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Original scientific paper

HETEROSIS AND ANTIOXIDANT COMPOUNDS OF SWEET CORN BREEDING LINES AND THEIR F_1 HYBRID

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In this research, the mid-parent heterosis and the levels of phenolic, flavonoids compounds and antioxidants activity among two selected sweet corn parental lines and their F_1 hybrid Zaharina were evaluated. Substantial positive mid-parent heterosis (MPH) was found for parent-hybrid triplet for ear weight and for plant height. Among all other traits, insertion height had the greatest heterosis and ear diameter had the lowest. Negative mid-parent heterosis exhibited only the trait 1000-kernel weight. Data analysis indicated significant differences in the contents of total phenolic content, total flavonoids content, water–soluble antioxidant capacity, lipid-soluble antioxidant capacity, and antioxidant activity among parent-hybrid triplet. The results suggest that F_1 hybrid Zaharina can be considered as good source of natural antioxidants since it extracts were found to possess high antioxidant activity.

Key words: α-tocopherol; ascorbate; flavonoids; phenols; sweet corn; *Z ea mays*

INTRODUCTION

Sweet corn (*Zea mays* L. *saccharata*) is one of the most popular vegetable crops in countries like USA and Canada. It is characterized by translucent, horny appearance of kernel when matures and wrinkled when it dries. The research reports have indicated that the sweet corn and field corn are nearly identical genetically, except the mutation at the sugary (*Su*) locus on chromosome 4 that prevents the conversion of sugar to starch. At the milky stage, this mutation causes greater accumulation of sugars and water soluble polysaccharides, than normal field corn resulting in specific sugary texture and flavor (SRDIĆ *et al*., 2011). Flavor is the largest factor affecting marketability of sweet corn and according to food scientist it is defined as the total experience from sweetness, texture, and odor, with sweetness being the most important factor in sweet corn (LERTRAT and PULAM, 2007). For food, sweet corn must be harvested when kernels are

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fully developed, but still at an immature stage of the cob, otherwise they lose sweetness and become though. At optimum harvest maturity, the kernels are sweet, milky, tender and nearly optimum size. Over-mature corn is rather starchy than sweet, tough and the kernels are often dented (MOTES *et al*., 2007). While both fresh and processed sweet corn is very popular in the USA and Asia, it is a relatively new vegetable in Europe. In Bulgaria, in the recent years, both consumptions and production area of sweet corn have constantly and consistently increased.

The present work is a part of a maize research program with the objective of characterizing and evaluating the germplasm bank of available sweet corn breeding lines and hybrids developed at the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria, providing useful information on plant genetic resources. In our previous study we reported on genotypic correlation and path-coefficient analysis of some productivity elements in sweet corn (VASSILEVSKA-IVANOVA *et al*., 2007), combining ability in diallel crosses among sweet corn inbred lines (VASSILEVSKA-IVANOVA *et al*., 2007), and capacity for callus formation in inbred lines and F_1 hybrids (NEDEV *et al*., 2008). Recent study reported on a new sweet corn hybrid with high productivity and favorable taste (KRAPTCHEV *et al*., 2010).

The objective of the current study was to characterize the F_1 sweet corn Zaharina and its parental breeding lines accessed from Institute of Plant Physiology and Genetics, BAS, Sofia, by their agronomical traits and to determine the levels of phenolic, flavonoids compounds and antioxidants activity of parent-hybrid triplet.

MATERIALS AND METHODS

Plant materials. A heterotic F_1 hybrid named Zaharina was produced after cross among the two sweet corn lines 6-13 and C-6 (C-6-pollen source). Both inbreds used as parents are part of a set of selfed lines developed at the Institute of Plant Physiology and Genetics, BAS, Sofia following a program of production highly homogeneous, homozygous inbred lines. Trials, consisting of randomized block design were sown at the Experiment Field of the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia (42°50′N, 23°40′E, and 595 m above the sea level). The soil type at Sofia is sandy loam (0 - 15 cm, top soil). Seeds from each sample were sown 4-6 cm deep by hand as the single-row plots were approximately 10 m long, spaced 0.70 m apart and hills 0.30 m. Hills were thinned to one plant to achieve final plant density of 5500 - 6500 plants per 1000 m^2. The sowing date covered the range of those normally used for corn in Bulgaria and was performed in early-May (1 to 7 May). In all three years of the trial, the preceding crop was cereals. Standard agronomic practices were used.

