Non-Optimal Growth Temperatures and Antioxidants in the Leaves of *Sorghum bicolor* (L.) Moench. I. Long Term Acclimation

By

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Summary


The foliar antioxidant status and the photosynthetic capacity were compared in two sorghum [*Sorghum bicolor* (L.) Moench] cultivars of different agroclimatic provenance, namely Aralba and ICSV 112, which were grown at near-optimal, 27±0.3 °C, suboptimal, 17±0.4 °C, or supraoptimal, 37±0.1 °C, temperatures. Both non-optimal growth temperatures, although unable to cause visible symptoms of stress, affected gas exchange parameters and antioxidant levels both in cv. Aralba and in cv. ICSV 112. Compared to controls, plants grown at 17±0.4 °C or at 37±0.1 °C had higher contents of photosynthetic pigment, an increased size of the ascorbate pool and an enhanced monodehydroascorbate reductase activity. On the other hand, suboptimal and supraoptimal growth temperatures, respectively, decreased and increased the glutathione pool and on the capacities of ascorbate- and guaiacol peroxidases, and of catalase. In cv. Aralba, but not in cv. ICSV 112, the expression of superoxide dismutase, in terms of both enzymic activity and mRNA transcripts abundance, was downregulated by the growth at non-optimal temperature. Adaptation to non-optimal growth temperature might involve antioxidant responses which could be different in part from those evoked by genuine temperature stress.

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Introduction

In order to evaluate the resulting functional impairment and/or to evoke the maximal expression of adaptative responses, the studies on temperature stress have been frequently conducted by exposing plant material to thermic regimes which are rather distant, both in physical and physiological terms, from those considered optimal for growth (Feierabend & al. 1992, Kraus & Fletcher 1994). Also, many experimental protocols purposely adopted ex abrupto exposure of plant material to disthermia, thus departing to a considerable extent from the real-world circumstances. The massive elicitation of stress responses obtained through the above treatments, however, could hinder the recognition of those early and subliminal adjustment processes allowing plant metabolism to gradually prepare to face adverse thermic regimes. The ubiquitous and perceptive nature of the plant antioxidant systems renders them good candidates to play a role in the abovedicted preparatory responses. Therefore, it is of interest to study the antioxidant status of photosynthetic tissues during the transition from optimal to adverse temperatures, i.e. in the presence of non-optimal temperatures which, though not yet stressing, could act in nature as signals inducing specific paths of metabolic changes. In the present work, the foliar antioxidant status and the photosynthetic capacity were compared in two sorghum [Sorghum bicolor (L.) Moench] cultivars of different agroclimatic provenances, namely Aralba and ICSV 112, which were grown at near-optimal, 27±0.3 °C, suboptimal, 17±0.4 °C, or supraoptimal, 37±0.1 °C, temperatures, under moderate light intensity and ad libitum water and mineral nutrition.

