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Original scientific paper

ANTIOXIDANT ACTIVITY IN SEEDS OF MAIZE GENOTYPES WITH DIFFERENT PERCENTAGE OF EXOTIC GERMPLASM

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In order to broaden the genetic base of maize (*Zea mays* L.) germplasm, it is necessary to integrate exotic materials into adapted breeding materials. The aim of the study was to compare antioxidative systems of two adapted maize inbred lines (A and B) with exotic germplasm, Drought Tolerant Population (DTP), and their backcrosses with DTP (A1, A2 and B1, B2). The content of low-molecular weight antioxidants, proline and phenolics, as well as antioxidant capacity, detected as free radical scavenging activities against DPPH radical, were measured in maize seeds. Proline content in both, embryo and endosperm was higher in backcrosses than in inbred lines and DTP, and increased in

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embryo by getting higher percentage of exotic germplasm. Contrary, phenolic content and DPPH radical scavenging activity of seeds, which were higher in adapted inbred lines than in DTP, were slightly decreased in their backcrosses with DTP.

Key words: antioxidant activity, proline, phenolics, exotic germplasm, *Zea mays* L.

INTRODUCTION

Drought is one of the most important abiotic stresses that seriously decreases final grain yield in maize. Since the occurrence of drought is not predictable, breeders have to produce maize genotypes able to withstand stress and have stable yield under no stressed conditions. Drought Tolerant Population (DTP) was formed in CIMMYT (International Maize and Wheat Improvement Center), Mexico It is formed from components that have shown superior performances in the conditions of drought and high stress tolerance. Based on the reaction in the drought conditions, introgression of new components was also made, so the final population had predominantly tropical, less subtropical and the least amount of the germplasm from the temperate zone (Beck et al., 1997). The point of forming this population was to provide and ensure wide and new genetic base for the drought tolerance, based on combinations of variety germplasm with different adaptation levels (ANDJELKOVIC, 2000).

It is well known that dehydration stress, like other stress conditions, induces increased production of reactive oxygen species (ROS), which in excess could be harmful to plant cells. Natural plant antioxidants, such as proline and phenolics are involved in regulation of ROS content. Thus, proline accumulation, as a common metabolic response of higher plants to water deficits (STEWART, 1981), performs an important function as protective compatible osmolyte in scavenging free radicals and facilitates a correction of altered redox potential (HARRE *et al*., 1999). Antioxidant properties of phenolic compounds abundant in plants were also reported (RICE-EVANS *et al.* 1997; TAKAHAMA, 2004).

In seed physiology it is known that although production of ROS may have a beneficial role in embryo growth, prevention of oxidative damage, occurring during germination, is required to allow the embryo to produce a viable and vigorous seed. At low moisture content of seeds prevention of oxidative damage might be more likely related to ROS scavenging by antioxidative compounds than by enzymatic activities (BAILLY, 2004). Since both antioxidants, proline and phenolics, are compounds found in grains, their protective role could be supposed. Literature data mostly refer to the role of proline in protein synthesis during germination, while antioxidant activity of proline in mature seeds has not been yet discussed. In maize kernel, proline is one of the most abundant amino acids, essential for further growth and plant development (DATA *et all*., 1983). It is predominantly found in its bound form as a storage protein zein and released during germination, thus providing amino nitrogen and energy needed for protein

synthesis. Genotypic differences among maize inbred lines in respect to protein content and quality, as well as the variability of free proline content in maize endosperm tissue has been established (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ and JELENIĆ, 1984).

In the present work we investigated the influence of exotic germplasm on the content of low-molecular weight antioxidants, proline and phenolics, as well as antioxidant capacity, of maize seeds.

MATERIALS AND METHODS

Plant material

The study was carried out on seven maize genotypes: two local inbred lines (**A** and **B**), their backcrosses with exotic germplasm (drought tolerant population-DTP) **A1** ((AxDTP))xA), **A2** (((AxDTP)xA)xA) and **B1** ((BxDTP))xB) and **B2** (((BxDTP)xB)xB), as well as **DTP**.

Free proline determination

Concentration of free proline was determined in embryo and endosperm of analyzed maize genotypes. After soaking seeds in distilled water at 4°C over night, the endosperm and embryo were separated and dried at room temperature. Endosperms were ground in a cyclone mill, 1 g of flour was extracted with 10 volumes of 3% sulfosalicylic acid by continuous shaking for one hour and filtrate used for proline determination. The embryo (0.1 g) was homogenized with mortar and pestle in 10 volumes of the same extraction solution and filtered homogenate used for proline determination. In both cases the content of proline was determined according to BATES (1973) and expressed as $\mu g\ g^{-1}$FW. The assays were done in duplicate, from three separate extractions.