At harvest, 30 randomly selected plants from each genotype were used for recording plant height (centimeters from the soil to the tassel top), length of tassel (cm), size of the nearest to the ear leaf (length and width, cm), number of internodes, ear characteristics such as insertion height (from the soil to the ligulae of the leaf subtending the ear, cm), number of kernel rows per ear, ear length (cm), ear max. diameter (cm), number of kernels per row, 1000-kernels weight (g) and ear weight (g). Harvest was made at dry grain stage instead of at the normal eating stage to estimate more closely physiological efficiency and to reduce the coefficient of heterosis calculations. Mid-parent heterosis (MPH) (relative heterosis) was calculated by formulae $MPH = F_1 - (P_1 + P_2)/2$, where F_1 is the numerical value trait measurement in the hybrid and P_1 and P_2 are the measurements in the parents.

Antioxidant activity. The total antioxidant capacity (free radicals scavenging activity) as well as the presence of the antioxidants ascorbate, tocopherols, phenols and flavonoids was

measured in dry leaves from both parental inbred lines 6-13 and C-6 and their F_1 hybrid. All methods used were previously described (STANCHEVA *et al*., 2011). Spectrophotometric quantification of water-soluble antioxidant capacity (WS – AOC) and lipid-soluble antioxidant capacity (LS – AOC) (expressed as equivalents of ascorbate and α-tocopherol) was performed through the formation of phosphomolybdenum complex (PRIETO *et al*., 1999). The assay was based on the reduction of Mo (VI) to Mo (V) by the sample analysis and the subsequent formation of a green phosphate/Mo (V) at acidic pH.

Plant dry material (0.5 g) was ground with a mortar and pestle to a fine powder. Next, 3 mL of dH_2O was added and the suspension was homogenized, transferred to tubes, and shaken for 1 h at room temperature for 1 h in the dark. The suspension was filtered and extraction was repeated with 3 mL of dH_2O. The pellet was washed again with 2 mL of dH_2O. For lipid-soluble antioxidant capacity (expressed as α-tocopherol) the procedure was the same but the extraction was carried out with hexane as a solvent. The method has been optimized and characterized with respect to linearity interval, repetitively and reproducibility, and molar absorption coefficients for the quantitation of water-soluble and lipid-soluble antioxidant capacities, expressed as equivalents of ascorbate and α-tocopherol (PRIETO *et al*., 1999). Absorption coefficients were $(3.4 \pm 0.1) \times 103$ M^{-1} cm^{-1} for ascorbic acid and $(4.0 \pm 0.1) \times 103$ M^{-1} cm^{-1} for α-tocopherol.

The total antioxidant capacity was measured from bleaching of the purple coloured methanol solution of free stable radical (2, 2-diphenyl-1-picryl-hydrasyl, DPPH) by the method of TEPE *et al*. (2006). $DPPH^{\bullet}$ is a stable radical with a maximum absorption at 517 nm that can readily undergo reduction by an antioxidant. Antioxidant activity of the sample was calculated as percentage inhibition of oxidation versus control sample (blank), using the equation

% antioxidant activity (I) $= (A_{blank} - A_{sampe}/A_{blank}) \times 100$,

where A_{blank} is the absorbance of the control sample (containing all reagents except the test compound), and A_{sample} is the absorbance of plant extracts.

For determination of the phenols and flavonoids, dry leaf samples (1 g) were ground and exhaustively extracted with 96% (v/v) methanol. Contents of phenolic compounds were determined spectrophotometrically using Folin-Ciocalteu reagent and calculated as caffeic acid equivalents (PFEFFER *et al*., 1998). Flavonoids in plant tissues were measured by the method of ZHISHEN *et al.* (1999) spectrophotometrically using the standard curve of catechin.

