Contents of Total Phenolics and Ferulic Acid, and PAL Activity during Water Potential Changes in Leaves of Maize Single-Cross Hybrids of Different Drought Tolerance

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Introduction

Drought is one of the stress factors causing an increase in the emission of blue fluorescence from leaf tissues, after they are excited by visible light or UV radiation (Schweiger et al. 1996, Hura et al. 2006, 2007b). The presence of phenolic compounds is the reason behind the formation of the blue fluorescence (Lichtenthaler and Schweiger 1998), mainly ferulic acid belonging to biochemically active phenylpropanoids. By absorbing radiation, the phenolic compounds transform short-wave, high-energy and highly destructive radiation into the blue radiation of a longer wavelength and, therefore, is less destructive to the cellular structures of the leaf, including the photosynthetic apparatus (Bilger et al. 2001). Additionally, phenolic compounds possess an adequate chemical structure capable of scavenging free radicals, and they have also been found to be effective antioxidants (Blokhina et al. 2002).

Drought stress increases the sensitivity of the photosynthetic apparatus to the radiation reaching it (García-Plazaola and Becerril 2000). Thus, water deficit in the leaf tissue can induce protective mechanisms involving the synthesis of phenolic compounds and subsequently, the neutralization of the radiation by its absorption and transformation into blue fluorescence. Therefore, phenolics can function as a filter absorbing radiation and limiting the excitation of chlorophyll during conditions unfavourable for the photosynthetic apparatus (Nogués and Baker 2000).

The aim of this study was to determine whether resistance and/or sensitivity to drought stress can be attributed to the level of phenolic compounds in the leaves of maize genotypes. The experiments were carried out on seedlings of three maize genotypes characterized by different levels of drought resistance. Experiments with three periods of drought were conducted (8, 11 and 14 days), to obtain plants with different levels of water potential in leaves, which induced changes in the total phenolic content and ferulic acid, and L-phenylalanine ammonia-lyase (PAL) activity. Only for the drought-resistant genotype Tina, was the low water potential found to be correlated with the high level of the total phenolic content and ferulic acid, which is the main source of blue fluorescence emissions. Moreover, only for Tina were the highest intensities of blue fluorescence emission correlated with the low water potential in leaves. The phenolic compounds present in leaf tissues can protect the deeper situated mesophyll, by absorbing light reaching the leaf and transforming it into a blue fluorescence. Phenolic compounds can, in this way, function as photoprotectors limiting the excitation of chlorophyll during conditions of water deficit in leaves.

Abstract

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synthesis of phenolic compounds (Strack 1997), was also estimated. At the leaf level, the emission of blue fluorescence, the main source of which is ferulic acid, was analysed. Moreover, measurements of the emissions of red fluorescence originating in chlorophyll $a$ of photosystem II (PSII) (Buschmann and Lichtenthaler 1998), which enabled one to determine the overall state of the photosynthetic apparatus during drought stress, were performed.

Materials and Methods

Plant materials

Three maize (Zea mays L.) single-cross hybrids (Ankora – a sensitive genotype; Nova – a moderately resistant/sensitive genotype; Tina – a resistant genotype) were included in this study. Each of the chosen genotypes had a different drought susceptibility index (DSI), calculated by Grzesiak (2004) and Grzesiak et al. (2007) according to Fischer and Maurer (1978). The genotype Tina was classified as drought-resistant (DSI = 0.381), Ankora as drought-sensitive (DSI = 0.650) and Nova as moderately resistant/sensitive (DSI = 0.562).

Plant growth conditions

Maize plants were grown in an air-conditioned greenhouse chamber at a temperature 23/18 °C (±2 °C) day/night, with a 16-h photoperiod, 50 ± 5 % relative humidity and at a light intensity of 450 μmol m$^{-2}$ s$^{-1}$. Plants were grown in Mitscherlich pots (six plants per pot) of 20 cm internal diameter and 20 cm height, filled with a mixture of soil, peat and sand (1 : 1 : 3, v/v/v).

