Korean J. Soil Sci. & Fert.(2000)
Vol. 33(2) : 85~91

Effects of NPK Fertilizers on Antioxidant Activity of Corn(*Zea mays L.*)

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ABSTRACT

Antioxidant activity and related components including tocopherols, phenolic compounds and carotenoids in the corn(*Zea mays* L.) kernels were measured to understand effects of fertilizer application. Hybrids used were Chalok 2, a waxy corn, and Suwon 19, a dent corn. The standard fertilizer level of N-P-K was 14.5-3-6 for Chalok 2 and 17.4-3-6.9 for Suwon 19. The treatments of the fertilizer levels were the standard level, half and two fold amounts of N, P, and K, and no fertilizer. The antioxidant activity was determined by measuring electron donating ability. The antioxidant activity of Chalok 2 was higher than Suwon 19. The antioxidant activity was the heighest at the standard fertilizer treatment for Chalok 2, but was statistically not significant. The antioxidant activity and the related compounds content in Suwon 19, however, were the highest in the two fold nitrogen treatment, and the differences were significant. The contents of tocopherols, phenolic compounds and carotenoids were 30.0~38.1, 104.7~118.8, 0.1 $\mu g ml^{-1}$, respectively for Chalok 2 and 16.7~20.1, 59.9~72.7, 35.5~50.0 $\mu g ml^{-1}$, respectively for Suwon 19. The antioxidant activity was positively correlated with the grain yield in both cultivars. The shorter the ASI the higher the yield of the plant. The difference in genetic factors would affect greater influence than fertilizer effect on the antioxidant activity of corn.

Key words : Corn(*Zea mays* L.), Antioxidant activity, Fertilizer, Tocopherols, Phenolic compounds.

Introduction

Antioxidant in the corn kernel is an indicator of the quality factor of corn. Kurilich and Juvik(1999) analyzed carotenoid and tocopherol antioxidants in sweet corn and Seo et al.(1999a,b) reported the antioxidant activity and contents of tocopherols, phenolic compounds and carotenoids in waxy corn. Tocopherols function as the main antioxidants in biological lipid membranes, protecting against peroxyl radicals and nitrogen oxide species(Christen et al., 1997). In addition, they provide antiproliferative effects(Azzi et al., 1995), anticlotting activity(Dowd and Zheng, 1995), and immunoprotection(Maydani, 1995). Phenolic compounds, secondary metabolites which are ubiquitous in plant food, are classified to flavonoides, phenolic acids, coumarins and tannins. While many reports have described the effect of fertilizers on yield(Shin et al., 1983; Heckman and Kamprath, 1992), seedling growth(Choi and Kim, 1993;Kim and Oh, 1994; Oh et al., 1996), utilization efficiency(Seo et al., 1997), and nitrate

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accumulation(Yoon et al., 1990) in corn, little informations were available to understand the effect of fertilizers on antioxidant activity. This paper was aimed to investigate the effect of nitrogen, phosphorus, and potassium applications on antioxidant activity and contents of tocopherols, phenolic compounds and carotenoids in corn.

Materials and Methods

Chalok 2, a waxy corn, and Suwon 19, a dent corn, hybrids used planted in the field plots of the Maize Experimental Station of Kangwon-do Agricultural Research and Extension Services, which located in the Hongcheon. The chemical characteristics of the soil and the treatment level of fertilizers were in Table 1 and Table 2, respectively. The plot size was 3m by 4m. Each treatment had three replications. Basal fertilizers were applied, corns were planted on Apr. 27, 1999 at the rate of 56,000 plants per ha. Half amount of the nitrogen and all of the phosphorus and potassium fertilizers were broadcasted to the soil before planting. The remainder of the nitrogen was applied when the corn height was knee high. In order to eliminate xenia effect, each plant was pollinated by hand. The kernels in the middle part of ear were used for analysis.

The content of chlorophyll of ear leaf was measured by SPAD-502 Chlorophyll Meter(Minolta, Japan) at flowering stage, since linear relationship could be obtained between the SPAD values and the total chlorophyll calculated by conventional methods in maize leaves(Krugh et al., 1994).

Antioxidant activity was analyzed as Blois(1958) described. The 1g of crushed corn samples were extracted with 10ml of methanol. Solutions of DPPH were prepared in methanol at a concentration of 1×10^{-4}M. For evaluation, 0.2ml of methanolic extract was mixed with 2.8ml of DPPH solution for 10 sec. After allowing the mixture to stand at room temperature for 10 min, the absorbance was determined at 525nm with a UV/vis spectrophotometer (UVIKON942, KONTRON). The antioxidant activity was calculated by following equation;

$$A_a = \frac{(A_o - A_i)}{A_o} \times 100$$

where A_a was the antioxidant activity, A_o was absorbance without sample, and A_i was absorbance with sample.