Statistical analysis. Twenty plants were raised for each treatment and all the experiments were repeated twice. Data were subjected to one-way ANOVA analysis of variance for comparison of means, and significant differences were calculated according to Fisher LSD test at the 5% level using a statistical software package (Statigraphics Plus, version 5.1 for Windows). Data were reported as means ± standard error.

RESULTS AND DISCUSSION

Morphological data were evaluated in the inbred lines and in F_1 hybrid (Table 1). Both parents differed for all traits thus exhibiting high morphological variability. For example, line C-6 (pollen source) is approximately 205 cm high at maturity, whilst 6-13 plants are much shorter. Similarly, the line 6-13 had the lowest insertion height, with lower ear characteristics observed for the sweet corn parental genotypes. Kernel row number usually behaves as an additive trait, but the F_1 hybrid plants were similar to the male parent C-6 for that trait (Table 1). Substantial positive mid-parent heterosis (MPH) was found for parent-hybrid triplet for ear weight (62%) and for plant height (44.4%). Among all other traits, insertion height had the greatest heterosis (18.45%) and ear

diameter had the lowest (0.4%) (Table1). For 1000-kernel weight, mid-parent heterosis equaled -23.2. Negative heterosis indicates smaller kernels than both inbred parents. In sweet corn production, harvestable maturity is defined by grain moisture associated with optimal flavor, tenderness, and texture (TRACY, 1999). The growing period between germination and harvest maturity can be divided into two growth phases, vegetative, from emergence to pollen shed; and reproductive, from silk emergence to maturity (RITCHIE *et al*., 1997). In temperate sweet corn, the length of the vegetative phase has a greater role in determining maturity than does the length of the reproductive phase (HUELSEN, 1954). In this study, the average time from sowing to male flowering was about 82 and 88 days for 6/13 and C-6 breeding lines. In F_1, male flowering was just delayed by three days from 6/13 line but increased up to three days from C-6 (Table 1).

Mid-parent heterosis for ear weight was 62% (Table 1). This value is much greater than many of the sweet corn values reported (DICKERT and TRACY, 2002).

Table 1. Agronomic traits of two sweet corn lines and their hybrid. Each value is expressed as mean ± standard deviation (n=3)

a) Agronomic traits

Genotype	Plant height (cm)	Length of tassel (cm)	Size of the nearest to the ear leaf		Number of internodes
			Length (cm)	Width (cm)	
6/13 ♀	120.0 ± 1.7[a]	32.3 ± 0.7[a]	48.7 ± 0.6[a]	5.3 ± 0.1[a]	6.5 ± 0.1[a]
C-6 ♂	205.3 ± 2.2[b]	36.0 ± 1.5[c]	82.5 ± 1.2[c]	9.0 ± 0.3[c]	10.5 ± 0.2[c]
Zaharina	207.8 ± 4.5[c]	36.7 ± 0.7[b]	82.0 ± 0.9[b]	8.6 ± 0.2[b]	9.2 ± 0.1[b]
MPH	44.4	2.1	16.4	1.45	0.7

b) Ear characteristics

Genotype	Insertion height (cm)	Length (cm)	Diameter (cm)	KR/E	KN/R	1000-kernels weight (g)
6/13 ♀	25.1 ± 0.3[a]	14.9 ± 0.7[a]	4.3 ± 0.4[a]	14 -16	23.0 ± 1.2[a]	216.2
C-6 ♂	62.0 ± 0.8[b]	19.8 ± 0.9[b]	4.9 ± 0.8[b]	16 -18	50.0 ± 0.9[b]	178.4
Zaharina	62.0 ± 1.3[c]	20.7 ± 0.8[c]	5.0 ± 0.7[c]	18 - 20	50.5 ± 1.1[c]	174.2
MPH	18.45	2.65	0.4	3.0	14.0	-23.2

KR/E - kernel row per ear; KN/R - kernel number per row

There were significant differences in total phenolic content, total flavonoids content, WS-AOC, LS-AOC, and antioxidant activity among parent-hybrid triplet (Table 2). The total phenolic content of the plant extracts were determined using the Folin–Ciocalteu phenol reagent giving a crude estimate of the amount of phenolic compounds present in an extract (WONG *et al*., 2006). From Table 2, inbred line C-6 (pollen source) had the lowest amount of polyphenols while the highest was observed in F_1 hybrid. Generally, extracts that contain a high amount of polyphenols also exhibit high antioxidant activity. Maize (*Zea mays* L.) is one of the world's most important cereal crops that display antioxidant activity in its grains (SALAR *et al*., 2012). Maize phenolics are powerful antioxidants through radical scavenging (ADOM and LIU, 2002), and thus have great potential in the development of rich of antioxidants whole grain products protective against certain chronic diseases, cardiovascular diseases, diabetes, obesity and cancer (FLORA and WILEY, 1974).