Drought treatment was initiated by the withdrawal of the water supply at the five-leaf stage, corresponding to 3-week-old plants. For the control plants 70 % of field water capacity (FWC) and for water-stressed plants 30–35 % of FWC for 2 weeks were applied. The pots were weighed every day, and the amount of the water lost through transpiration was refilled to maintain the appropriate weight of the pots for each treatment. After 8, 11 and 14 days of stress, 10-cm-long fragments from the middle part of the sixth leaf were taken for measurements. The plants were irrigated with a full-strength Hoagland’s nutrient solution once a week. Each treatment (control, drought) included 10 pots. In total, there were 60 samples within the treatment for each genotype.
The analyses of both physiological and biochemical parameters for each genotype were performed in five replicates.

Water potential

The measurements were completed with a dew point microvoltmeter (model HR-33T with C-52 sample chambers; Wescor, Inc., Logan, UT, USA). Leaf discs (diameter 0.5 cm) were cut at noon from the middle part of fully expanded leaves and immediately placed inside the psychrometer chamber and left to balance the temperature and water vapour equilibrium for 60 min, before water potential measurements.

Spectrofluorescence

Fluorescence spectra were measured using a Perkin-Elmer LS 50B spectrofluorometer (Perkin Elmer, Norwalk, CT, USA). Emission spectra of red fluorescence were recorded between 650 and 800 nm. The leaf samples were excited at 450 nm, and excitation and emission slit widths were set at 10 nm. Emission spectra of blue fluorescence were recorded between 380 and 600 nm. The excitation wavelength was set at 350 nm. The slit widths for excitation were set at 15 nm and for emissions at 20 nm.

PAL activity

The measurement of PAL activity was carried out according to the modified methods described by Peltonen and Karjalainen (1995). All procedures were carried out at +4 °C. The reaction mixture contained 2.5 ml of a 0.2 % solution of L-phenylalanine in 50 mM Tris-HCl (pH 8.5) and 0.5 ml of supernatant. The incubation of the reaction mixture was set for 24 h at 38 °C and the absorbance at 290 nm was measured. The enzyme activity was expressed as nanograms of cinnamic acid produced during 1 min per mg of the protein. In enzyme assays the protein contents were determined by the method of Bradford (1976).

Phenolics analysis

For both total phenolics and ferulic acid measurements, the lyophilized material was homogenized in 80 % ethanol. The total phenolic content was determined using the Folin-Ciocalteau method of Singleton and Rossi (1965). The absorbance was measured at 760 nm and chlorogenic acid was used as a standard.

Ferulic acid contents were analysed with a PerkinElmer LS 50B spectrofluorometer. Before measurements, chlorophyll was removed by several extractions with hexane until no green colour was visible. The samples were excited at 243 nm and the detection was carried out at 434 nm. The slit widths for both excitation and emission monochromators were adjusted to 10 nm.

Statistical analysis

STATISTICA 5.0 software for Windows was used. Correlations between measured parameters were tested at a probability of \( P < 0.05 \). The line in the figures represent the best linear adjustment.

Results

Emission of blue and red fluorescence

Figure 1 shows the correlation between the intensity of blue fluorescence (Fig. 1a), red fluorescence (Fig. 1b)
and the water potential in leaves of maize hybrids. The values indicated in the figure represent the measured parameters and the noticeable differences are the result of the varied length of the drought periods (8, 11 and 14 days). The above-mentioned treatments were applied to obtain plants of a different level of the water potential in leaves which induced changes in blue and red fluorescence emission, in the content of phenolic compounds and ferulic acid, and PAL activity. For Ankora, the highest intensity of blue fluorescence was detected at medium and high values of the water potential (Fig. 1a). For Tina, the relationship was the opposite, with the highest values of the emission of blue fluorescence observed at the low water potential. The positive correlation obtained for Nova was statistically insignificant. A statistically significant correlation between the intensity of red fluorescence and leaf water potential, for all maize genotypes examined, was observed (Fig. 1b). In terms of the dependence of the intensity of red fluorescence on the emission of blue fluorescence, a statistically significant correlation was found for Tina (Fig. 2). For Ankora, the low intensity of blue fluorescence was significantly correlated with the high emission of red fluorescence. In the case of Nova, the dependence was statistically not significant.

