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Maize seedling performance as a potential index for drought tolerance

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Abstract: Twenty-six maize landraces were tested in order to evaluate maize seedling performance as an index for drought tolerance in adult plants. Samples were subjected to polyethylene glycol-induced osmotic stress at the early seedling stage. Grain yield was obtained in field experiments under well-watered (OC) and a combination of drought and high plant density (HD) conditions. Osmotic stress caused a reduction in seedling growth (length, fresh and dry weight), and increase in the shoot and in particular the root proline contents in the majority of landraces, and variations in root peroxidase (POD) activity. Genotypes displaying more pronounced root growth reduction and higher proline contents exhibited decreased POD activity under osmotic stress. Direct positive correlations between the proline content and growth inhibition, and between the proline and soluble protein content were established. Correlations between the changes in POD activity and growth parameters were significant and positive, and significant but negative with the changes in the proline content. In the field, water stress led to a reduction in grain yield in all of the tested landraces. Correlations between grain yield from both experimental sets (OC and HD) and osmotic-induced changes in seedling root growth were negative, which was opposite to the highly significant and positive correlations between the changes in the seedling root proline content and yield. Also, genotypes with the highest seedling root proline content increase under osmotic stress, exhibited the highest stress tolerance index (STI) based on grain yield achieved under both field conditions. Our results indicate that lower changes in POD activity and especially an increased proline content after exposure to osmotic stress during the early seedling stage could be considered as useful indices to facilitate selection efficiency for drought tolerance in adult plants.

Key words: drought; grain yield; POD activity; proline content; *Zea mays* L.

INTRODUCTION

Identification of traits related to drought tolerance through the responses of plants to water deficits includes analyses of the physiological, cellular, biochemical and molecular basis of the traits [1]. Water deficit, as an unfavorable environmental condition, disrupts cellular homeostasis that can lead to oxidative stress due to enhanced generation of reactive oxygen species (ROS) in plants. The ROS that are produced in both unstressed and especially in stressed plant cells are important signals and mediators in the biosynthesis of complex organic molecules, the polymerization of cell wall constituents and of the defenses against various abiotic and biotic stresses. Since oxidative damage to lipids, proteins and DNA occurs under an excess of ROS, the balance between their production and removal, which is disturbed under stress conditions, is critical for the maintenance of active growth and metabolism of the plant and overall stress tolerance [2,3]. In order to regulate and remove excess ROS, plant cells possess a complex antioxidant system, consisting of low-molecular mass antioxidants and antioxidant enzymes [4,5].

Proline, which is widely found in higher plants, accumulates primarily in the cytosol under dehydration and excessive osmotic pressure stress, contributing to osmotic adjustment as a key osmolyte [6]. Proline accumulation can influence stress tolerance in multiple ways. Apart from being an important molecule in redox signaling and contributing to the stabili-

zation of cellular homeostasis during stress conditions, proline can influence cell proliferation or cell death, protect subcellular structures and macromolecules exposed to osmotic stress and trigger specific gene expression, which can be essential for plant recovery from stress [7]. Besides its protective functions, proline has been shown to possess an antioxidant capacity, either as an effective quencher of ROS [8], or by protecting and stabilizing ROS-scavenging enzymes and activating alternative detoxification pathways [7].

Among the antioxidant enzymes involved in the alleviation of the effect of oxidative stress, class III peroxidases (POD, EC 1.11.1.7), defined as secretory peroxidases, assume a major role as they are directly involved in ROS scavenging through catalyzing the reduction of H_2O_2 by a wide range of electron donor molecules, such as phenolics, lignin precursors, auxin and secondary metabolites [9]. Since in the standard peroxidative cycle they can oxidize different substrates in the presence of H_2O_2, and also produce ROS in their oxidative cycle, peroxidases are considered to be bifunctional enzymes [10]. In response to biotic and abiotic stresses, several roles have been attributed to plant peroxidases, including cell wall modification such as suberin polymerization, crosslinking of structural nonenzymatic proteins, the ability to cleave cell wall polysaccharides, growth regulation by controlling hormonal and cell wall metabolism and antioxidant defense [11,12]. Moreover, a correlation between the antioxidant capacity of peroxidases and drought tolerance has been reported in several plant species [13].

The susceptibility of plants to drought stress varies depending on the degree of stress and the plant species. Considering the developmental stage of plant, the damaging effects of water deficit are more notable when it coincides with a particular growth stage, such as germination, seedling and flowering. Studies examining the effect of water deficit on different plant species point to the existence of stress-tolerant mechanisms at the seedling stage [14]. When exposed to such conditions, plants respond by various adaptive mechanisms, ranging from whole-plant responses to changes in cellular-level functions, such as osmoregulation [15].

A number of genes influencing the anatomy, biochemistry and/or physiology of functions important for agronomic performance may be expressed at an early stage of development. As an example, significant associations were found in maize between the traits of seedlings grown under controlled laboratory conditions and traits of adult plants grown in the field, especially with regard to grain yield [16] and root strength [17]. Analogously to maize, significant associations were also found in wheat (*Triticum aestivum* L.) between seedling root characteristics and root system traits of the adult plant, particularly under conditions of limited water availability [18].