Materials and Methods

The sorghum cultivar Aralba is a low-tannin, intermediate class, commercial F1 hybrid normally grown in Mediterranean environments; the cv. ICSV 112 is a "tan" cultivar developed in India by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). The sorghum seedlings were grown from seeds sown on a mixture of agriperlite and coarse vermiculite (1:1, vol:vol). Water and nutrients were supplied in the form of aerated Hoagland solution (Hoagland & Arnon 1939), given twice a week during seedlings growth. The seeds were germinated under controlled environmental conditions at a constant temperature regime of 27±0.3 °C, a relative humidity of 60±5%, a 16 h photoperiod, and a photosynthetic photon flux density at plant height of 500-550 μmol m⁻² s⁻¹, obtained through a set of Sylvania Grolux wide spectrum incandescent lamps. Established seedlings (5-7 d-old) were either left at the optimal temperature of 27±0.3 °C or gradually exposed, at a 2 °C per day change rate, to the suboptimal temperature of 17±0.4 °C or to the supraoptimal temperature of 37±0.1 °C until the fifth leaf was fully expanded. Each of the above treatments was replicated in three separate experiments. Maize (Zea mays L. cv. Samantha) plants to be used for the extraction of genomic DNA (see below) were grown at 27±0.3 °C under the aforementioned controlled conditions. Non-destructive measurements (see below) were conducted on the third, fourth and fifth fully expanded leaf belonging to 6 representative plants, uniform in size, for each sorghum cultivar. Then, the same leaves were excised at their collar from each individual plant, pooled together, wrapped in aluminum foil, dipped in liquid N₂ within 30 s from excision and finally stored at -80 °C until needed for destructive measurements (see below).
Equipment, protocol, and microenvironmental conditions adopted for measuring gas exchange rates and chlorophyll a fluorescence have been given in a previous paper (BADIANI & al. 1993b). The determinations of: chlorophyll (Chl) and total carotenoids (Car); ascorbic (AsA) and dehydroascorbic (DHAsA) acids; reduced glutathione (GSH) and glutathione disulfide (GSSG); total ascorbate (APX, EC 1.11.1.11) and guaiacol (GPX, EC 1.11.1.7) peroxidases, catalase (CAT, EC 1.11.1.6), DHAsA reductase (DHAR, EC 1.8.5.1), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), GSSG reductase (GR, EC 1.6.4.2), and total superoxide dismutase (SOD, EC 1.15.1.1) activities; water soluble protein content (TSP); thiobarbituric acid-reactive substances (TBARS), and leaf dry weight (DW) were performed following the procedures reported in previous papers (BADIANI & al. 1993a, 1993b). Each analyte was measured at least in triplicate. For each of the above measurements, the same number of sample replicates was adopted at 27±0.3, 17±0.4 and 37±0.1 °C. Total RNA from sorghum leaves was extracted by using the RNA Fast kit (Molecular System, San Diego, CA, USA). Poly(A)+ RNA was purified by using the PolyATtract mRNA Isolation System (Promega, Madison, WI, USA). Both procedures were carried out according to the manufacturer’s instructions. Genomic DNA from sorghum and maize leaves was extracted as reported by D’OVIDIO & al. 1992. DNA and mRNA (8 μg) electrophoretic fractionation, transfer to nylon membrane, prehybridization, hybridization and washing of the nylon filters were performed following standard procedures (SAMBROOK & al. 1989). Southern and Northern blot hybridizations were performed by using as a probe the 711bp EcoR I Sod2.1 insert in pUC12. This full-length cDNA encodes one of the four cytosolic Cu,Zn-isofoms of the enzyme (SOD-2) in maize leaves (SCANDALIOS 1990). pUC12 DNA (50-100 ng) was labelled with digoxigenin by the Polymerase Chain Reaction following the procedure reported by D’OVIDIO & ANDERSON 1994.

Results and Discussion

 Constitutive differences among cv. Aralba and cv. ICSV 112 plants grown at of 27±0.3 °C (optimum) are shown in Table 1. Under non-optimal thermic regimes, the plants of both cvs. lacked any visible symptom of stress or damage and were visually indistinguishable from their control counterparts grown at 27±0.3 °C. Thus, the adopted gradual and limited deviations from the optimal growth temperature apparently failed to cause manifest symptoms of temperature stress. Figure 1 coherently shows that neither reductions of leaf DW and TSP nor pigments bleaching and changes in Chl a fluorescence characteristics (as reported by SCHÖNER & KRAUSE 1990, PAULSEN 1994) occurred in sorghum plants grown at 17±0.4 °C or at 37±0.1 °C. In spite of this, the growth at non-optimal temperatures affected both the gas exchange parameters and the antioxidant status of the sorghum plants (Fig. 1) Unadvertant exposure of the plant material to excess radiative energy, interfering with the S. bicolor responses to non-optimal growth temperature, appeared unlikely. In fact, on a DW basis, the growth at both non-optimal temperatures led to a general increase in the levels of photosynthetic pigments, most conspicuous for Chl b (Fig. 1). Moreover, no temperature-dependent effect was observed on the main Chl a fluorescence characteristics. Indeed, the mean values obtained for the ratios Fₘ/F₀ and Fₐ/Fₘ were similar to those obtained from plants grown at 27±0.3 °C (Fig. 1). These results tend to exclude the occurrence of both photoinhibitory and photooxidative processes and
% change respect to value at 27 °C

