randomized design with two genotypes: tolerant (NSX 062030) and suceptible (30B80(Pioneer) as main plot, 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 supplot, having four pots per an experimental unit and replicated four times. The pot experiment was conducted in an open-ended outdoor greenhouse. with daily maximum and minimum termperature of 33 °C and 23 °C. Seeds of each genotype were planted in a 46 cm-diameter pot contained 7 kg pot^{-1} of paddy soil having the following chemical properties; pH= 5.2, % OM = 0.89, aval.P = 5.01 ppm, exch. K = 66.2 ppm. Seven days after emergence, plants were thinned and left four healthy plants per pot, and the onset of chitosan application was firstly done according to the treatment details.

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Chitosan at the concentration of 80 ppm was sprayed four times (7, 14, 21 and 28 days after planting) on corn leaves. After one day of the last chitosan spraying; corn seedlings were subjected to transient waterlogging (hypoxia) by adding water to the pots until the water level reached 5.0 cm above the soil surface and maintained its level at 2.5-5.0 cm for nine days. After the ninth day of waterlogging, two plants per plot were sampled. The newly generated roots (young white root) were counted. Percentage of aerenchyma development was evaluated using the methods described by IRRI (1995). The young nodal roots were done by free-hand cross-sections at 5 cm from root tips. The root cross sections were photographed with the Olympus compound microscope at 10X. For each photograph the whole root cross-sectional region was cut and weighed (A) and, the aerenchyma region was cut and weighed (B) separately. Then B/A ratio was calculated and finally converted to percentage of aerenchyma development. For the leaf blade, leaf greenness was measured on one side of the midrib, midway between leaf base and tip by chlorophyll meter. Leaf nitrate reductase activity was analysed by the method of Jaworski (1971) and soluble sugar in leaves was investigated by the method of Yoshida *et al.*,(1976). All data obtained were subjectd to Analysis of Variance (ANOVA) and treatment mean comparision was done by the use of Least Significant Difference (LSD).

RESULTS and DISCUSSION

Morphological responses

New root development

After nine days of flooding , root systems of each treatment were taken to count only new root developement. The numbers of new root development (NNR) of both NSX 062030 and 30B80 (Pioneer) observed in C-H was significantly grether than I-NC and H-NC. All new roots generated were thicker than the existing roots (figure 1).Field corn genotype did not affect NNR in this study. This finding suggested that chitosan may cause NNR under hypoxia resulting in increased root surface exposed to air and consequently increased aerobic respiration. This result was supported by the work of Chibu *et al.*,1999, who revealed that the numbers of first order, thick lateral roots and second order, lateral roots of radish (*Raphenus sativus*, radicula group) were apparently increased with chitosan application at the concentration of 0.1 %.

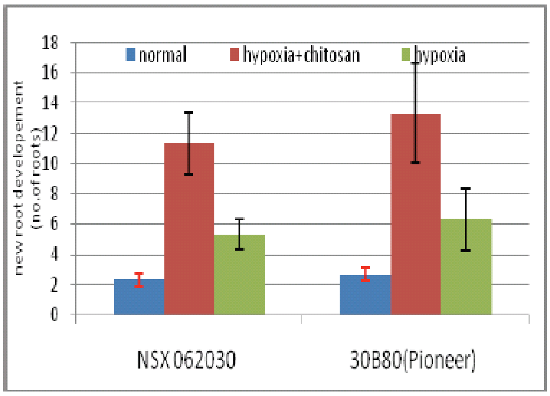

Figure 1. Effect of chitosan on numbers of new root development of two field corn genotypes under various treatment conditions .

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Aerenchyma developement

The aerenchyma tissue in root cortex of NSX062030 genotype dramatically developed under C-H; the percentage of aerenchyma development was higher than that of 30B80 (Pioneer) genotype under the same condition. It may be conceivable that 30B80 (Pioneer) genotype was susceptible to hypoxia, thus it poorly adapted to generate aerenchyma under hypoxia, even though it was applied with chitosan. This results were suported by the work of Pourabdal *et al,* 2008 who indicated that development of aerenchyma and adventious roots are more recessive factors that increases hypoxic tolerance in *Zea mays.* Considering within genotype , it was found that C-H affectd aerenchyma development of NSX062030 genotype, the tolerant hybrid, but did not affect that of 30B80 (Pioneer). However, aerenchyma development under hypoxia of both genotypes was higher than that of under I-NC(figure 2,3,4).