The analysis of phenolic compounds was followed method described by Rhee et al.(1981). Methanolic extract(50μl) was combined with 2.0ml of 2% Na_2CO_3. After 2 min, 0.2ml of 2N Folin-Ciocalteu's reagent was added and the mixture was incubated at room temperature for 30 min. The absorbance was read at 750nm. Chlorogenic acid was used for constructing a standard curve. The content of tocopherols was measured by the method of Tsen(1961).

Table 2. Treatment level of fertilizer applied

Cultivar	Treatment	Treatment level(kg ha^{-1})		
		N	P_2O_5	K_2O
	None	-	-	-
Chaok 2	Standard	145	30	60
	1/2N	73	30	60
	2N	290	30	60
	1/2P	145	15	60
	2P	145	60	60
	1/2K	145	30	30
	2K	145	30	120
Suwon 19	Standard	174	30	69
	1/2N	87	30	69
	2N	348	30	69
	1/2P	174	15	69
	2P	174	60	69
	1/2K	174	30	35
	2K	174	30	138

Table 1. Chemical characteristics of the experimental field soil before applications of fertilizers at time of corn planting

pH	EC (dS m^{-1})	OM (g kg^{-1})	Av. P_2O_5 (mg kg^{-1})	Exch. cations (cmol$^+$ kg^{-1})		
				Ca	Mg	K
4.8	0.14	20	56	1.63	0.29	0.71

The bathophenanthroline reagent(0.5ml of 6.0×10^{-3}M) was added to 0.2ml of methanolic extract in a 10ml amber bottle, followed by adding with 3ml of ethanol and the bottle was gently swirled for a few seconds. At 15 second after mixing of 0.5ml of 1×10^{-3}M ferric chloride solution, 0.5ml of 4×10^{-2}M phosphoric acid solution was introduced. The absorbance of the solution was measured at 534nm. Since most carotenoids absorb at a wavelength of 450nm with high extinction coefficients(E 140,000/M · cm), the total carotenoid content of plant extracts in organic solvent can be reliably estimated by the optical density at 450nm(Handelman, 1994). The content of carotenoids was measured by reading the absorbance of methanolic extract at 450nm.

The content of amylopectin in waxy corn was determined by adapting the procedureof Gilbert and Spragg(1964). Crushed Chalok 2 sample(50mg) was mixed with 10ml of distilled water and 5ml of 1N NaOH in 100ml volumetric flask. After standing the mixture on boiling water bath for 5 min, 5ml of 1N HCl was added. The potassium hydrogen tartrate(0.14g), 70ml of distilled water and 1ml of iodine solution(2mgml^{-1} iodine + 20mgml^{-1} KI) were added and the volume was made up to 100ml with distilled water. After mixing thoroughly, the mixture was kept at room temperature for 20 min. The absorbance was measured at 680nm.

Results and Discussions

Table 3 shows the agronomic characters under different level of fertilizer treatment. Days to silking were delayed by low application of fertilizers. The days to silking in the two fold application of N, P and K were shorter by two to four days than no fertilizer application. A typical observation under stress at flowering was slowed ear growth. It might caused delay in silk emergence. The anthesis-silking interval(ASI) was the period between pollen shed(anthesis) and silk emergence. This interval could be used as a sensitive measure of susceptibility/tolerance to stresses which reduce photosynthesis at flowering(Kling and Edmeades, 1997). The ASI of Suwon 19 was comparatively shorter than that of Chalok 2, indicating that Suwon 19 was more tolerable to mineral nutrition stress than Chalok 2. The ASI of Chalok 2 was shortened to 2.7 days by nitrogen application. Plant height, ear height and number of leaves, which were affected by day length and

Table 3. Agronomic characters of Chalok 2 and Suwon 19 under different level of NPK treatment