Measuring the traits at the seedling stage in hydroponic culture (in particular those characterized by low heritability), offers the advantage of growing a large number of plants under relatively uniform conditions, and is an important prerequisite for investigating quantitative changes in traits. These findings prompted us to evaluate a set of 26 maize landraces by: (i) studying root and shoot parameters of growth and the free proline content, as well as activities of soluble root peroxidases (POD; class III; EC 1.11.1.7) at the early seedling stage under optimal and water stress conditions imposed by a polyethylene glycol (PEG) solution in the laboratory, and (ii) by evaluating their yield performance under optimal (OC) and a combination of drought and high plant density conditions (HD) in the field. The aim of this work was to determine whether the observed morphological and physiological parameters measured at the seedling stage and their interrelations could be used as reliable prediction criteria for plant performances, i.e. a higher grain yield, under different water-supply conditions.

MATERIALS AND METHODS

Plant materials

After a two-year screening for drought tolerance in Egypt in a managed stress environment (MSE), a drought-tolerant *core* collection of 571 accessions from the Maize Research Institute Zemun Polje (MRI) gene bank was created and further tested in temperate climate conditions (Serbia and Macedonia). Field trials included the evaluation of secondary traits that were relevant for drought tolerance (i.e. the anthesis-silking interval (ASI), leaf rolling, barrenness, seed set, grain filling and staying green), and testing for general combining ability. As a result, a drought-tolerant mini

core collection of 41 accessions (15 inbred lines, 13 local and 13 introduced maize landraces) was established. A set of 26 maize landraces were evaluated in the present study. Landraces designated as local were collected from all agronomic and ecological sites (of former Yugoslav territories), and their origin was as follows: LL1, LL4, Ll7 and LL8 from Serbia, LL2, LL3, LL6, LL9, LL11 and LL12 from Bosnia and Herzegovina, LL5, LL10 and LL13 from Slovenia, Macedonia and Croatia, respectively. The country of origin for the introduced landraces is as follows: IL14 and IL15 are from Iran, IL16 is from France, IL17 and IL18 are from Russia, IL19 is from Bulgaria, IL20 is from Argentina, IL21 and IL22 are from Italy, IL23, IL24 and IL25 are from the United States and IL26 is from Pakistan (www.mrizp.rs/emdb/default-en.htm).

Field experiment and grow conditions

The experiment was carried out in 2015 in Zemun Polje, Serbia (44°52´N, 20°19´E, 81m a.s.l.). Although the average temperature during the vegetative period (April-September) was optimal (21°C), high air temperatures characterized the flowering (June-July) and grain filling (July-August) stages, e.g. 18 days in June and 10 days in July had maximal temperatures above 30°C and above 35 °C, respectively; 12 days in August had maximal temperatures above 35°C. Since the estimated optimal precipitation for maize growth in the region where the trial was performed is 459.0 mm, this experimental year had sufficient precipitation; however, the distribution of precipitation was mostly unfavorable for maize production. Excessive precipitation that occurred in May (80.7 mm) contributed to shallow rooting. This was followed by periods of extremely low precipitation during the two subsequent crucial phenophases, the flowering (June-July with only 49.2 mm rainfall) and grain filling periods (July-August with 60.1 mm of rainfall). The stress in this period was severe, particularly because of the previously developed shallow root system. More excessive precipitation was recorded in September (101.4 mm), which contributed to the total amount of precipitation but had no significant impact on yielding.

A high density (high number of plants per unit area) presents general stress that includes light and nutrient shortage, sometimes water stress and/or high temperature stress, so that conventional breeding programs consider imposing higher plant densities as the standard test for abiotic stress tolerance. Thus, the landraces were grown in two sets of field experiment, well-watered and low plant density – the optimal condition (OC), and under a combination of drought and high-density stress (HD). The plants of each maize landrace were sown in a single 4 m-long row plot for the OC set (which was equivalent to 66700 plants ha^{-1}), and in a 3 m-long row plot for the HD set (equivalent to 88800 plants ha^{-1}), with an inter-row separation of 0.75 m. After the seedlings were established, the plots were overplanted and thinned to two plants per hill. A randomized complete block design (RCBD) with three replications was used in the experiments. The plants were harvested manually, and after drying to 14% of water content, the yield was determined and presented as the average grain yield per plant (g $plant^{-1}$).

Laboratory growth conditions

Seeds of the chosen maize landraces were germinated for three days on moistened filter paper and then transferred to a constantly aerated nutrient solution with 30 plants in one 2-L pot. Seedlings were grown for the following 6 days in a growth chamber under a 12-h photoperiod at 22/18°C, with an irradiance of 190 μE $m^{-2}{\cdot}s^{-1}$ PAR and relative humidity of 70%, in an aerated quarter-strength Knop solution with a modified nitrogen content [19]. Nitrogen was supplied as KNO_3, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ as a mix of nitrate (3.6 mM) and ammonium (1.8 mM). The initial pH of the solution was adjusted to 5.6 and did not change significantly during the experiment. For the terminal 48 h of the growing period, treated seedlings were grown on a nutrient solution supplemented with 4% polyethylene glycol (PEG, Mr 10000), parallel with control seedlings grown on a nutrient solution without PEG. The roots and shoots of each plant were sampled and used for the analyses. Dry weight (DW) was determined after drying the samples at 104°C for 24 h to a constant weight.

Free proline content determination

Seedling roots and shoots were homogenized in 3% sulfosalicylic acid (1:10 w/v). The filtered homogenate was mixed with acid ninhydrin solution and boiled for 15 min. After extraction with toluene, the absorbance

of the reaction product was determined at 520 nm [20]. The proline content in the sample was calculated from the proline standard curve and expressed in μg g^{-1} fresh weight (FW).