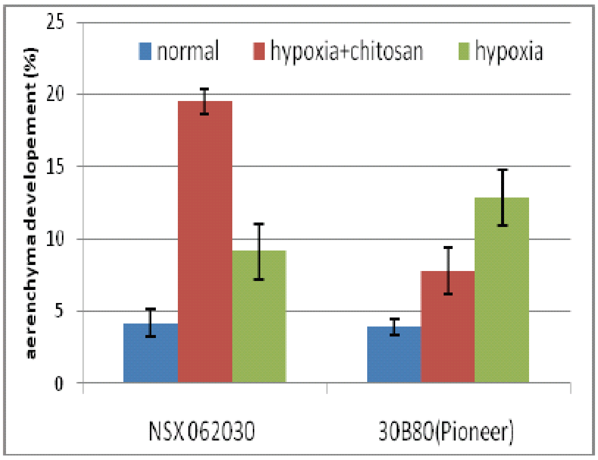

Figure 2. Effect of chitosan on aerenchyma development of two field corn genotypes under various treatment conditions.

(a) (b) (c)

Figure 3. Aerenchyma development of NSX 062030 genotype under (a) I-NC, (b) C-H and (c) H-NC conditions.

(a) (b) (c)

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Figure 4. Aerenchyma developement of 30B80 (Pioneer) genotype under (a) I-NC, (b) C-H and (c) H-NC conditions.

Leaf greenness

Under normal irrigation, leaf greenness values of both genotypes were dramatically higher than those of under hypoxic condition (figure 5). C-H did not significantly affect leaf greenness. However, chitosan application tended to retain leaf greenness of the two field corn genotypes under hypoxia. Chibu and Shibayama (1999) reported that tomato and lettuce leaves turned darker green with increasing chitosan concentrations.

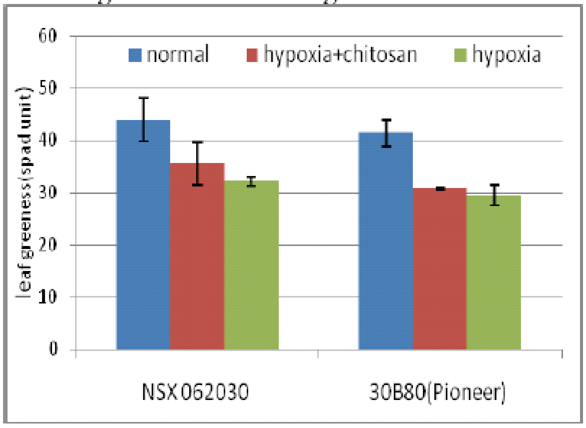

Figure 5. Effect of chitosan on leaf greenness of two field corn genotypes under various treatment conditions.

Physiological responses

Soluble sugar accumulation

The soluble sugar content in corn leaves of two genotypes was not significantly different in all treatment conditions (figure 6). C-H tended to accumulate higher level of soluble sugar in both NSX 062030 and 30B80 (Pioneer) genotypes. The results were in line with Shou-Qiang and Langlai (2003) who showed that the content of soluble sucrose in non-heading Chinese cabbage leaves was increased after seed dressing and leaf spraying with chitosan. However, the level of soluble sugar of NSX 062030 genotype did not differ between H-NC and I-NC whereas 30B80 (Pioneer) genotype showed poor adaptation to accumulate soluble sugar under hypoxia due to its susceptible nature. The amount of soluble sugar had been increased in both the roots and shoots of maize seedlings under flooding (Pourabdal *et al.*, 2008).

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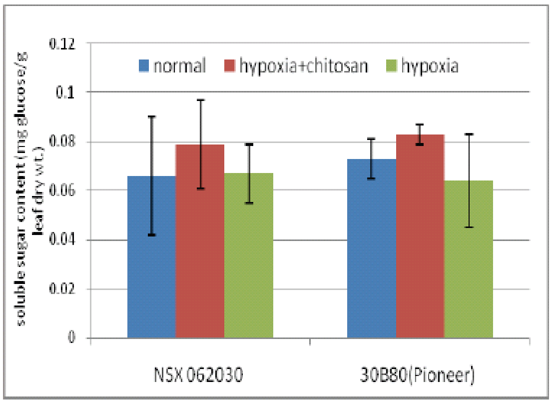

Figure 6. Effect of chitosan on soluble sugar content in leaves of two field corn genotypes under various treatment conditions.