Treatment	Days to silking		ASI		Plant height(cm)		Ear height(cm)		No. of leaves		Chlorophyll[3]		Yield (Mg ha^{-1})	
	C[1]	S	C	S	C	S	C	S	C	S	C	S	C	S
None	85a[2]	87a	7.3a	4.3a	150a	243a	65.7a	116a	15a	20a	38.5a	38.1a	4.3a	6.1a
1/2N	80ab	84a	5.3b	2.7a	152a	254b	65.2a	123bc	14a	20a	59.0b	56.7b	5.7ab	8.1ab
Standard	80ab	86a	4.7b	3.7a	152a	247ab	64.2a	121ab	15a	20a	61.1b	60.4c	6.3b	8.3ab
2N	78b	84a	2.7c	2.3a	153a	253b	70.6a	129c	15a	20a	60.5b	60.2c	5.6ab	9.0b
None	85a	87a	7.3a	4.3a	150a	243a	65.7a	116a	15a	20a	38.5a	38.1a	4.3a	6.1a
1/2P	82ab	86a	5.7b	3.7a	154a	249b	64.9a	129b	15a	20a	62.1c	59.3b	5.9b	8.9b
Standard	80ab	86a	4.7b	3.7a	152à	247b	64.2a	121a	15a	20a	61.1bc	60.4b	6.3b	8.3ab
2P	79b	83a	5.0b	2.0a	152a	259b	71.1a	134b	15a	20a	59.6b	60.9b	5.7b	9.7b
None	85a	87a	7.3a	4.3a	150a	243a	65.7a	116a	15a	20a	38.5a	38.1a	4.3a	6.1a
1/2K	80ab	83a	4.3b	2.7a	157a	251b	74.1b	128b	15a	20a	60.1c	59.6b	5.7ab	10.5c
Standard	80ab	86a	4.7b	3.7a	152a	247ab	64.2a	121a	15a	20a	61.1bc	60.4b	6.3b	8.3b
2K	76b	85a	3.3b	2.7a	164a	254b	77.8b	131b	15a	20a	61.0b	58.8b	6.8b	9.2bc

[1] C : Chalok 2, S : Suwon 9

[2] Means within column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test

[3] Values measured with SPAD 502

temperature(Kling and Edmeades, 1997), were not affected by mineral nutrition in this study. Chlorophyll content of Suwon 19 grown under standard treatment was significantly higher than under 1/2 N treatment and there was no difference between standard treatment and 2 N treatment. The yield was highest at standard application level of nitrogen and phosphorus in Chalok 2, and at two fold level of nitrogen and phosphorus in Suwon 19.

Between fertilizer level, the antioxidant activity showed little difference in Chalok 2(Table 4). The antioxidant activity and the related compounds content in Suwon 19 with the two fold nitrogen treatment were higher than other N levels. Antioxidant activities of Chalok 2 and Suwon 19 were 19.0 ~22.2% and 9.7~11.6%, respectively. Antioxidant activities of F1 and inbred in waxy corn were 9.0~48.2%(Seo et al., 1999a) and 15.5~65.0%(Seo et al., 1999b), respectively. It showed that difference in genetic factors would affect greater influence than fertilizer effect on the antioxidant activity of corn. Hybrids were more important than growing conditions such as fertilizer treatments and planting rates as a source of variation in oil content of corn(Genter et al., 1956). Similarly, fatty acid composition was influenced more by genetic difference than by environmental factors such as planting dates and ear position(Jellum and Marion, 1966), and the gene action was more additive(Seo et al., 1999c). The gene action of antioxidant activity in waxy corn was also more additive(Seo et al., 1999d). The antioxidant activities of Chalok 2 were about 2 times as high as Suwon 19 due to high contents of tocopherols and phenolic compounds.

The contents of tocopherols, phenolic compounds and carotenoids were 30.0~38.1, 104.7~118.8, 0.1 $\mu g ml^{-1}$, respectively in Chalok 2, and 16.7~20.1, 59.9~72.7, 35.5 ~50.0 $\mu g ml^{-1}$, respectively in Suwon 19. About 67% of corn tocopherols was γ-tocopherol(Kurilich and Juvik, 1999), and this form was more effective at protecting against nitrogen oxide species than α- and β-form(Christen et al., 1997). The carotenoid level of Suwon 19, yellow dent corn, was much higher than that of Chalok 2 but antioxidant activity was low, indicating that the effect of carotenoids on antioxidant activity was comparatively low in corn. Lutein and zeaxanthin, the dominant carotenoids in corn(Kurilich and Juvik, 1999), had no pro-vitamin A activity(Handelman, 1994). Kurilich and Juvik(1999) investigated carotenoids and tocopherols in sweet corn as antioxidant, but phenolic compounds were considered to possess more strong antioxidant properties than carotenoids. Rhee et al.(1981) proposed that flavonoids and phenolic acids would be the

Table 4. Antioxidant activities and related components of Chalok 2 and Suwon 19 under different level of NPK treatment

