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Major Agricultural Characteristics and Antioxidants Analysis of the New Developed Colored Waxy Corn Hybrids

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This study was carried out to develop the new colored waxy corn hybrids with high yield and functional characteristics using the domestic collected genetic resources. Botanical characteristics and antioxidant activities of the developed CNU (Chungnam National University) waxy corn hybrids were analyzed and evaluated for selection of superior hybrids with high yield including high table quality. Stem height of CNU19 hybrid among developed hybrids were higher as 242 cm and its ear height were also higher as 52 cm than check, Chalok 1. Tiller per plant of CNU19 was high as 1.5 compared to 0.8 of check. Days to tasseling appeared as 74 days and ear length of CNU19 were longer than check, Chalok 1. Lodging and insect resistance of CNU19 was 2 and 3, respectively, stronger than other hybrids. Pericarp thickness of CNU19 in yellow waxy hybrids was thinner than 40 μm which was a selection criterion of edible waxy corn hybrid. Sugar content (Brix) of the developed CNU19 waxy corn hybrid was comparatively lower than check. In table quality, CNU12 and CNU19 hybrids were good than check in sugar content and tenderness.

In functional analysis, 1,1–diphenyl–2–picryhydrazyl (DPPH) radical scavenging effect, xanthine oxidase (XO) activity and catalase activity by methanol extracts were comparatively appeared high in CNU19. CNU19 and CNU 153 hybrids from ethanol extracts showed also higher antioxidant activities.

INTRODUCTION

Recently agricultural products and cultivation area of the edible waxy and sweet hybrid corn were gradually increased by favor of well–being food due to consumer awareness of their various health and nutraceutical benefits. By these trends, most of us regarded as important parts more quality than quantity among colored foods and vegetables but colorless as like colored soybean, rice, corn and vegetable (Kim *et al*., 1994; 2000).

Besides, these all agricultural products were plays well as antioxidant materials against aging and various diseases. In spite of many important results, there was no study of functional and antioxidant materials in colored waxy corn. A few researchers have studied the prospects for development of maize hybrids with high functional and antioxidant materials.

Purple corn has been cultivated for centuries in the Andean Region and used to color foods and beverages. The already–known antioxidant and anti–carcinogenic properties of purple corn, in addition to its coloring attributes, make it an attractive crop for the Nutraceutical and Functional Food Market (Cevallos–Casals and Cisneros–Zevallos, 2004). More recently, purple corn extracts were tested for antiobesity activity and amerlioration of hyperglycemia (Tsuta *et al*., 2003). In addition, purple corn color did not showed any hepatotoxicity or nephrotoxicity in mice depleted of glutathione by pretreatment with buthionine sulfoximine at a dose of 4,500 mg/kg (Kawazoes *et al*., 2000).

Previous investigations of purple corn bioactivities have mainly been focused on its anthocyanins. A purple corn color extract has been shown to inhibit colorectal carcinogenesis in male F344 rats pretreared with 1,2–dimethylhydrazine and PhIP. The inhibition was attributed only to anthocyanins present in the purple corn color. However, purple corn has a significant amount of phenolic compounds other than anthocyanins, including mainly phenolic acids and flavonols (Pedreschi and Cisneros–Zevallos, 2007). The roles of other phenolic compounds present in the purple corn color extract have been ignored up to now.

Pedreschi and Cisneros–Zevallos (2006) demonstrated that phenolic compounds present in Andean purple corn have antimutagenic properties. The phenolic compounds present in the ethyl acetate fractions were composed of phenolic acids and flavonols and were more potent antimutagenes than the anthocyanins present in the water fraction. The antimutagenic mechanism of action of purple corn phenolic compounds involved enzyme inactivation and scavenging of electrophiles and depended on the phenolic fraction.

Blue, purple and re–pigmented corn kernels are also rich in anthocyanins with well established antioxidant and bioactive properties (Adom and Lie 2002; Matsumoto 2004; Tsuda *et al*., 2003)

The objective of our study was to know botanical characteristics and biological activity of colored waxy corn.