Enzyme assays and protein content determination

Root tissue was homogenized in 10 volumes of 50 mM K-phosphate buffer, pH 7.5, centrifuged at 20000 *g* for 15 min. The supernatant representing the soluble fraction was used to assay POD activity and the protein content. POD activity was determined as the oxidation of 0.1 mM ferulic acid with 1 mM H_2O_2 and 3.6 μg of sample protein in 50 mM K-phosphate buffer, pH 6.0 at 30°C [16]. The protein content was determined according to the Lowry procedure, using bovine serum albumin as the standard [21].

Statistical analysis

The analyses for seedling evaluation were performed in four measurements (n=4) and the results were presented as the mean±standard error (SE). For each trait, the coefficient of variation (CV%) was determined for both control and treated seedlings of each landrace. Significant differences between genotype means were determined by Fisher's least significant difference (LSD) test at a 0.05 probability level after analysis of variance (ANOVA) using one-factorial RCBD. The differences between the mean values with $P \leq 0.05$ were considered as significant. Correlation analyses were performed using Pearson's correlation coefficient. Drought-tolerance indices for grain yield obtained under different water-supply conditions were calculated using the stress tolerance index (STI) [22].

RESULTS

The effect of osmotic stress on growth parameters

Measurements of root and shoot characteristics were performed on 9-day old seedlings before the onset of secondary root growth. Significant genotypic variations ($P \leq 0.05$) of all morphological, physiological and biochemical parameters were revealed, and they were more apparent under the applied PEG-induced osmotic stress. Application of this mild osmotic treatment (osmotic pressure ~ 0.1 MPa) caused a reduction in seedling growth, i.e. of the length (L), fresh (FW) and dry weight (DW) in all genotypes. Growth reduction was more pronounced in the roots than in the shoots (Table 1). When compared to the control, the average decreases in root and shoot lengths of PEG-treated plants were 19.3% and 13.9%, respectively. Further, the osmotic stress brought about an average decline in the root and shoot fresh weights by 22.1% and 20.7%, respectively, and in the dry weight: from 0.4% to 39.9% for the roots, and from 0.2% to 37.4% for the shoots, depending on the genotype. The reduction in growth parameters (up to 10%) in roots was recorded for genotypes L8, L9 and L10, whereas genotypes L2 and L6 exhibited the smallest growth reduction.

The effect of osmotic stress on the proline content

The growth reduction caused by the PEG treatment was followed by an increase in the free proline contents of both roots and shoots in the majority of evaluated landraces. In control plants, a higher proline content was determined in the roots as compared to the shoots, with a much more pronounced increase in roots after the PEG treatment. Under osmotic stress, the increase in the proline contents ranged from 0.7% up to 95.7% in the shoots (data not presented), and from 0.2% up to 168.7% in the roots when compared to the control (Table 2). A genotype-specific response to PEG treatment was recorded in genotype L6, which displayed 21.7% of proline reduction in the root, and a very low level of increase (0.7%) in the shoot, whereas genotype L10 displayed a small proline increase of 9.9% in the root, and a 6.4% reduction in the shoot.

The effect of osmotic stress on the soluble protein content and root peroxidase activities

As the PEG-induced osmotic stress caused more pronounced changes in the proline content in the roots than in the shoots in most of landraces, analyses of the protein content in the soluble fraction and of peroxidase (POD) activity were performed in seedlings roots (Table 2). with regard to the protein content, significant genetic variations ($P \leq 0.05$) were observed. In response to the applied osmotic stress, changes in protein content ranged from 37% reduction to 15% stimulation, depending on the genotype. Among the

Table 1. The effect of PEG on root and shoot length, fresh and dry weight of maize landraces (Ls) at the early seedling stage, expressed as % of the control.