Nitrate reductase activity

The NR activities in both genotypes under H-NC and C-H were higher than those under I-NC (figure 7). It could be explained that under waterlgging, the most important compound utilized by soil microorganisms as alternative electron acceptors for respiration is nitrate, which is rapidly reduced to be nitrite resulted in increased NR activity. Garcia-Novo and Crawford (1973). Lambers(1976) reported that the activity of NR in roots of flooded-tolerant plants increased rapidly during flooding. 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 detcted. C-H did not contribute to NR activity increases in NSX 062030 genotype whereas 30B80 (Pioneer) genotype was quite affected by C-H. In contrast, Xian-Ling *et al.* (2002) revealed that seeds of mulberry cv. Sha2 coated with chitosan solution at 0.5-2.0 % could increase NR activities.

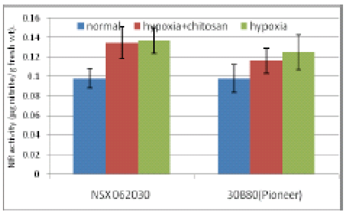

Figure 7 Effect of chitosan on nitrate reductase activity in leaves of two field corn genotypes under various treatment conditions.

ACKNOWLEDGES

The authors would like to thank Thailand Research Fund (TRF) and the Commission of Higher Education (CHE) for the research budget.