MATERIALS AND METHODS

Plant materials: The materials were white, yellow and purple colored waxy corn hybrids. These hybrids were developed at Corn Genetics and Breeding Laboratory of

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Chungnam National University in Korea and they named as CNU (Chungnam National University) and other hybrid corn included; Chalok 1 as check. The corn seed planted two kernels per hill as 70 cm by 30 cm in Randomized Complete Block Design (RCBD) on 25th April, 2007 at Chungnam National University Agriculture Farm and thinned after 4 to 5 leaves. Fertilizer was 20–10–10 kg (as components of $N–P_2O_5–K_2O$) per 10a. Other cultivation and management followed standard cultivation methods of corn of RDA (Rural Development Administration), Suwon.

Measurement methods: Plant characteristics measured at flowering stages as days to tasseling, stem and ear height, ear length and pericarp thickness, and sugar content (Brix) were surveyed at harvest stages.

For antioxidant analysis, pre–treatment procedure of waxy corn hybrid was as follows; 1: anthocyain extract, 2: qualitative experiment (Chromatography) and 3: antioxidant analysis; 1,1–diphenyl–2–picryhydrazyl (DPPH) free radical scavenging activity, superoxide dismutase (SOD) activity, xanthine oxidase (XO) inhibitory activity and catalase activity were measured by extraction for 2 hours with high pressure and 70% methanol 50 mL.

1. DPPH radical scavenging activity

DPPH free radical scavenging activity was according to the method of Yoshida *et al.* (1989). Thus, 2.5 mL of 0.35 mM DPPH and 0.25 mL of 1% sample solution were homogenized and allowed to react for 10 min at room temperature, and then the optical density was measured at 517 nm. For the control, ethanol was used. The activity was calculated against control.

2. XO inhibitory activity

XO inhibitory activity, XOI (%), was expressed as the percentage inhibition of XO in the above assay system, calculated as (1–B/A) x 100, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material.

3. SOD activity

The determination of SOD activity was assayed by their capacity to compete with native or partially succinylated ferricytochrome c for O_2^- radicals generated by the xanthine/xanthine oxidase system (McCord and Fridovich, 1969). One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of the rate of nitroblue tetrozolumn (NBT) reduction.

4. Catalase activity

Catalase activity was measured in 50 mM sodium phosphate buffer (pH 7.0) using 50 mM hydrogen peroxide as substrate (Abei *et al.*, 1974).

RESULTS AND DISCUSSION

1. Agronomical characters of hybrids

Days to tasseling of new colored waxy corn hybrid, CNU 19 showed moderate value as 74 days and stem height was 242 cm and ratio of stem height to ear height appeared as 50% below. Growth characters, ear morphological characters and ear height about new yellow waxy corn hybrid, CNU 19 is showed in Table 1.

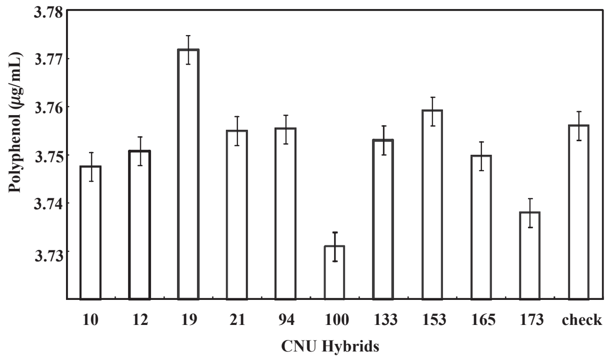

Fig. 1. Comparison of polyphenol compounds among the selected CNU colored waxy corn hybrids.

Ear height is one of the most important selection criteria in most maize breeding program. Especially, ear height is of importance when it comes to lodging. High ear position is generally like to become more susceptible to lodging (Ji *et al.*, 2006). Ear height of CNU19 was higher as 83 cm above compared to Chalok 1 as 67 cm. The main reason that plant height of CNU 19 was higher than that of check, Chalok1. Especially, research on plant and ear height need to more continue in the future, and statistical access method should be considered due to need more active access method of data analysis.