Genotypes	Length		Fresh weight		Dry weight	
	Root	Shoot	Root	Shoot	Root	Shoot
LL1	88.4^{b}	83.9^{j}	76.1^{n}	80.7^{m}	76.1^{j}	79.7^{o}
LL2	89.2^{k}	98.2^{k}	82.2^{w}	97.7^{s}	88.6^{n}	97.0^{p}
LL3	82.6^{b}	87.7^{fgh}	81.0^{i}	82.8^{m}	82.5^{d}	84.9^{h}
LL4	84.7^{cd}	83.1^{ij}	69.8^{r}	74.8^{n}	65.6^{l}	74.7^{ij}
LL5	96.5^{a}	87.5^{a}	90.5^{a}	76.6^{a}	85.4^{a}	83.0^{a}
LL6	94.5^{c}	95.7^{gh}	93.6^{h}	96.4^{d}	99.6^{e}	98.2^{c}
LL7	87.6^{c}	90.2^{ij}	83.1^{g}	89.5^{l}	79.2^{i}	86.4^{jkl}
LL8	97.8^{c}	90.1^{efg}	95.9^{f}	84.0^{d}	89.6^{f}	81.8^{c}
LL9	91.9^{cde}	92.6^{gh}	93.0^{s}	78.6^{k}	90.0^{m}	78.1^{jk}
LL10	89.7^{gh}	92.1^{ij}	90.9^{o}	90.8^{p}	91.4^{l}	89.3^{klm}
LL11	84.1^{hi}	81.5^{l}	89.3^{w}	84.4^{t}	83.8^{o}	83.7^{p}
LL12	82.8^{i}	79.0^{hi}	68.1^{t}	59.2^{n}	65.5^{m}	62.6^{mn}
LL13	91.9^{b}	92.7^{b}	77.8^{e}	83.5^{b}	81.6^{c}	84.2^{b}
IL14	76.9^{def}	74.7^{gh}	56.7^{m}	64.9^{g}	60.1^{h}	64.7^{ef}
IL15	41.3^{m}	86.3^{fgh}	62.7^{r}	77.0^{o}	65.8^{jk}	76.4^{lm}
IL16	88.4^{cde}	80.1^{gh}	88.4^{m}	67.6^{m}	74.5^{i}	68.1^{hij}
IL17	60.0^{hi}	84.3^{cde}	65.5^{j}	78.0^{g}	64.7^{h}	74.9^{fg}
IL18	70.0^{j}	80.3^{k}	65.1^{p}	74.1^{q}	64.1^{k}	72.1^{o}
IL19	81.9^{b}	82.0^{gh}	77.2^{h}	77.2^{h}	77.9^{g}	77.5^{g}
IL20	62.5^{m}	83.1^{k}	73.4^{u}	76.5^{j}	69.1^{m}	75.6^{jkl}
IL21	72.9^{b}	89.4^{bc}	74.5^{d}	93.4^{e}	87.5^{e}	99.8^{cd}
IL22	70.2^{ef}	89.9^{bcd}	71.4^{k}	81.7^{c}	74.6^{h}	87.4^{b}
IL23	79.1^{b}	88.9^{def}	76.0^{c}	87.8^{d}	75.6^{cd}	90.1^{c}
IL24	92.2^{a}	84.3^{efg}	75.6^{b}	72.9^{f}	79.9^{b}	71.8^{de}
IL25	63.0^{l}	78.4^{k}	73.2^{q}	68.9^{r}	69.5^{l}	67.7^{no}
IL26	78.2^{fg}	81.8^{bcd}	75.0^{l}	62.2^{i}	72.3^{i}	65.6^{hi}
CV%	8.35	8.25	0.23	0.49	1.55	3.10
$LSD_{0.05}$	0.939	0.475	0.609	2.127	0.218	1.025

The results are presented as means obtained from at least twenty plants and four measurements (n=4). Means in a column followed by the same letter were not significantly different ($P \leq 0.05$) according to the LSD test. *LL – local landrace; IL – introduced landrace.

genotypes with decreased protein contents, three landraces (L13, L14 and L26) exhibited low rates of protein reduction (up to 6%), whereas the protein contents in landraces L1 and L3 were almost similar to the control. On the other hand, changes in POD activity varied from approximately 80% stimulation to 20% of reduction. Stimulation was observed in about 2/3 of the samples. Apart from genotype L17 which exhibited the highest suppression of POD activity (up to 19.6%), four genotypes (L3, L4, L13 and L21) exhibited about a 10% reduction in POD activity, whereas in three landraces (L14, L15 and L18) less than 5% reduction was observed in comparison to control plants.

Correlations within seedling traits

Correlations between the percentage changes of the control in all seedling growth parameters were highly significant ($P \leq 0.001$) and positive for both roots and shoots (Table 3). Correlation analysis of the root proline content changes (percentage of control) with growth parameters revealed a significant negative correlation with changes in L, FW and DW ($P \leq 0.05$, $P \leq 0.001$ and $P \leq 0.01$, respectively). Similar trends were found in the changes in the same parameters in shoots ($P \leq 0.01$).

Highly significant and negative correlations were found between the changes in the protein contents and L, FW and DW in roots ($P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.05$, respectively). Interestingly, a significant and positive correlation ($P \leq 0.01$) was observed between changes in the protein and proline contents. Correlations between POD activity and changes in growth parameters were significant and positive, and significant ($P \leq 0.05$) and negative with the changes in the proline content (Table 3).

Grain yield and its correlations with seedlings traits and stress tolerance index

The combined occurrence of water shortage and high temperatures during the vegetative period, along with the application of higher plant density, resulted in a reduction in the grain yield in all of the maize landraces. Compared to optimal growing conditions (OC), the reduction of grain yield under HD ranged from 21.6% to 43.7% depending on the genotype. Seven genotypes (L1, L3, L14, L15, L17, L25 and L26) achieved the highest grain yield under both OC and HD conditions, thus having the highest STI (Fig. 1).

Correlation analyses between grain yield obtained under OC and changes in seedling root L, FW and DW showed significant ($P \leq 0.05$) and negative correlations (r=-0.384*, r=-0.460*, r=-0.411*, respectively). Moreover, correlations between the measured growth parameters of seedling roots and grain yields obtained under HD conditions were significantly higher ($P \leq 0.01$) as compared to those under OC (Fig. 2A). Similar correlations were found for changes in seedling shoot L and DW and grain yields for both OC and HD, being significant and negative ($P \leq 0.01$ and $P \leq 0.05$, respectively), yet slightly weaker under HD conditions (Fig. 2B).

Table 2. The effect of PEG treatment on the proline content (μg g^{-1} FW), peroxidase (POD) activity (μmol ferulic acid oxidized mg^{-1}prot. min^{-1}) and protein content (mg prot g^{-1} FW) in the soluble fractions of seedling roots from maize landraces (Ls).