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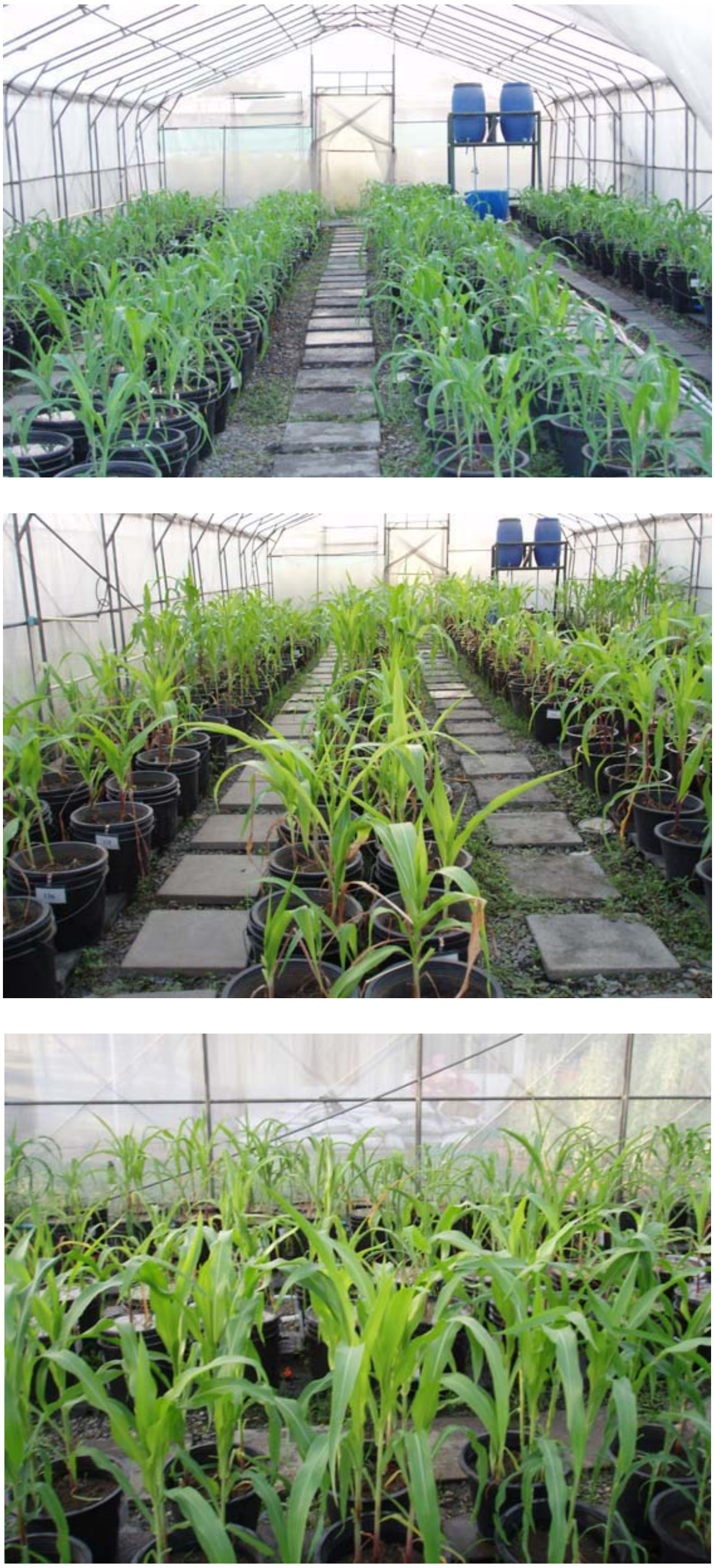
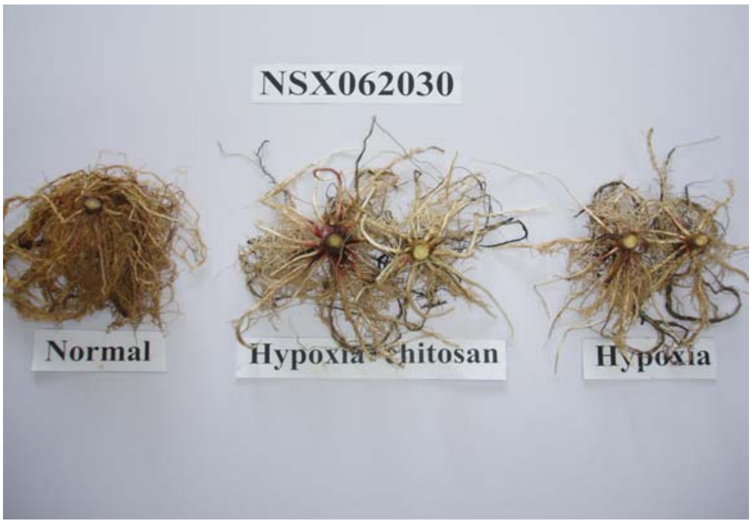
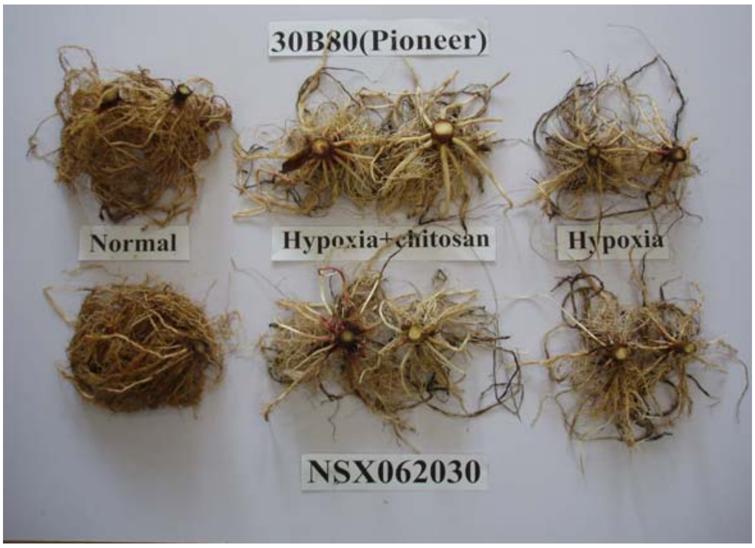
Pictures showing experimental activities

Appendix figure 1. Pot experiment in a greenhouse (experiment #1)



Appendix figure 2. Corn seedlings before and after subjecting to waterlogging (Experiment#1)





Appendix figure 3. Effects of chitosan on new root development. (Experiment #2)

Appendix figure 4. Steps of soluble sugar analysis. (Experiment#2)

Appendix figure 5. Steps of nitrate reductase activity (Experiment#2)

Appendix figure 6. Steps of chlorophyll content analysis (Experiment #1 and #2)