17 vs 27 °C[●]
37 vs 27 °C[●]
thus suggest that the observed effects on $P_n$-associated parameters and antioxidants levels were specifically due to the thermic treatments adopted. In previous work on non-hardened seedlings of a C3 Gramineae, *Triticum durum* Desf. cv. Duilio (BADIANI & al. 1993b, PAOLACCI & al. in press), it was found that, compared to the optimum of 25 °C, both a suboptimal (10 °C) and a supraoptimal (30 °C) growth temperature, in the absence of stress symptoms, evoked a common set of antioxidant responses consisting of: a) accumulation of photosynthetic pigments, especially Chl b; b) enhanced consumption of non-enzymic antioxidants, such as AsA and GSH, and c) decrease or steadiness in the extractable capacities of ROS-scavenging enzymes, such as GPX, CAT, and SOD. It was suggested that non-optimal, non-stressing growth temperatures might induce overproduction of ROS, especially $H_2O_2$, dealt with by non-enzymic antioxidants, acting as a first defense line, and without the intervention of ROS-scavenging enzymes. It was also speculated that the above responses might be the first step of a rather efficient biphasic adaptative strategy, aimed both at saving resources in the presence of moderate metabolic perturbations and at producing the maximal compensative effort only if and when it is effectively needed. According to this hypothesis, should a further deviation from the normative growth temperature enhance the risk of oxidative stress, then more robust but more energetically-expensive responses would be required, such as the induction of scavenging- and antioxidant-regenerating enzymes. Indeed, the induction of antioxidant enzymes has been repeatedly reported to be caused by, and to take part in, the acclimation to both low (SCHÖNER & KRAUSE 1990) and high (KRAUS & FLETCHER 1994) temperature stress.

Unlike in *T. durum*, in *S. bicolor* the patterns of antioxidants responses to suboptimal and supraoptimal growth temperatures were mostly divergent (Fig. 1). However, an analysis of these responses reveals that the above biphasic adaptation hypothesis might be still tenable. In fact, moderate hyperthermia, unable to affect the $P_n$ and to stimulate lipid peroxidation (Fig. 1), caused in both sorghum cultivars notable increases in pigments levels, especially Chl b, and in the pool sizes of AsA and GSH, more accentuated for the oxidized forms, DHAAsA and GSSG, respectively (Fig. 1). In further agreement with the *T. durum* results (see above), $H_2O_2$-scavenging enzymes, as well as DHAR, were either unaffected of

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Fig. 1. Comparison between the mean relative changes induced in the leaves of two sorghum cultivars by the growth at 17±0.4 °C or at 37±0.1 °C with the values obtained from control plants grown at 27±0.3 °C made = 100. Each result is the mean ± standard deviation (vertical bars) of the values obtained from third, fourth and fifth fully expanded leaves and measurement replication is the same as reported in Table 1 for each plant parameter. The asterisks denote statistically significant differences from control values (P<0.05). Acronyms for plant parameters are the same as in Table 1.
Table 1. Mean values (± standard deviation of the mean) measured in the leaves of cv. Aralba and cv. 1CSV 112 sorghum seedlings grown at the optimal temperature of 27± 0.3 °C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>cv. Aralba</th>
<th>cv. 1CSV 112</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pn</td>
<td>mmol</td>
<td>15.42 ± 3.63</td>
<td>19.69 ± 6.11</td>
</tr>
<tr>
<td>Pn</td>
<td></td>
<td>1.54 ± 0.27</td>
<td>1.76 ± 0.34</td>
</tr>
<tr>
<td>Pn</td>
<td></td>
<td>33.7 ± 3.6</td>
<td>58.3 ± 3.8</td>
</tr>
<tr>
<td>Py</td>
<td>umol CO2 m-2 s-1</td>
<td>16.66 ± 3.98</td>
<td>18.98 ± 3.07</td>
</tr>
<tr>
<td>Py</td>
<td></td>
<td>18.5 ± 3.0</td>
<td>19.3 ± 3.1</td>
</tr>
<tr>
<td>TSP</td>
<td>nmol CO2 m-2 s-1</td>
<td>139 ± 3.96</td>
<td>178 ± 3.84</td>
</tr>
<tr>
<td>TSP</td>
<td></td>
<td>14.4 ± 3.0</td>
<td>14.7 ± 3.1</td>
</tr>
<tr>
<td>TPFAS</td>
<td>nmol CO2 m-2 s-1</td>
<td>60 ± 6.69</td>
<td>60 ± 6.98</td>
</tr>
<tr>
<td>TPFAS</td>
<td></td>
<td>6.6 ± 0.2</td>
<td>6.9 ± 0.3</td>
</tr>
</tbody>
</table>

n.s. indicates not significant at P = 0.05. For parameters acronyms, see the text.