**Phenolics and ferulic acid content**

Statistically significant associations between the total phenolic content and water potential were observed (Fig. 3a). For Tina, a decrease in the leaf water potential induced an increase in the total phenolic content. However, in Ankora and Nova, a decrease in the leaf water potential did not increase the total phenolic content. Similarly, for both Ankora and Nova, a decrease in the water potential was accompanied by a decrease in the content of ferulic acid (Fig. 3b), and the associations obtained were statistically significant. For Tina, the high content of ferulic acid was accompanied by low water potential. Moreover, an increase in the content of ferulic acid was significantly correlated with an increase in the total phenolic content (Fig. 4).

For all hybrids studied, a statistically significant correlation between the total phenolic content and emission of blue fluorescence was observed (Fig. 5a). An increase in the intensity of blue fluorescence was accompanied by an increase in the total phenolic content. Additionally, for Tina, a statistically significant correlation was found between the content of ferulic acid and the intensity of blue fluorescence (Fig. 5b). A similar result was obtained for the Nova and Ankora genotypes.

![Fig. 3](image-url)  
**Fig. 3** Correlations between total phenolics content (a), ferulic acid content (b) and leaf water potential for leaves of maize single-cross hybrids with different drought tolerance. *Statistically significant correlations between measured parameters at a probability of P < 0.05.
genotypes, although for Ankora the correlation was not statistically significant.

An increase in the content of ferulic acid corresponded statistically significantly to a decrease in the emissions of red fluorescence in the Ankora genotype (Fig. 6a). For Tina, the observed dependence had a statistically significant correlation, where an increase in the content of ferulic acid was correlated with an increase in red fluorescence emissions. Similar results were obtained for the dependence of the total phenolic content and the intensity of the red fluorescence (Fig. 6b).

PAL activity

A correlation between the activity of PAL and leaf water potential of the genotypes studied is shown in Fig. 7. For all the hybrids, statistically significant relations were obtained. For Nova and Tina, the high activity of PAL was accompanied by the low water potential in leaves. However, for Ankora, the low activity of the enzyme was correlated with the low water potential in leaves. For Ankora, statistically significant correlations were found between the total phenolic content and the PAL activity (Fig. 8a). Only in the case of Ankora and Tina, was the activity of PAL significantly correlated with an increase in the content of ferulic acid (Fig. 8b). In the case of Tina, a statistically significant correlation between the activity of PAL and the intensity of blue fluorescence was observed (Fig. 9) and high values of blue fluorescence intensity were accompanied by higher activity of PAL.

Discussion

It is well established that the origin of blue fluorescence are phenolic compounds, and that ferulic acid predominately occurs in leaf tissues (Schweiger et al. 1996, Lichtenthaler and Schweiger 1998, Meyer et al. 2003). In the present study for all maize genotypes, an increase in the emission of blue fluorescence corresponded to an increase in total phenolic content (Fig. 5a) as well as ferulic acid (Fig. 5b). These data are in agreement with the observation that phenolics in the chlorophyll-free epidermis can attenuate light reaching the leaf by its absorption before transformation into blue fluorescence (Bilger et al. 1997). Other investigations have shown that, phenolic compounds can function as photoprotectors limiting light penetration into the mesophyll cells and in this way reduce the excitation of chlorophyll during the leaf water deficit (Nogués et al. 1998, Nogue´s and Baker 2000). The reduction in leaf water potential observed here induced an increase in emissions of blue fluorescence (Fig. 1a), total phenolic content (Fig. 3a) and ferulic acid levels (Fig. 3b) only for the resistant genotype Tina. Lang et al. (1996) discovered that when the water content in green tobacco leaves decreased below 82 %, an increase in the emission of blue fluorescence resulted. In another experiment, Lang et al. (1996) studied the effect of simultaneous application of drought, high temperature and irradiation on emission of blue fluorescence from the leaves of Rhododendron. The observed emission of blue fluorescence was attributed to the increase in the concentration of phenolic compounds, analysed with a spectrophotometric method. In our experiment, this accumulation of phenolic compounds may indicate activated defence reactions in the drought-resistant genotype Tina. Moreover, these phenolic compounds, as results showed, may originate from activation of the PAL (Fig. 7), the key enzyme in the synthesis of phenols (Strack 1997, Lee et al. 2007). These findings are consistent with our previous study (Hura et al. 2007a), which found an increase in PAL activity and accumulation of high levels of phenolics in some drought-resistant genotypes of winter triticale.