Treatment	Antioxidant activity(%)		Tocopherols ($\mu g ml^{-1}$)		Phenolic compounds($\mu g ml^{-1}$)		Carotenoids ($\mu g ml^{-1}$)	
	C[1)]	S	C	S	C	S	C	S
None	19.0a[2)]	9.8a	34.7a	16.9a	104.7a	63.9a	0.1a	35.5a
1/2N	19.6a	10.7ab	32.7a	18.4ab	111.4a	64.3a	0.1a	39.6a
Standard	22.2a	9.7a	38.1a	16.7a	118.8a	59.9a	0.1a	39.3a
2N	19.6a	11.6b	35.3a	20.1b	109.4a	66.5a	0.1a	49.8a
None	19.0a	9.8a	34.7a	16.9a	104.7a	63.9a	0.1a	35.5a
1/2P	20.4a	9.7ab	30.0a	17.1ab	108.5a	61.5a	0.1a	43.2a
Standard	22.2a	9.7a	38.1a	16.7a	118.8a	59.9a	0.1a	39.3a
2P	21.6a	10.9a	36.8a	18.3a	106.7a	61.9a	0.1a	43.8a
None	19.0a	9.8a	34.7a	16.9a	104.7a	63.9ab	0.1a	35.5a
1/2K	21.4a	11.6b	34.8a	18.2a	105.6a	61.8a	0.1a	50.0c
Standard	22.2a	9.7a	38.1a	16.7a	118.8a	59.9a	0.1a	39.3ab
2K	20.5a	10.6ab	32.2a	17.4a	107.3a	72.7b	0.1a	43.7bc

1) C : Chalok 2, S : Suwon 9

2) Means within column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test

Table 5. Correlation coefficients between agronomic characters and antioxidant activity

	ASI	Chlorophyll	Antioxidant activity	Tocopherols	Phenolic compounds	Carotenoids
					Chalok 2	
Chlorophyll	-0.64**					
Antioxidant activity	-0.26	0.47*				
Tocopherols	-0.08	0.15	0.22			
Phenolic compounds	-0.11	0.38	0.41*	0.50*		
Carotenoids	0.26	-0.22	0.16	-0.09	-0.26	
Yield	-0.52**	0.67**	0.48*	0.11	0.42*	-0.09
					Suwon 19	
Chlorophyll	-0.51*					
Antioxidant activity	-0.28	0.28				
Tocopherols	-0.24	0.06	0.69**			
Phenolic compounds	-0.12	-0.04	0.47*	0.54**		
Carotenoids	-0.19	0.42	0.38	0.29	0.08	
Yield	-0.40*	0.59**	0.43*	0.22	0.14	0.49**

Table 6. Amylopectin contents of Chalok 2 under different level of NPK treatment

Treatment	Amylopectin(%)
None	94.5a[1)]
Standard	94.7a
1/2N	94.8a
2N	94.7a
1/2P	94.8a
2P	94.8a
1/2K	94.7a
2K	94.8

1)Means within column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test

most important antioxidant compounds in seeds.

As shown in Table 5, a positive correlation was found between the antioxidant activity and the grain yield in both cultivars. The antioxidant activity was correlated with the level of tocopherols and phenolic compounds but not with the contents of carotenoids. The shorter the ASI the higher the yield of the plant.

The contents of amylopectin, measure of waxy corn quality, in Chalok 2 were also unaffected by the level of fertilizer application(Table 6).

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韓土肥誌(2000)
Vol. 33(2) : 85~91

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옥수수의 항산화 활성에 대한 3요소 비료의 효과

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옥수수의 항산화 활성에 대한 N-P-K의 시비의 효과를 알아보고자, 찰옥 2호와 수원 19호 두 품종을 대상으로 시비량에 따른 옥수수의 항산화 활성과 tocopherols, 페놀화합물, carotenoids의 함량을 분석하였다. 시비 수준은 N, P, K 각각에 대한 표준 시비량과 이의 1/2 및 2배, 그리고 무비이었다. 찰옥2호의 항산화 활성은 19.0~22.2%로, 수원19호보다 높았다. 찰옥 2호의 항산화 활성은 표준 시비구에서 가장 높았으나, 처리간의 유의성은 없었고, 수원19호의 항산화 활성과 항산화 성분 함량은 질소 2배 시용구에서 높았다. Tocopherols, 페놀화합물, carotenoids의 함량은 찰옥2호의 경우 각각 30.0~38.1, 104.7~118.8, 0.1 ㎍ ml^{-1}였고, 수원19호는 각각 16.7~20.1, 59.9~72.7, 35.5~50.0 ㎍ ml^{-1}로 항산화 활성과 마찬가지로 변이폭이 작고, 시비량에 따른 뚜렷한 경향을 보이지 않았다. ASI와 항산화 활성은 옥수수의 수량과 정상관이 있었으며, 항산화 활성은 tocopherols, 페놀화합물의 함량과 정상관이 있었으나 carotenoids와는 상관관계가 없었다. 옥수수의 항산화 활성과 항산화성분 함량은 품종간 변이의 영향이 시비량에 의한 환경변이의 영향보다 큰 것으로 보인다.

Key words : Corn(*Zea mays* L.), Antioxidant activity, Fertilizer, Tocopherols, Phenolic compounds.

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