Manuscript for submitting to ScienceAsia (not completed)

Physiological and Morphological Responses of Field Corn Seedlings Applied with Chitosan under Hypoxic Condition

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Abstract

Chitosan acts as an elicitor in many plant species. It not only activates the immune system of plants, but also increases the yields. The objective of this study was to investigate the effect of chitosan on physiological and morphological responses of two field corn genotypes, tolerant and susceptible, under hypoxia. The pot experiment was conducted using a split plot in completely randomized design with four replications. Main plots were two genotypes of field corn: NSX 062030 (tolerant) and 30B80 (Pioneer) (susceptible), and sub plots 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 difference was observed for aerenchyma development under hypoxia. However, various treatment conditions influenced activity of nitrate reductase (NR), leaf greenness, number of new roots and aerenchyma development. C-H had positive effects on number of new roots and aerenchyma development and also tended to retain leaf greenness. The highest leaf NR activity was detected under H-NC. However, C-H tended to show positive effect on soluble sugar accumulation, but did not show any significant differences from H-NC.

Keyword: chitosan, hypoxia, physiological and morphological response, field corn seedling

Introduction

Chitosan is a natural biopolymer found inexoskeletons of crustaceans and insect cuticles as well as cell walls of fungi and some algae. Chitosan can be used in agriculture with many aspects. It has been shown to induce defence mechanism in many plant species (Ben-Shaloam *et al.* 2003; Photchanachai *et al.* 2006) and increase the activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) ,the key enzymes of phenylpropanoid pathways associated with synthesis of secondary plant metabolites under unfavorable condition (Khan *et al,* 2003). Furthermore, chitosan also promotes plant growth and enhances yield of many crop species such as *Oryza sativa* L.and *Eustoma grandiflorum* (Boonlertnirun *et al*., 2008; Ohta *et al*., 1999) as well as accelerates germination rate and germination index (Xue-Yan *et al.,* 2002). Hypoxia describes the status of cell or tissue which is deficient or low in O_2 concentration (<20%). This term is different from anoxia, in which the O_2 concentration is zero (Pradet and Bomsel, 1978). Plant survival under hypoxic condition is a major problem affecting agricultural productivity (Saglio *et al*., 1988) due to many factors related to this condition, for example , inhibition of N uptake and redistribution of N within the shoot are important factors contributing to early senescence of leaves and shoot growth (Drew and Sisworo, 1977). Hypoxia also reduces shoot and root growth, dry matter accumulation and final yield (Malik *et al*., 2002). It is estimated to reduce yields by 20 to 25% but the loss may exceed 50% depending on the stage of plant development (Setter *et al.,* 1999).

Water logging tolerance is likely to be a complex trait which is related to many morphological and physiological traits (Collaku and Harrison, 2005). It negatively affects shoot and root growth and chlorophyll content (Pang *et al.,* 2005). Reduction of root respiration is one of the earliest responses of plants under flooding stress, regardless of whether the plants are flooding-tolerant or intolerant (Carpenter and Mitchell,1980). The most conspicuous anatomical response of crop roots to soil waterlogging is the development of an extensive aerenchyma system in their cortex (Konings and Lamber, 1991). Moreover, changes in hormonal regulation have been reported that ABA concentrations were increased in roots of pea plants during the 2^{nd}, 3^{rd} and 4^{th} days of flooding, causing stomata to partially close and enriching the leaves with the hormone (Zhang and Davies,1987). Among the biological changes of plants under flooding, a high level of fermentative metabolism is important for plant survival because it supplies a high enough energy charge that can sustain metabolism in roots(Jackson and Drew, 1984; Mohanty *et al.,* 1993). The composition and quantity of protiens and amino acids,and the activities of related enzymes are important. In particular, nitrate reductase and glutamine synthetase, the two key enzymes in nitrate reduction and ammonia assimilation influencing the total nitrogen balance, are affected by flooding(Buwalda *et al.,*1988 ;Garcia-Novo and Crawford, 1973; Riggiani *et al*.,1988).

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 condition.