Genotypes	Proline		POD activity		Protein content	
	Control	Treatment	Control	Treatment	Control	Treatment
LL1	27.66±2.1^{kl}	74.32±4.3^{fg}	5.36±0.1^{hij}	5.91±0.2^{hij}	3.02±0.0^{f}	3.00±0.1^{d}
LL2	35.65±0.7^{ghij}	36.61±0.3^{n}	3.61±0.2^{mn}	4.74±0.1^{klm}	3.81±0.0^{b}	2.99±0.1^{d}
LL3	26.88±0.8^{klm}	62.37±2.8^{h}	7.45±0.3^{e}	6.68±0.1^{fghi}	2.71±0.1^{h}	2.69±0.0^{f}
LL4	33.63±2.9^{ij}	45.20±1.1^{k}	5.63±0.2^{fghi}	4.97±0.3^{jkl}	3.03±0.1^{f}	3.14±0.2^{c}
LL5	31.23±1.7^{jk}	36.37±5.7^{no}	6.02±0.3^{fg}	8.38±0.3^{de}	2.46±0.1^{j}	1.74±0.0^{l}
LL6	40.35±8.1^{efg}	31.59±7.1^{o}	5.29±0.0^{ij}	6.15±0.1^{ghi}	2.88±0.0^{g}	2.40±0.0^{h}
LL7	37.45±0.3^{fghi}	39.42±2.2^{mn}	5.58±0.2^{ghij}	9.09±0.8^{d}	2.75±0.1^{h}	2.38±0.2^{hi}
LL8	39.01±7.5^{fgh}	43.98±5.1^{klm}	4.48±0.2^{kl}	6.84±0.0^{fgh}	3.93±0.1^{a}	2.83±0.0^{e}
LL9	18.82±0.7^{n}	18.86±1.2^{q}	6.88±0.1^{e}	12.19±0.6^{c}	2.61±0.0^{i}	1.77±0.1^{l}
LL10	23.14±6.0^{lmn}	25.43±3.5^{p}	8.22±0.3^{d}	12.60±0.1^{c}	2.34±0.1^{k}	1.67±0.0^{l}
LL11	51.80±7.4^{bc}	69.84±1.2^{g}	5.94±0.1^{fgh}	6.99±0.1^{fg}	3.67±0.1^{c}	3.16±0.0^{c}
LL12	25.32±0.6^{lm}	43.71±0.6^{klm}	16.46±1.5^{c}	24.87±2.8^{a}	1.62±0.1^{m}	1.21±0.1^{n}
LL13	47.14±4.5^{cd}	78.29±0.6^{ef}	21.88±0.8^{a}	19.78±1.9^{b}	1.37±0.0^{n}	1.33±0.1^{m}
IL14	45.32±1.1^{de}	95.54±0.8^{b}	5.17±0.3^{ij}	4.98±0.2^{jkl}	2.51±0.2^{ij}	2.35±0.0^{hi}
IL15	39.52±1.4^{fg}	85.45±1.3^{c}	3.17±0.1^{no}	3.02±0.1^{n}	3.59±0.0^{c}	3.74±0.1^{a}
IL16	52.16±1.6^{b}	57.52±1.6^{i}	2.85±0.0^{o}	3.83±0.1^{mn}	3.15±0.0^{e}	1.97±0.0^{k}
IL17	36.72±1.3^{ghi}	79.94±2.9^{de}	6.20±0.1^{f}	4.99±0.1^{jkl}	1.99±0.0^{l}	2.30±0.0^{ij}
IL18	42.03±0.6^{ef}	44.77±1.5^{kl}	6.19±0.1^{f}	6.19±0.2^{ghi}	2.91±0.0^{g}	2.94±0.0^{d}
IL19	22.03±1.8^{mn}	40.11±1.2^{lmn}	7.00±0.3^{e}	7.55±0.2^{ef}	2.47±0.1^{j}	2.22±0.0^{j}
IL20	83.67±3.1^{a}	103.62±1.5^{a}	4.12±0.0^{lm}	4.54±0.1^{lm}	3.80±0.0^{b}	3.45±0.1^{b}
IL21	34.37±2.0^{hij}	47.75±2.5^{jk}	6.95±0.2^{e}	6.18±0.0^{ghi}	2.27±0.0^{k}	2.51±0.0^{g}
IL22	51.92±4.2^{bc}	72.55±6.6^{g}	5.57±0.1^{ghij}	6.29±0.1^{ghi}	3.44±0.0^{d}	2.64±0.0^{f}
IL23	45.33±1.5^{de}	51.43±1.9^{j}	6.20±0.2^{f}	6.66±0.2^{fghi}	2.75±0.0^{h}	2.35±0.1^{hi}
IL24	51.92±1.3^{bc}	83.87±2.3^{cd}	4.33±0.1^{l}	5.73±0.1^{ijk}	3.04±0.0^{f}	2.39±0.0^{hi}
IL25	39.85±0.7^{fg}	93.77±1.2^{b}	5.00±0.1^{jk}	6.91±0.1^{fgh}	3.11±0.0^{ef}	2.53±0.0^{g}
IL26	50.51±4.7^{bc}	103.54±8.3^{a}	17.26±1.1^{b}	19.45±0.4^{b}	1.30±0.1^{n}	1.22±0.0^{n}
CV %	8.90	5.64	5.92	8.71	2.49	2.98
$LSD_{0.05}$	4.983	4.781	0.586	1.018	0.099	0.099

The analyses were performed in four measurements (n=4) and the results were presented as the mean±SE. Means in a column followed by the same letter were not significantly different ($P \leq 0.05$) according to the LSD test. *LL – local landrace; IL – introduced landrace.