The biochemical meaning of phenolics as antioxidants should also be considered here. The production of
Fig. 5 Correlations between total phenolics content (a), ferulic acid content (b) and emission of blue fluorescence for leaves of maize single-cross hybrids with different drought tolerance. *Statistically significant correlations between measured parameters at a probability of P < 0.05.

Fig. 6 Correlations between the emission of red fluorescence and ferulic acid content (a), total phenolics content (b) for leaves of maize single-cross hybrids with different drought tolerance. *Statistically significant correlations between measured parameters at a probability of P < 0.05.
reactive oxygen species (ROS) under drought stress was observed in several investigations (Bartoli et al. 1999, Navari-Izzo and Rascio 1999, Alscher et al. 2002, Noc-tor et al. 2002, Luna et al. 2005, Zhang and Nan 2007). ROS production by the photosynthetic electron transport chain is exacerbated by drought (Sairam and...
Saxena 2000, Bagci et al. 2007), as light levels that are optimal for photosynthesis in well-watered plants become excessive for the photosynthetic apparatus in water-deprived plants (Noctor et al. 2002, Luna et al. 2005). Plants respond to diverse environmental signals in order to survive stresses such as drought, and as suggested by Sgherri et al. (2004), phenolics can be involved in scavenging ROS during dehydration and releasing oxidative stress during recovery. Under increasing conditions of water deficit, it was observed that the emission of red fluorescence is enhanced for all maize genotypes examined (Fig. 1b).

Based on these results, two tentative mechanisms to explain such phenomenon can be given. For drought-sensitive genotypes, the emission of red fluorescence may occur at the expense of the photosynthetic conversion of light quanta and could involve functional disturbances of PSII and light-harvesting complex (LHC) and changes in the activity of electron carriers (Schweiger et al. 1996, Tambussi et al. 2000). On the other hand, high emissions can also be a consequence of the discharging of high-energy states, produced in the photosynthetic apparatus and adaptation of the drought-resistant genotype Tina to drought conditions (Lang et al. 1996, Buschmann et al. 2000). Studies carried out in vivo conditions proved that water stress resulted in injuries to both the oxygen-producing complex of PSII and the reaction centre of PSII. Giardi et al. (1996) and He et al. (1995) linked structural injuries to the PSII with the degradation of D1 proteins. Only for the drought-resistant hybrid Tina, was an increase in the emission of red fluorescence correlated with an increase in the content of the total phenolics and ferulic acid (Fig. 9a,b).

Based on measurements of the concentration of phenolics, blue and red fluorescence under drought treatment, it seems that for the resistant genotype Tina, the positive correlation between emission of the blue and red fluorescence is in part a result of the re-absorption of blue fluorescence by chlorophylls and carotenoids possessing blue absorption bands (Lang et al. 1996). We suggest that the photosynthetic apparatus in the resistant genotype Tina was able to partially utilize blue fluorescence, in the process of photosynthetic light conversion and the re-emission as red fluorescence.

To summarize, for the resistant maize genotype, the observed increase in the total phenolic content, including ferulic acid, as an effect of a decrease in the water potential in leaves, could be the result of protective mechanisms being triggered. Phenolic compounds can play the role of a filter protecting the photosynthetic apparatus against excess radiation and can be effective antioxidants. Their increased level and synthesis during drought can be an indicator of the resistance to drought stress. More studies are needed involving testing a greater number of genotypes of different resistance levels to drought, during various stages of a plant’s development.

References