Table 2. Antioxidant content in the leaves of two sweet corn lines and their hybrid. Each value is expressed as mean ± standard deviation (n=3)

Genotype	Phenols mg gDW^{-1}	Flavonoids mg gDW^{-1}	WS – AOC µmol ASC g DW^{-1}	LS – AOC µmol ASC g DW^{-1}	DPPH %
6-13 ♀	5.39 ± 0.15^{b}	6.66 ± 0.17^{b}	119.65 ± 4.20^{ab}	0.43 ± 0.02^{b}	65.93
C-6 ♂	3.72 ± 0.01^{a}	4.89 ± 0.13^{a}	126.94 ± 5.04^{b}	0.30 ± 0.01^{a}	61.84
Zaharina	12.50 ± 0.20^{c}	13.18 ± 0.22^{c}	114.24 ± 4.36^{a}	0.97 ± 0.04^{c}	83.49
LSD	0.5264	0.6178	10.8755	0.0573	

Flavonoids are the most common and widely distributed group of phenolic compounds, occurring in sweet corn. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Based on the results, total flavonoids contents were markedly higher in F_1 hybrid plants than in both parental lines (2-fold in 6-13 and 2.7-fold in C-6) (Table 2). Quantification of lipid soluble antioxidant capacity (LS-AOC) expressed as equivalent of α-tocopherol in parent-hybrid triplet revealed that the level of α-tocopherol was increased 2-fold and 3-fold, respectively, in the F_1 hybrid compared with parental lines (Table 2). By contrast, the water soluble antioxidant capacity (WS-AOC) expressed as equivalent of ascorbate of the F_1 hybrid was actually slightly reduced, compared with 6-13 and C-6 lines (Table 2). Indeed, the effect of hybridization was as dramatic on the polyphenols and flavonoids levels as on the LS-AOC (α-tocopherol) content. The higher level of antioxidant compound is beneficial for edible plant species such as sweet corn while the antioxidant activity of plants is mainly contributed by the active compounds present in them (CHANWITHEESUK *et al*., 2005).

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 517 nm. The DPPH quenching activity of parent – hybrid triplet is shown in Table 2, where DPPH radical scavenging varied from 61.84 to 83.49%. The highest radical scavenging activity was found in the F_1 hybrid genotype, while the line C-6 had the lowest quenching capacity.

In summary, the results show that there are differences in the contents of antioxidant compounds of the parent – hybrid sweet corn triplet. The results suggest that the F_1 hybrid Zaharina can be considered as a good source of natural antioxidants since it extracts were found to possess high antioxidant activity. This information may have a significant impact on evaluation of nutritional value of sweet corn and could increase interests on consumer's food selection by increasing their consumption of sweet corn. If we also consider the wider implication of this work, it is clear that the sweet corn F_1 hybrid Zaharina has an altered phenotype with sticking changes in a wide range of antioxidant compound and thus, it may benefit the health and food industries.

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HETEROSIS I ANTIOKSIDANTI KOD LINIJA ŠEĆERCA I NJIHOVIH F_1 HIBRIDA

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Izvod

Vršena su ispitivanja heterozisa dve samooplodne linije kukuruza šećerca i njihovog F_1 hibrida Zaharina i nivoa fenola, flavonoida i aktivnost antioksidanata kod njih. Utvrđen je pozitivan heterotični efekat težine klipa i visine biljaka. Dobijeni rezultati sastava ekstrakta ukazuju da hibrid Zaharina može da se koristi kao dobar izvor prirodnih antioksidanata jer je utvrđena njihova visoka aktivnost u analiziranom ekstraktu

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