Determination of antioxidant activity using DPPH radical scavenging method

For the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test the maize seeds extract was prepared by mixing 0.3 g of whole grain flour with 10 mL of 70% (v/v) acetone. After continuous shaking for 30 min at room temperature, the solution was centrifuged for 20 min at 20 000g. The obtained supernatant was used for DPPH radical scavenging activity according to modified Abe et al. (1998) assay. An aliquot of extract (0.1 ml) was mixed with ethanolic DPPH solution (0.5 mM, 0.25 ml) and acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results are expressed as IC_{50} value that represents the amount of flour (in mg DW) providing 50% inhibition of DPPH radicals. (ABE *et al*., 1998.; KOLEČKÁŘ *et al*., 2007). All the assays were done in duplicate, from two separate extractions.

Determination of the total phenolic content

Total phenolics were determined by the method of SINGLETON and ROSSI (1965) by using the same extract as for DPPH test. Briefly, 0.1 ml of extract was mixed with 0.25 ml Folin reagent, 1.25 ml 20% sodium carbonate, and 0.4 ml deionized water. After standing for 40 min. at room temperature, the absorbance was measured at 725 nm. Total phenolics content was calculated as a catechin equivalent from the calibration curve of catechin standard solutions and expressed as mg catechin g^{-1}flour. All the assays were done in duplicate, from two separate extractions.

RESULTS AND DISCUSSION

In order to broaden the genetic base of ZP maize germplasm, the back cross method was used to integrate DTP as exotic material into local adapted breeding material. To investigate the effect of different percentage of exotic germplasm on antioxidant activity in seeds, we used two adapted maize local inbred lines (A and B), their backcrosses with DTP (A_1, A_2, B_1 and B_2) and DTP. Kernel mass, the total phenolic content and DPPH radical scavenging activity in the investigated material are presented in Table 1. There was no significant difference in kernel mass between two inbred lines (A and B) and DTP. Crosses A_1 and B_1 showed decrease, while crosses A_2 and B_2 showed increase in kernel mass in regard to corresponding lines and DTP.

In our experiments the content of phenolics was determined in whole grain flour, because they are abundant in pericararp and aleuron layer. Although generally the differences between genotypes were not significant, the lowest content of total phenolic compounds was detected in DTP genotype. The phenolics content in backcrosses decreased by getting lower percentage of exotic germplasm, except in the B_2 genotype, which was higher than in both parental genotypes (Table 1). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, chelators of metal catalyst and singlet oxygen quenchers (SHAHIDI and WANASUNDRA, 1992). According to the literature data, the antioxidant ability of seeds is highly positively correlated with phenolics content (MALENČIĆ *et al*., 2007; VERMA *et al*., 2008). In our experiments antioxidant potential of seeds has been determined as the free radical scavenging ability using a stable DPPH radical. IC_{50} values presented in Table 1, which refer to the mass of flour at which DPPH radicals were scavenged by 50%, are negatively correlated to antioxidative ability. Thus, the lowest antioxidative activity was demonstrated in DTP and antioxidant ability followed the same tendency as phenolic content in the case of A backcrosses, while in any B backcross with DTP, antioxidant ability decreased in respect to inbred line.

In maize kernel, proline is one of the most abundant amino acids, predominantly found in its bound form as a constituent of the storage protein zein, making about 10% of zein molecule (REF). Also, it accounts a considerable

portion of free amino acid pool in mature endosperm of maize (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, 1983). Compared to endosperm, embryo tissue contains significantly higher level of free proline. Furthermore, free proline is more abundant in the embryo than any other amino acid (AGUILAR and JIMENEZ, 1984). We have determined free proline content in both, endosperm and embryo tissue of mature seeds. The content of proline ranged from 40 to 135 µg g^{-1} and from 1542 to 5329 µg g^{-1} of endosperm and embryo, respectively (Fig 1 a, b). Our previous investigations performed on 20 local maize populations from the collection of the Maize Research Institute, showed free proline content in endosperm from 19 to 79 µg g^{-1} (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ and JELENIĆ, 1984). Also, the content of free proline in the embryo of several maize inbred lines ranged from 1414 to 3900 µgg^{-1} (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, 1990).