Table 3. Correlation analysis between changes (% of control) in morphological, physiological and biochemical parameters measured in roots (below the diagonal) and in shoots (above the diagonal) of selected maize landraces at the early seedlings stage.

Traits	Length	Fresh weight	Dry weight	Proline	Proteins
Length	-	0.840***	0.838***	-0.598**	not measured
Fresh weight	0.729***	-	0.966***	-0.591**	not measured
Dry weight	0.664***	0.883***	-	-0.574**	not measured
Proline	-0.485*	-0.632***	-0.562**	-	not measured
Proteins	-0.550**	-0.634***	-0.408*	0.548**	-
POD/prot	0.480*	0.607***	0.403*	-0.467*	-0.836***

*, **, *** – correlations are significant at 0.05, 0.001 and 0.001 probability levels, respectively.

Correlation analyses of the changes in seedling root proline contents and the grain yield from both experimental sets revealed highly significant ($P \leq 0.001$) and positive correlations (r=0.725*** for OC), although they were slightly weaker under HD (Fig. 3). A similar trend in the correlations was determined between the changes in the shoot proline content and grain yield, being significantly higher under HD (r=0.545**) in comparison to OC conditions (r=0.392*).

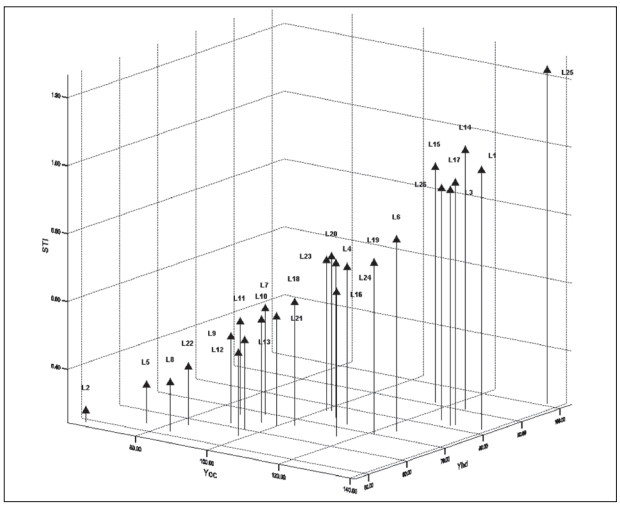

Fig. 1. Three-dimensional plot of evaluated maize landraces (Ls) based on grain yield achieved under optimal (OC) and the combination of drought and high-density stress conditions (HD), and the stress tolerant index (STI). L1-L13 – local landrace; L14-L26 – introduced landrace.

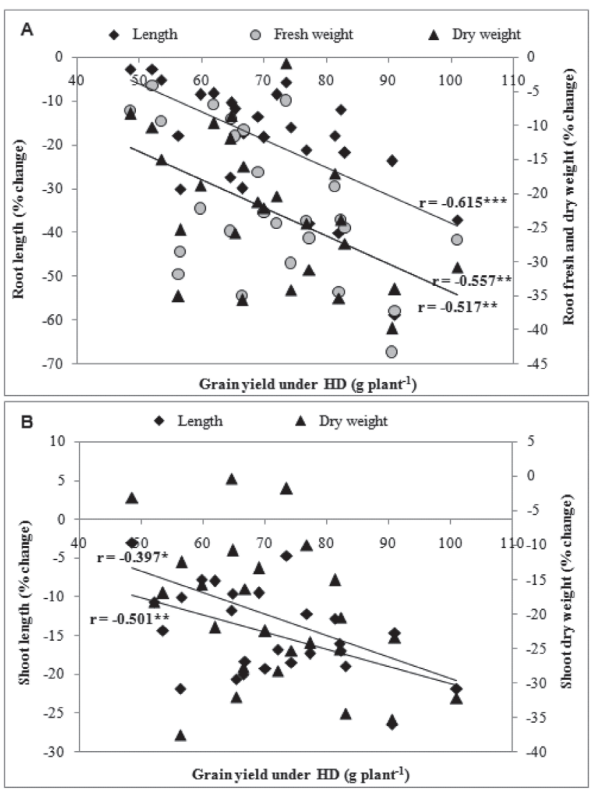

Fig. 2. Correlations between grain yield obtained under a combination of drought and high-density stress conditions (HD), and the % change (relative to the control) in root (**A**) and shoot (**B**) growth parameters (length, FW and DW) of maize landraces at the early seedling stage. *, **, *** – correlation is significant at 0.05, 0.001 and 0.001 probability levels, respectively.

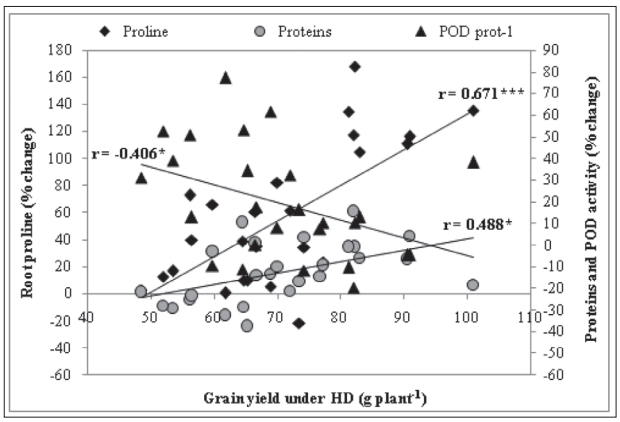

Fig. 3. Correlations between grain yield obtained under a combination of drought and high-density stress conditions (HD) and the % change (relative to the control) in proline content, peroxidase (POD) activity and the protein content in the soluble fraction measured in roots of maize landraces at the early seedling stage. *, *** – correlation is significant at 0.05 and 0.001 probability levels, respectively.