Materials and methods

This experiment was conducted using a split plot in completely randomized design with two genotypes: tolerant (NSX 062030) and suceptible (30B80(Pioneer) as main plot, 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 supplot, having four pots per an experimental unit and replicated four times. The pot experiment was conducted in an open-ended outdoor greenhouse with daily maximum and minimum termperature of 33 °C and 23 °C. Seeds of each genotype were planted in a 46 cm-diameter pot contained 7 kg pot^{-1} of paddy soil having the following chemical properties; pH= 5.2, % OM = 0.89, aval.P = 5.01 ppm, exch. K = 66.2 ppm. Seven days after emergence, plants were thinned and left four healthy plants per pot, and the onset of chitosan application was done according to the treatment details. Chitosan at the concentration of 80 ppm was sprayed four times (7, 14, 21 and 28 days after planting) on corn leaves. After one day of the last chitosan spraying; corn seedlings were subjected to transient waterlogging (hypoxia) by adding water to the pots until the water level reached 5.0 cm above the soil surface and maintained its level at 2.5-5.0 cm for nine days. After the ninth day of waterlogging, two plants per plot were sampled. The newly generated roots (young white root) were counted. Percentage of aerenchyma development was evaluated using the methods described by IRRI (1995). The young nodal roots were done by free-hand cross-section at 5 cm from root tips and photographed with the Olympus compound microscope at 10X. For each photograph the whole root cross-sectional region was cut and weighed (A) and, the aerenchyma region was cut and weighed (B) separately. Then B/A ratio was calculated and finally converted to percentage of aerenchyma development. For the leaf blade, leaf greenness was measured on one side of the midrib, midway between leaf base and tip by chlorophyll meter. Leaf nitrate reductase activity was analysed by the method of Jaworski (1971) and soluble sugar accumulation in leaves was investigated by the method of Yoshida *et al.*,(1976). This experiment was carried out at Rajamangala University of Technology Suvarnabhumi during September to November 2008. All data obtained were subjectd to Analysis of Variance (ANOVA) and treatment mean comparision was done by the use of Least Significant Difference (LSD).

Results and disscussion

1. Morphological responses

1.1 Number of new root development (NNR)

The number of new root development (NNR) of both NSX 062030 and 30B80 (Pioneer) observed in C-H condition was significantly grether than I-NC and H-NC. All new generated roots were thicker than the existing roots (figure 1). Different field corn genotypes did not affect NNR in this study. This finding may suggested that chitosan may stimulate NNR under hypoxia resulting in increased root surface exposed to air and consequently increased aerobic respiration. This result was supported by the work of Chibu *et al.,*1999, who revealed that the numbers of first order, thick lateral roots and second order, lateral roots of radish (*Raphenus sativus*, radicula group) were apparently increased with chitosan application at the concentration of 0.1 %. Similar results were reported by Shou-Qiang and Lang-Lai (2003)who indicated that root length of chinese cabbage (*Brassica campestris*) cv. Dwarf hybrid No. 1 was increased after chitosan was applied by seed dressing with 0.4-0.6 mg chitosan g^{-1}seed and leaf spraying with 20-40 µg chitosan ml^{-1}.

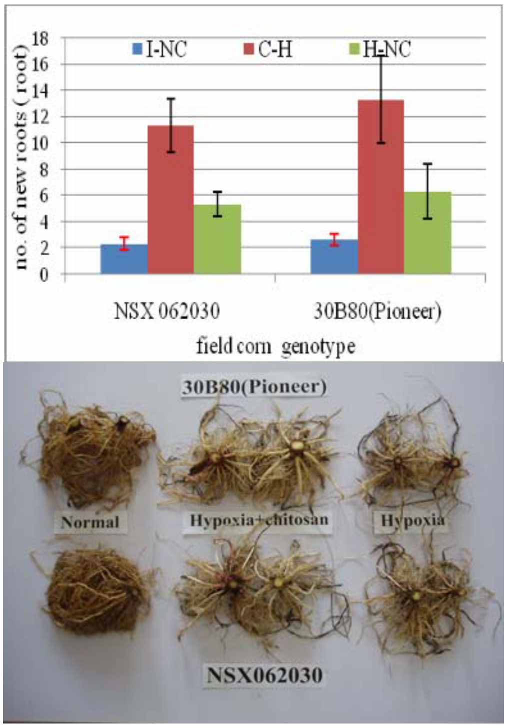

Figure 1. Effects of chitosan on number of new root development of two field corn genotypes under hypoxic condition.