As new varieties are developed, breeders are considered not only morphological traits, but also other traits. Number of tillers per plant of CNU19 was high as 1.5 compared to 0.8 of check, Chalok 1. Ear length of CNU19 was longer than check. Maize tillering is one of the plant characteristics that have been neglected and unwanted for breeding purpose. However, it has been continued the studies on some tillering maize (Ji and Choe, 1998) and some of tillering hybrids produced greater dry matter than non–tillering maize.

Lodging and insect resistance of CNU19 was 2 and 3, respectively, stronger than other hybrids (Table 1). Seed coat color of new waxy corn hybrid, CNU 19, was yellow and white. Most of domestic leading varieties were white color and some of them was purple color. Ear length, diameter and seed coat color for domestic leading waxy corn varieties showed in Table 2.

Besides, ear diameter of CNU19 hybrids appeared about 4.8 cm longer than 44 cm of Chalok 1 and grain yield of CNU19 and Chalok 1 showed 736 kg/10a, 746 kg/10a, respectively (Table 2).

From these results, tolerance about environmental stress of this hybrid regarded as very high.

2. Physical characters of hybrids

Comparision among hybrid for major factors related to table qualities of edible waxy corn is shown in Table 1. Sugar content (Brix) measured at 30 days after tasseling

Table 1. Comparison of the major characteristics among domestic colored waxy corn hybrids

Hybrids	Stem ht. (cm)	Ear ht. (cm)	Days to tasseling	Tillers/ plant	Lodging (1–9)*	Insect (1–9)*	Sugar Content Brix(%)	Pericarp Thickness (μm)
Chalok1	190de	67^{bc}	66^{b}	0.8^{a}	7^{bc}	5^{a}	14.8^{a}	48.5^{b}
CNU10	208^{c}	74^{bc}	78^{ab}	1.6^{a}	6^{bc}	3^{a}	10.3^{d}	36.8^{d}
CNU12	232^{b}	78^{ab}	78^{ab}	1.2^{a}	6^{bc}	3^{a}	14.2^{a}	34.7^{c}
CNU19	242^{a}	83^{ab}	74^{ab}	1.5^{a}	2^{a}	3^{a}	13.8^{b}	35.8^{c}
CNU21	246^{a}	87^{ab}	75^{ab}	1.3^{a}	4^{b}	5^{a}	12.7^{bc}	63.8^{a}
CNU94	214^{c}	44^{c}	78^{ab}	1.7^{a}	7^{bc}	3^{a}	10.3^{d}	58.5^{ab}
CNU100	180^{fg}	50^{bc}	81^{a}	1.5^{a}	8^{c}	5^{a}	11.1^{bc}	60.8^{ab}
CNU133	187^{ef}	103^{a}	80^{a}	1.2^{a}	8^{c}	5^{a}	10.2^{d}	60.1^{ab}
CNU153	175^{g}	80^{ab}	82^{a}	1.5^{a}	8^{c}	5^{a}	11.7^{bc}	48.3^{b}
CNU165	196^{d}	101^{a}	78^{ab}	1.6^{a}	8^{c}	3^{a}	10.9^{d}	62.8^{a}
CNU173	194de	102^{a}	78^{ab}	1.5^{a}	8^{c}	3^{a}	12.5^{bc}	58.2^{ab}

*1: strong, 9: weak. In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 2. Comparison of the ear and yield characters among domestic colored waxy corn hybrids

Hybrids	No. of ear/100plt.	Ear length (cm)	Ear dia. (mm)	Ear tip rate (%)	No. ears per 10a	Grain yield (kg/10a)
Chalok1	96^{a}	15.6^{b}	44.0^{ab}	81^{b}	5,712^{a}	746^{a}
CNU10	97^{a}	17.4^{ab}	48.2^{a}	90^{ab}	3,570^{b}	657^{b}
CNU12	96^{a}	20.4^{a}	49.3^{a}	96^{a}	4,641^{ab}	685^{b}
CNU19	98^{a}	18.6^{ab}	48.8^{a}	97^{a}	4,750^{ab}	736^{a}
CNU21	98^{a}	19.1^{ab}	47.6^{a}	96^{a}	4,683^{ab}	715^{a}
CNU94	100^{a}	16.8^{ab}	40.2^{ab}	93^{ab}	3,570^{b}	339^{e}
CNU100	95^{a}	15.9^{b}	41.4^{ab}	93^{ab}	4,132^{ab}	527^{d}
CNU133	90^{b}	16.3^{ab}	34.8^{b}	94^{ab}	4,038^{ab}	426^{c}
CNU153	95^{a}	15.5^{b}	31.5^{b}	96^{a}	4,172^{ab}	387^{e}
CNU165	97^{a}	15.8^{b}	36.8^{b}	92^{ab}	3,988^{ab}	435^{d}
CNU173	93^{b}	17.0^{ab}	34.1^{b}	95^{ab}	4,215^{ab}	422^{d}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