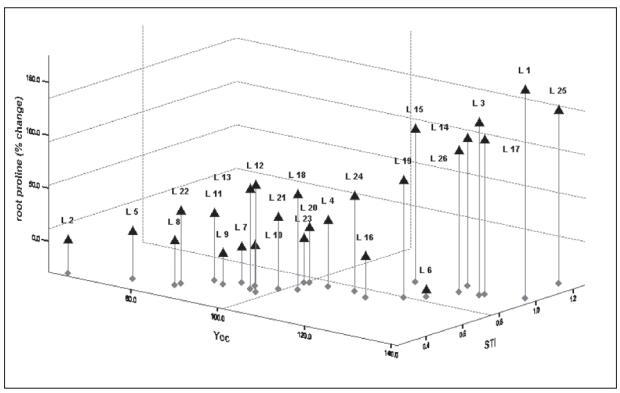

Fig. 4. Three-dimensional plot based on the grain yield achieved under optimal conditions (OC), stress tolerant index (STI) and the % change (relative to the control) in root proline content of maize landraces (Ls) at the early seedling stage. L1-L13 – local landrace; L14-L26 – introduced landrace.

In comparison to the grain yield under OC (r=0.454*), a more pronounced significant and positive correlation ($P \leq 0.05$) was observed between changes in the protein content and grain yield under HD conditions (Fig. 3). Changes in POD activities in the soluble fraction revealed a significant ($P \leq 0.05$) and negative correlation with the grain yield in plants that were grown under HD conditions (Fig. 3). Also, we observed the same grouping of the most drought-tolerant genotypes according to the changes in the seedling root proline contents and grain yields achieved under OC and STI (Fig. 4).

DISCUSSION

Whether they were grown in hydroponics or in the field, inhibition of root and shoot growth in response to a water deficit was reported in different plant species [23]. The adverse effect of water deficit (i.e. applied osmotic stress) on seedling length could be due either to a decrease in cell elongation resulting from possible suppression of growth promoting hormones, and/or due to a blocking up of xylem and phloem vessels that hinders any translocation [24,25]. In addition, PEG-induced osmotic stress led to a decrease in both fresh and dry weights of each genotype, and the reduction could largely be due to the loss of water, which contributes to the FW. Evaluation of the physiological characteristics in 42-day-old maize and triticale seedlings grown under a water deficit in the field and their comparison with the drought-tolerance index based on grain yield (DTI_{GY}) revealed highly significant and negative correlations between root and shoot dry weight and grain yield (r=-0.793*** for root DW and r=-0.706 for shoot DW, respectively) [23]. This is in agreement with the fact that positive relationships between root ramification and yield under drought have not always been established and that a significant association between lower root mass and increased growth was observed under drought in a tropical maize population [26]. In our experiment, the same trend between changes in seedling root length and grain yield obtained under HD is consistent with previously reported findings that genotypes with a poorer early root development grew better under drought conditions than those with a better developed root at the seedling stage [27]. Since growth is generally an energy-requiring process, growth inhibition in the maize landraces can be considered to represent an adaptive mechanism that allows plants to withstand abiotic environmental stress.

PEG-induced osmotic stress led to an increased proline content in the majority of tested landraces, being more pronounced in seedling roots. Proline accumulation during osmotic stress is mainly linked to its increased biosynthesis and reduced degradation. In maize seedlings, these processes were shown to be regulated by H_2O_2 as a signaling molecule, which caused proline accumulation [28]. Significant negative correlations between changes in seedling growth parameters and the proline content support a direct correlation between the degree of osmotic stress and proline accumulation. This is in line with previous findings that the level of proline content in plants increased in parallel with the severity of environmental stress [29]. Based on our data obtained in the early developmental stage, the increased proline accumulation that is observed during osmotic stress could act to save energy by inhibiting seedling growth and as a readily utilizable source of energy and amino groups once stress is relieved. Moreover, significant and negative correlations between changes in seedling growth parameters and proline content ($P \leq 0.01$), as well as the highly significant and positive correlation between the changes in the root and shoot proline contents and grain yields under more intensive stress (HD) in the field, indicate that the increased capacity for proline synthesis during the early seedling stage could represent a stimulatory adaptive mechanism for overcoming limited water-supply conditions. Our findings are in agreement with those for screening wheat genotypes for drought tolerance, where the presence of a positive correlation between the proline content and grain yield suggests that the proline content remains an important trait in enhancing the capacity of genotypes to optimize grain yields under drought stress [30].