Genotype	Kernel mass (g)	Total phenolics (m g catechin g^{-1} DW)	DPPH radical scavenging activity (IC_{50} mg DW)
A	0.369±0.01	1.96±0.12	3.19±0.11
A_1	0.337±0.01	1.85±0.07	3.69±0.03
A_2	0.406±0.01	1.54±0.06	3.81±0.22
B	0.368±0.01	1.68±0.15	3.09±0.05
B_1	0.315±0.01	1.57±0.16	3.51±0.09
B_2	0.443±0.01	1.86±0.05	3.32±0.17
DTP	0.379±0.01	1.42±0.10	4.26±0.20

Table 1. The effect of different percentage of exotic germplasm on kernel mass, content of total phenolics and radical scavenging activity

Our results demonstrated that proline content was significantly higher in backcrosses compared with DTP and corresponding lines (Fig 1). Although differences between backcrosses were not so pronounced, embryo proline content was higher in backcrosses with the higher amount of DTP (BC1) than in BC2 (Fig 1a). In the endosperm proline content followed the same tendency in line B crosses, while in the case of line A it was opposite (Fig 1b). Anyhow, from our results it is evident that introduction of exotic germplasm into local materials increases the content of free proline in seeds. This increase of seed proline content indicates capability of higher protein synthesis rate during germination, being dependent on the endogenous amino acid pool (MARCH *et al.*, 1982), in which proline metabolism has an important role. Also, increased antioxidative ability of seed due to protective role of free proline against ROS could be supposed.

It could be supposed that higher level of seed proline could influence the plant response to stress since results of Raymond and Smirnoff (2002) demonstrated that proline accumulates in roots at low water potential as a result of its translocation from the endosperm of germinated seedlings. However, the

capacity for proline biosynthesis in root tips from older plants after exhaustion of the endosperm reserves has not yet been investigated. Future experiments with plants subjected to stress conditions could give evidence if seed proline content is correlated to plant capacity to accumulate proline and overall stress tolerance.

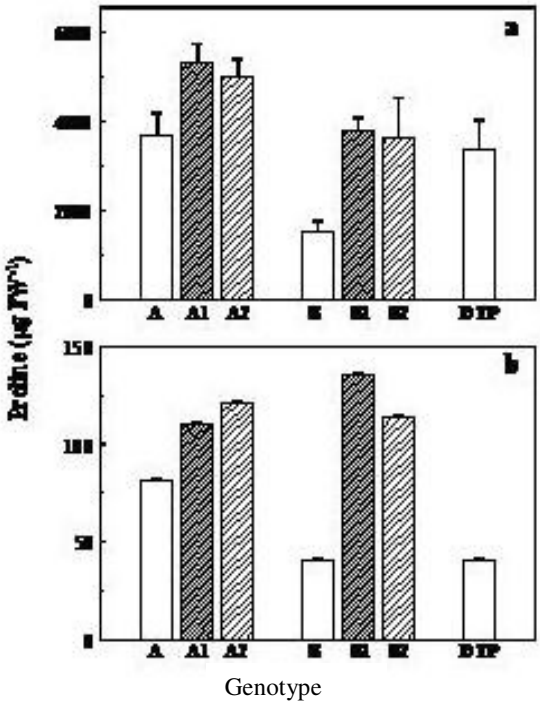

Figure 1. Embryo (a) and endosperm (b) proline content (μg g^{-1} FW) of seven maize genotypes

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SADRŽAJ ANTIOKSIDATIVNIH KOMPONENATA U SEMENU GENOTIPOVA KUKURUZA SA RAZLIČITIM UDELOM DTP-A

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I z v o d

U cilju proširenja genetičke osnove germplazme kukuruza (*Zea mays* L.), neophodna je inkorporacija egzotičnog u lokalni adaptirani selekcioni materijal.

U radu su upoređeni antioksidativni sistemi dve lokalne inbred linije (A i B) sa antioksidativnim sistemima egzotične germplazme (DTP) i povratnih ukrštanja tih inbred linija i DTP-a (A_1, A_2, B_1 i B_2). U semenu kukuruza meren je sadržaj antioksidanata male molekulske težine, prolina i ukupnih fenola, kao i antioksidativni kapacitet, izražen preko sposobnosti hvatanja slobodnih DPPH radikala.

Rezultati su pokazali porast nivoa prolina, kako u klici, tako i u endospermu, kod A_1, A_2, B_1 i B_2 genotipova, u odnosu na čiste inbred linije i DTP. Takođe, nivo prolina se povećavao sa povećanjem udela egzotične germplazme. Nasuprot tome, kod čistih inbred linija je bio veći nivo ukupnih fenola i stepen hvatanja slobodnih DPPH radikala nego kod DTP-a, i sa tendencijom neznatnog smanjenja kod A_1, A_2, B_1 i B_2 genotipova.

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