1.2 Aerenchyma developement

The aerenchyma tissue in root cortex of NSX062030 genotype dramatically developed under C-H; the percentage of aerenchyma development was higher than that of 30B80 (Pioneer) genotype under the same condition. It may be conceivable that 30B80 (Pioneer) genotype was susceptible to hypoxia, thus it poorly adapted to generate aerenchyma under hypoxia, even though it was applied with chitosan. This results were suported by the work of Pourabdal *et al,* 2008 who indicated that development of aerenchyma and adventious roots were more recessive factors that increased hypoxic tolerance in *Zea mays.* The aerenchyma formation and adventitious roots in the vicinity of cotyledonary nodes was an indicator of adaptive mechanisms present in flood-tolerant plants (Kawase, 1981). Considering within genotype , it was found that C-H affectd aerenchyma development of NSX062030 genotype, the tolerant hybrid, but did not affect that of 30B80 (Pioneer). However, aerenchyma development under C-H and H-NC of both genotypes were higher than that of under I-NC(figure 2,3,4).

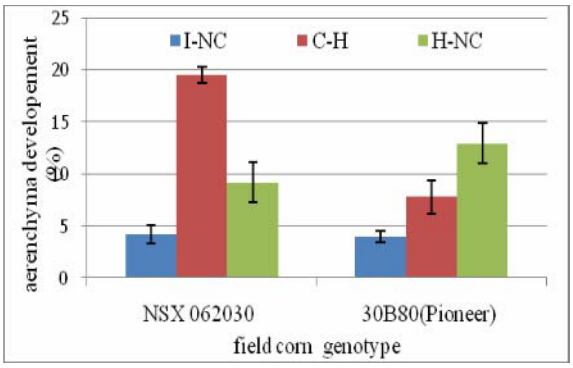

Figure 2 Effects of chitosan on aerenchyma development of two field corn genotypes under hypoxic condition.

(a) **(b)** **(c)**

NSX 062030 (tolerant genotype)

Figure 3 Aerenchyma development of NSX 062030 genotype under (a) I-NC (b) C-H and (c) H-NC conditions.

(a) **(b)** **(c)**

30B80 (Pioneer) (susceptible genotype)

Figure 4 Aerenchyma developement of 30B80 (Pioneer) genotype under (a) I-NC (b), C-H and (c) H-NC conditions.

1.3 <u>leaf greenness</u>

Under normal irrigation without chitosan (I-NC), leaf greenness values of both genotypes was dramatically higher than those of under hypoxic condition(figure 5).

Application of chitosan before hypoxia did not significantly affect leaf greenness. However, chitosan application tended to retain leaf greenness of the two field corn genotypes under hypoxia. Chibu and Shibayama (1999) reported that tomato and lettuce leaves turned darker green with increasing chitosan concentrations.

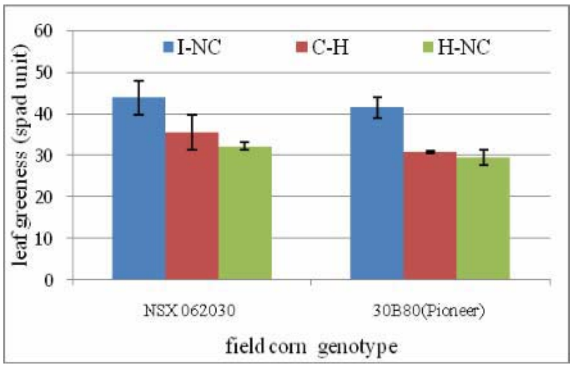

Figure 5 Effects of chitosan on leaf greenness of two field corn genotypes under hypoxic condition.

2. Physiological responses

2.1 soluble sugar accumulation

The soluble sugar content in corn leaves of two genotypes was not significantly different in all treatment conditions (figure 6). C-H tended to accumulate high level of soluble sugar in both NSX 062030 and 30B80 (Pioneer) genotypes. The results were in line with Shou-Qiang and Langlai (2003) who showed that the content of soluble sucrose in non-heading Chinese cabbage leaves was increased after seed dressing and leaf spraying with chitosan. Chitosan may induce plant cells to synthesize soluble sugar by increasing the activities of phenylalanine ammonia –lyase (PAL) and tyrosine ammonia-lyase (TAL), the key enzymes of phenylpropanoid pathway which associated to produce precursors of secondary metabolites (Khan *et al*., 2003). However, the level of soluble sugar of NSX 062030 genotype did not differ between H-NC and I-NC whereas 30B80 (Pioneer) genotype showed poor adaptation to accumulate soluble sugar under H-NC. The amount of soluble sugar had been increased in both the roots and shoots of maize seedlings under flooding (Pourabdal *et al*., 2008). The soluble sugar consumption under fermentative metabolism during flooding condition had been increased (Mohanty *et al.,*1993).