In previous studies of peroxidase activity, it has been shown that PODs from maize root tissue use H_2O_2 to oxidize a variety of phenolics [31]. By utilizing ferulic acid as a substrate in the crosslinking of the cell wall polymers that contributes to cell wall stiffening, PODs play a role in growth regulation. In our experiment, ferulic acid was used as a natural substrate for POD assay activity [32]. Our results show that changes in POD activities ranged from approximately 20% reduction to 80% stimulation. The decrease in POD activity in the soluble fraction of maize roots treated with the same PEG concentration was previously reported for maize seedlings grown under similar conditions [33,34]. In contrast, stimulated POD activity was observed in roots of different plant species under water deficit or under salt stress [35,36]. The reported positive correlation between changes in soluble POD activities in the roots of wheat genotypes with abiotic stress tolerance indicated the presence of adaptation to induced moderate drought stress in evaluated genotypes [13]. A significant positive correlation between changes in POD activity and changes in root growth parameters, as well as a significant negative correla-

tion between the changes in proline content and POD activity ($P \leq 0.05$), indicated that genotypes with a less-pronounced root growth reduction under osmotic stress exhibited increased POD activity and a lower level of proline accumulation. The relation between POD activity and proline content could be explained by H_2O_2-induced proline accumulation [28]. H_2O_2 is localized not only in the cytosol, vacuoles, cell wall and extracellular space, but also in the mitochondria and chloroplasts, and class III PODs are involved in the regulation of its concentration [37]. Lower activities of soluble POD under osmotic stress could be the consequence of an increased level of H_2O_2 in the cytosol that could lead to the activation of proline biosynthesis. Also, an increased concentration of H_2O_2 in the mitochondria could contribute to the inhibition of proline degradation due to its inhibitory effect on enzyme proline dehydrogenase [28]. Moreover, considering the displayed performances in yields, the higher drought tolerance of adult plants might refer to lower changes in POD activities under osmotic stress conditions at the early seedling stage. A negative correlation between POD activity and grain yield that was established in a study on different bread wheat varieties pointed to the possibility of developing a dwarf plant type with a low POD activity and higher grain yield [38], which is in accordance with our findings.

The soluble protein content, as one of the indicators of oxidative stress, was reported to decrease or remain unchanged in plants subjected to osmotic stress [39,40]. On the other hand, many different proteins are synthesized and/or accumulate in response to osmotic stress, including dehydrins, heat shock proteins and detoxifying enzymes [41]. As a result of the applied osmotic stress, changes in protein content varied among genotypes, being reduced by about 30%, remaining similar to the control or increased (by about 15%), thereby excluding serious oxidative damage of proteins in those genotypes. Variations in the soluble protein content under osmotic stress were consistent with previous findings [42,43]. Protein degradation might be the result of increased activity of proteases or other catabolic enzymes under drought stress, or due to fragmentation of proteins as a consequence of the toxic effects of ROS, resulting in a reduction of the protein content [44]. Although the relationship between proline accumulation and protein degradation has been widely reported on, in our study we found a significant positive correlation ($P \leq 0.01$) between changes in free proline and the protein content in the soluble fraction [45]. We also observed that most of the genotypes with a markedly increased proline content in PEG-treated roots had a similar, increased soluble protein content as compared to the control, suggesting that they possessed more effective dehydration and drought avoidance mechanisms.

The responses of plants to drought observed under field conditions are generally much more complex than those measured under controlled laboratory conditions because of other factors accompanying the water deficit that influence the nature of the stress response. Differences in performance and yield potential could be associated with the variability in quantitative traits and processes, which are more expressed under stress conditions. Previously it was reported that the yield potential (including heterosis) is a constitutive trait and that in drought-tolerant populations the reduction in yields was less than 50% [46]. Moreover, the average reduction in grain yield in maize hybrids under drought as compared to well-watered conditions is within a range of 20-30% [47]. Compared to optimal growth conditions, the response of individual landraces to HD stress was within this range, i.e. 9 landraces exhibited less than 30% of decrease in grain yield, while the remainder of the tested landraces exhibited a reduced grain yield of 30-45%. A high yield potential could be achieved under optimal and conditions of mild environmental stress; however, under more intensive stress (HD stress in the present study), only the germplasm with stress-adaptive genes maintained a stable yield [48]. The STI was projected as a selection criterion that identifies genotypes with stress-tolerance potentials. Thus, the highest STI values found in the local landraces L1 and L3, as well as in the introduced landraces, L14, L15, L17, L25 and L26, pointed to the superiority of these genotypes as regards both high yield potential and stress tolerance. Although they originated from different countries, all of the introduced landraces (L14-L26) were previously tested and chosen as being well adapted to temperate climatic conditions and as drought tolerant [49].

At the seedling stage, all of the genotypes with the highest STI expressed a highly pronounced increase

in the proline content after PEG-induced osmotic stress, especially in seedling roots, as well as growth reduction. In addition, the introduced landraces L14 and L15 exhibited the highest suppression of POD activity in response to osmotic stress. Our findings are in agreement with previous findings that some of the genes that contribute to seedling drought resistance can also contribute to later stage tolerance. According to [16], root quantitative trait loci (QTLs) from seedlings grown in hydroponics were related to QTLs of field-grown maize under drought stress, indicating constitutive trait expressions. Although all of the evaluated genotypes are valuable sources for drought-tolerance breeding programs, it should be emphasized that L14 and L15 are already included in commercial maize breeding because of their good drought tolerance and ability to combine with three heterotic groups (*BSSS*, *Lancaster* and *Iodent*) [49].

In conclusion, the overall performance of the tested genotypes under different water-supply conditions revealed the importance of root and shoot characteristics during the early seedling stage for drought tolerance in maturity. Thus, the lower level of changes in POD activity and the increased proline content after exposure to osmotic stress at the early seedling stage could be considered as useful indices to facilitate selection efficiency for drought tolerance and selection based on yield alone.

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Author contributions: NK, VA and VHTŠ designed the research; NK and JM performed the laboratory experiments; VB and VA performed the field experiments; VB and JS analyzed the data; NK wrote the paper. All authors read and approved the final manuscript.

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