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drastically depressed in sorghum leaves developed at 37±0.1 °C (Fig. 1). On the other hand, and again sticking to the biphasic adaptation hypothesis, Fig. 1 suggests that the growth temperature of 17±0.4 °C must be, for the species *S. bicolor*, at the boundary between moderate hypothermia and genuine cold stress. In fact, albeit the levels of light intercepting- and protective pigments were enhanced, the $P_n$ was severely decreased and oxidative stress was stimulated, at least by judging from AsA and GSH depletion and TBARS overproduction (Fig. 1). Being unable to cope with the temperature-dependent oxidative stress with the sole "first move", i.e. at the expense of non-enzymic antioxidants (see above), to sustain or even to increase the activities of $H_2O_2$-scavenging enzymes and of DHAR (Fig. 1) could have become mandatory for sorghum plants grown at 17±0.4 °C. The above interpretive framework does not apply to the results obtained for GR, MDHAR and SOD (Fig. 1). The first enzyme tended to reflect the effects of non-optimal growth temperatures on its substrate, namely GSSG, thus being unaffected or increased at 37±0.1 °C and clearly depressed at 17±0.4 °C. The temperature-dependent stimulation of MDHAR activity was dramatic, especially at 37±0.1 °C and in cv. ICSV 112, and deserves further investigation, particularly in the light of the accumulating evidence pointing to the relevance of the monodehydroascorbate radical as the main oxidation product arising from the complex AsA redox chemistry within the plant cell (SANO & ASADA 1994). The effects of non-optimal temperatures on the total SOD activity were mirrored by those on Sod transcripts abundance, albeit in cv. Aralba Sod expression was much more reduced at 37±0.1 °C than at 17±0.4 °C (Fig. 2).

![Fig. 2](image.png)

**Fig. 2.** The effects of different growth temperature on the expression of superoxide dismutase in two sorghum cultivars. Inner panel: typical Northern hybridization signals obtained by using the heterologous cDNA probe Sod2.1 from maize (see text for details); each lane contained 8 μg of total mRNA. Outer panel: total enzyme activity.

Although no attempt was made in the present work to discriminate among the different Sod genes, the results in Fig. 2 confirm the high cellular turnover of the enzyme (SCANDALIOS 1990). In the cv. Aralba the differences among the
profiles of enzyme activity and of mRNA population suggest that both transcriptional and post-translational regulation intervene in the response of the Sod gene(s) to non-optimal growth temperatures. Southern analysis of sorghum and maize genomic DNA (Fig. 3) suggests a certain degree of similarity in the organization of the Sod genes in the two C4 Gramineae. However, the hybridization signal with sorghum genomic DNA was more intense than that with maize, suggesting the presence of a higher number of SOD-coding sequences in the former species. Differences among cultivars in their response patterns to non-optimal temperatures were scantly, SOD expression being the exception (Fig. 1 and 2). This could imply that, because of the mildness of the temperatre treatments, the compensatory ability of both cv. Aralba and cv. ICSV 112 was never exceeded. However, it should be noted that at suboptimal temperature, and despite of its origin and of its intrinsically lower antioxidant potential (see Table 1), cv. ICSV 112 performed even slightly better than cv. Aralba in terms of $P_n$, lipid peroxidation, GSH level and total SOD activity. Also, the depressive effects of supraoptimal temperature on ROS-scavenging enzymes were less intense in cv. ICSV 112 than in cv. Aralba. Moreover, unless in cv. Aralba, no temperature-driven downregulation of the Sod gene(s) was observed in the cv. ICSV 112 leaves. It could be therefore suggested that, at least in $S. bicolor$ and in response to disthermia, the ability to control oxidative processes and to sustain endogenous defense systems is more important than the intrinsic, constitutive antioxidant level.
Acknowledgements

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References