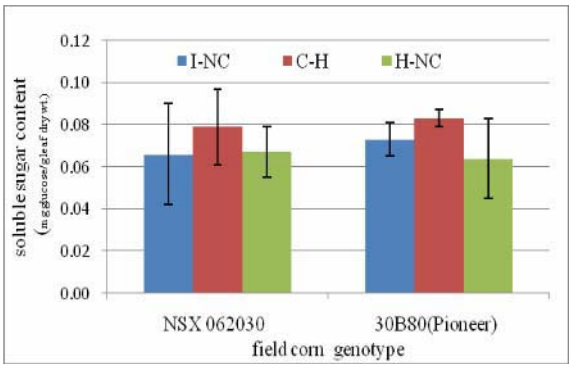

Figure 6. Effects of chitosan on soluble sugar content in leaves of two field corn genotypes under hypoxic condition.

2.3 Nitrate reductase activity

The NR activities in both genotypes under H-NC and C-H were higher than those under I-NC (figure 7). It could be explained that under waterlgging, the most important compound utilized by soil microorganisms as alternative electron acceptors for respiration is nitrate, which is rapidly reduced to be nitrite resulted in increased NR activity. Garcia-Novo and Crawford (1973) and Lambers(1976) reported that the activity of NR in roots of flooded-tolerant plants increased rapidly during flooding, as did the amino acid synthesis capability. For crop plants, some species are root-reducers for NO^{-}_{3} and species are leaf-reducers. No significant difference in terms of NR activity between two field corn genotypes was detcted. C-H did not contribute to NR activity increases in NSX 062030 genotype whereas 30B80 (Pioneer) genotype was quite affected by C-H. In contrast, Xian-Ling *et al*. (2002) revealed that seeds of mulberry cv. Sha2 coated with chitosan solution at 0.5-2.0 % could increase NR activities. On the other hand, the activity of NR in roots of wax- apple tree was significantly decreased with flooding (Hsu *et al.,*1999).

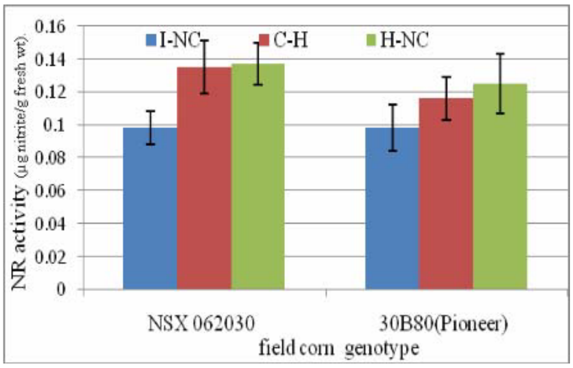

Figure 7 Effect of chitosan on nitrate reductase activity in leaves of two field corn genotypes under hypoxic condition.

Conclusions

This present study indicated that application of chitosan to corn seedlings before subjected to hypoxia showed many positive responses both physiologically and morphologically. Under C-H ,newly generated roots were significantly increased greater than those under I-NC and H-NC. Furthermore, chitosan also tended to contribute to maintaining leaf greenness under hypoxia. Aerenchyma in root cortex under C-H was rapidly developed over the other treatment conditions. Leaf NR activity and soluble sugar accumulation under C-H and H-NC were higher than those under I-NC but did not show any significant differences. Genotype effect was significantly found in terms of aerenchyma development; the tolerant one was better developed. According to this result, it could be suggested that chitosan had an ability to stimulate some responses of corn seedlings relating to survival under hypoxic condition.

Acknowledgements

The authors would like to thank Thailand Research Fund (TRF) and the Commission of Higher Education (CHE) for the research budget.

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