was higher in Chalok 1 and CNU12, while CNU 94 and CNU 133 were somewhat low as 10.3 and 10.2, respectively.

In the pericarp thickness (Table 1), CNU 12 and CNU 19 were thinner than check hybrid. Accordingly, CNU 12 and CNU 19 among used leading hybrids showed good characters in both sugar contents and pericarp thickness than those of check.

3. Functional analysis of new hybrid

1) Polyphenol compound

\Polyphenoal compound contents of the new waxy corn hybrids developed at Corn Genetics and Breeding Laboratory is shown in Fig. 1. That of the new developed CNU 19 waxy corn hybrid was higher than other hybrids. About results as like this, many researchers reported that there was positive correlation between polyphenol and antioxidant materials (Yoana *et al.*, 2006; Claudia *et al.*, 2008). Also, we identified the fact that function of CNU 19 hybrid was high in polyphenol compound.

2) XO inhibitory activity; XOI (%).

XOI (%) about the new developed waxy hybrids showed in Fig. 2. That of the CNU 19 hybrid among used hybrids was higher. From these results, we confirmed

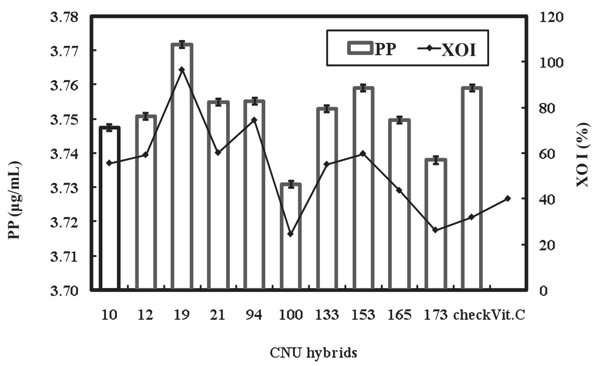

Fig. 2. Inhibition of xanthin oxidase on selected CNU colored waxy corn hybrids. XOI; Inhibitory activity (%) of xantine oxidase (units/mg protein), PP; polyphenol contents (μg/mL).

the fact that XOI (%) was also high in the CNU19 hybrid with high polyphenol compounds but XOI (%) of CNU 100, 173 and check, Chalok1 was lower than that of other CNU hybrids.

3) Catalase activity

Catalase activity was also high in CNU 19 hybrid shown in Fig. 3. But catalase activities of these hybrids were lower than vitamin C used as positive control. Catalase activity of most hybrids except CNU 100 hybrid appeared as about 60 to 80% of positive control.

4) SOD activity

As shown in Fig. 4, the high SOD activity in CNU 19 extract were also appeared highly in polyphenol compound contents, XOI (%) and catalase activity, but there was no hybrids better than vitamin C used as positive control. Among the 10 hybrids, CNU 100 extract showed the lowest value as 30% in the SOD activity

5) DPPH free radical scavenging activity

DPPH free radical scavenging for used hybrids were as shown in Fig. 5, those of used hybrids didn't directly related to polyphenol compounds, but most of hybrids highly appeared than positive check group.

DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986). In

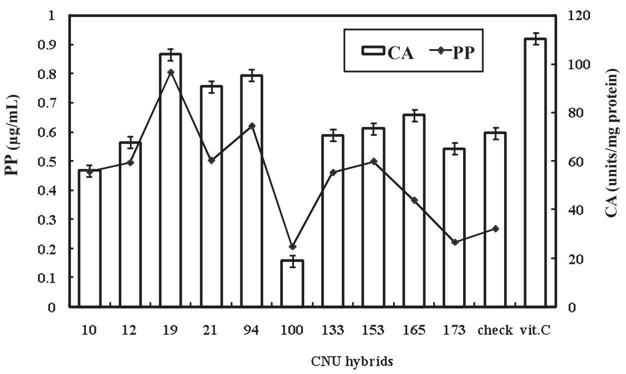

Fig. 3. Catalase activity of selected CNU colored waxy corn hybrids. CA; catalase activity (units/mg protein), PP; polyphenol contents (μg/mL).

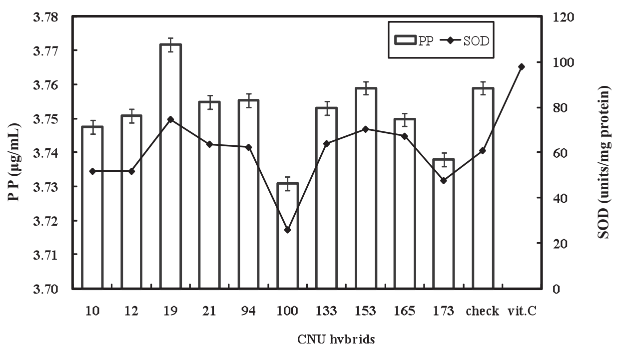

Fig. 4. Superoxide dismutase (SOD) activity of selected CNU colored waxy corn hybrids. SOD; SOD activity (units/mg protein), PP; polyphenol contents (μg/mL).

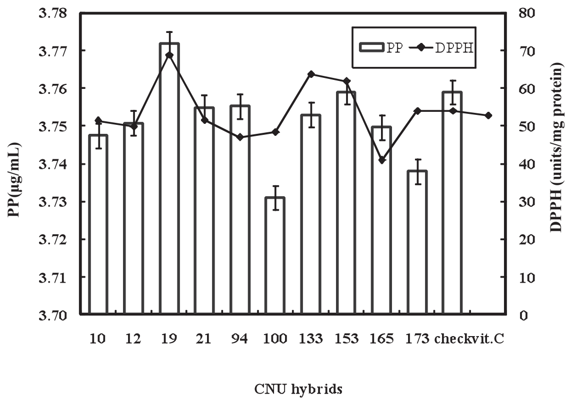

Fig. 5. DPPH radical scavenging activity of selected CNU colored waxy corn hybrids. DPPH; DPPH radical scavenging activity (units/mg protein), PP; polyphenol contents (μg/mL).

DPPH method, CNU 19 extract have the highest activity as 66% and CNU173 extract have the lower activity as 38%, but CNU 100 extract have the lowest activity as 25%.

4. Hybrid selection related table quality

Major components related to table qualities are exterior, stickness, pericarp thickness and sugar content. Most of the new colored waxy corn hybrid appeared strongly to environmental stress because of selection for lodging tolerance, pest–insect resistance and environmental stability. Quality table for these hybrids were as shown Table 3. Exterior of these hybrids were very good because of primary screening for ear size and ear form in field, while stickness, sugar content and tenderness were very variable according to hybrid combinations. Stickness and exterior were appeared highly in CNU 19 and CNU 153 hybrid.

From above total discussion, the yellow waxy CNU 19 hybrid was evaluated as a superior hybrid having functional materials as like higher polyphenol compounds, xanthin oxidase inhibitory activity, catalase activity, superoxide dismute activity and DPPH radical scaveng-

Table 3. Table quality of the developed colored waxy corn hybrids

Hybrids	Exterior (1–9)*	Stickness (1–9)	Sugar content (1–9)	Tenderness (1–9)
Chalok1	6	7	4	7
CNU10	6	7	9	9
CNU12	8	5	5	3
CNU19	9	8	7	9
CNU21	8	5	5	9
CNU94	7	7	7	4
CNU100	7	7	7	8
CNU133	6	6	7	8
CNU153	9	8	8	8
CNU165	8	6	5	6
CNU173	7	7	8	8

* 1: bad (low), 9: good (high).

ing activity. Therefore, this hybrid will be supply to farmers after local adapted test for 2 to 3 years.

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