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Tolerance to paraquat is correlated with the traits associated with water stress tolerance in segregating F_2 populations of barley and wheat

Ahu Altinkut[1], Kemal Kazan[2], Zeliha Ipekci[1] & Nermin Gozukirmizi[1,3*]
[1]*Tubitak, Marmara Research Center, Research Institute for Genetic Engineering and Biotechnology, P.O. Box 21, 41470, Gebze-Kocaeli, Turkey;* [2]*Cooperative Research Centre for Tropical Plant Pathology, The University of Queensland, John Hines Building, St. Lucia, Brisbane, Queensland 4072, Australia;* [3]*The University of Istanbul, Faculty of Science, Department of Biology, Vezneciler-Istanbul, Turkey; (*author for correspondence)*

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Summary

To identify scorable marker traits that can be used in cereal breeding programs for selecting drought tolerant individuals, we investigated the correlation among the drought-associated traits in two F_2 populations derived from the crosses made between drought tolerant and sensitive barley and wheat parental genotypes. The parental genotypes of these crosses also differed by at least three other traits – paraquat tolerance, leaf size, and the relative water content. These three traits were scored in two F_2 populations of 80 individuals for each barley and wheat cross. Analysis of results indicated that the enhanced tolerance to paraquat was correlated with reduced leaf size and increased relative water content, two traits associated with water stress phenotypes of the drought tolerant barley and wheat parents. Our results suggested that the selection based on paraquat tolerance is technically less demanding and thus useful for rapid screening of individuals for enhanced drought tolerance in segregating populations.
Abbreviations: PQ – Paraquat; RWC – Relative Water Content

Introduction

One of the most serious problems encountered in semi-arid and arid climatic regions is lack of water to obtain sufficient product from crop plants. Drought is a multi-dimensional stress, which causes various physiological and biochemical effects on plants. Such effects may include reduction in cell division and thus retardation of cellular growth, decrease in photosynthesis, closure of stomata and changes in the amount of chlorophyll (Turner, 1986; Tanaka et al., 1990; Irigoyen et al., 1992; Smirnoff, 1993; Bohnert & Jensen, 1996; Jamaux et al., 1997; Tabaeizadeh, 1998). The other cellular alterations such as decreased protein content, increased ribonuclease activity, protein hydrolysis, hydrogen peroxide concentration and dissociation of polyribosomes are also known to occur in plants exposed to water stress (Levitt, 1980; Mukherjee & Choudhuri, 1983).

Evidence from different lines of research suggests that water deficit also causes oxidative stress in plants (Moran et al., 1994; Foyer et al., 1994). Limitation of photosynthesis and exposure to high irridance during water stress can increase the rate of active oxygen formation in chloroplasts or hinder the activity of antioxidant defenses (Smirnoff & Colombe, 1988). This effect of water stress on the physiology of plants is very similar to the stress caused by PQ (a bypridlium herbicide), which leads to the production of highly toxic free radicals generated by reaction of molecular oxygen with PQ radicals formed in the chloroplast during photosynthesis (Dodge, 1971). Therefore, a close correlation is expected between the plant's tolerance to stresses imposed by water and PQ. It is also possible that drought tolerant plants can be selected based on their response to PQ in segregating breeding populations if an association between drought/water stress associated traits and PQ tolerance could be

found. Establishment of such correlation would be of value for rapid selection of drought tolerant individuals based on their tolerance to PQ in breeding programs. Selection of plants for drought tolerance is otherwise rather difficult due to genetic complexity of this trait.

In this study, homozygous plants from drought tolerant, and drought sensitive barley and wheat genotypes and their F_2 progenies originated from tolerant × sensitive crosses were analyzed for drought-associated morphological and physiological traits. The aim was to identify certain marker traits that can be potentially employed for rapid selection of drought tolerant genotypes. Our results suggested that the enhanced tolerance to PQ could be used for this purpose since this trait showed a significant correlation with a morphological and a physiological trait known to be associated with enhanced tolerance to water stress.

Materials and methods

Plant material and growth conditions

Two homozygous barley genotypes (*Hordeum vulgare* L. cv. Tokak – a known drought tolerant barley cultivar and *H. vulgare* ST 5819 – a highly drought sensitive line) and two homozygous wheat genotypes (*Triticum aestivum* L. cv. Kirac – a known drought tolerant wheat cultivar and *T. aestivum* Sultan – a drought sensitive cultivar were selected. Segregating F_2 populations were then obtained from the crosses between *H. v.* cv. Tokak × *H. v.* ST5819 and *T. a.* cv. Sultan × *T. a.* cv. Kirac. Seeds were sown in pots (150 × 160 mm) with a soil mixture containing soil/sand/organic matter in a ratio of 1:1:1. Pots (one plant in each) were irrigated with 1 ml of H_2O per 10 g of soil mixture twice a day. Once a month a commercial fertilizer solution (Sol-U-Gro) containing 12% N, 48% P, and 8% K and micronutrients was also applied. All plants were grown in a growth chamber at 23 °C, 70% humidity and 16 h light/8 h dark photoperiod.

Measurement of leaf size

The leaf sizes of the 2nd leaf from the top of the plants from parental barley and wheat genotypes and their F_2 progeny were calculated according to the following formula; leaf size = length × width × 0.7 (Jamaux et al., 1997). These calculations were performed during the preflowering stage, using the fully expanded 2nd leaf from the top.

PQ treatment of leaf tissue

PQ treatment was conducted on the second top leaf taken from the plants with the same developmental stage (see below). Leaf samples from the plants of drought tolerant and sensitive parental genotypes and their F_2 progenies were treated by floating the excised leaves on sterile water containing 100 μM PQ under a light intensity of 12,000 lux for 24 h. Leaf extracts were then analyzed for determination of chlorophyll content.

Determination of leaf chlorophyll content

The second half of the second leaf from the top was excised from the plants at the 5–6 leaf stage, treated with PQ as explained above, extracted with 80% acetone and absorbance of supernatant was measured spectrophotometrically at 652 nm (Linchtenthaler & Wellburn, 1983). Leaf chlorophyll content was then calculated according to the formula of Arnon (1949), namely (OD_{652} × 27.8 × 10/fresh weight (g) × 1000) and the results were expressed as mg/chlorophyll/fresh weight.

Application of water stress and measurement of Relative Water Content (RWC)

Plants from both drought tolerant and sensitive cultivars were exposed to water stress by not watering the pots and the RWCs were measured after 2, 4, 6 and 8 days of water stress and finally at the end of two days of rehydration. The RWC of the uppermost fully expanded leaf of each plant in the preflowering stage was estimated, using the following formula of Jamaux et al. (1997); RWC = (fresh weight-dry weight) / (rehydration weight-dry weight) × 100. Dry weight of the leaf tissue was measured after 48 hours of desiccation at 80 °C and the samples were subsequently allowed to rehydrate. Rehydration weight is measured after 19 hours of rehydration at 4 °C in the dark.

Results

Association between water stress and PQ tolerance

In this study, we first examined the association between drought-associated traits and PQ tolerance using the segregating populations derived from crosses between drought tolerant and sensitive barley and wheat genotypes. First, PQ tolerance of the parental

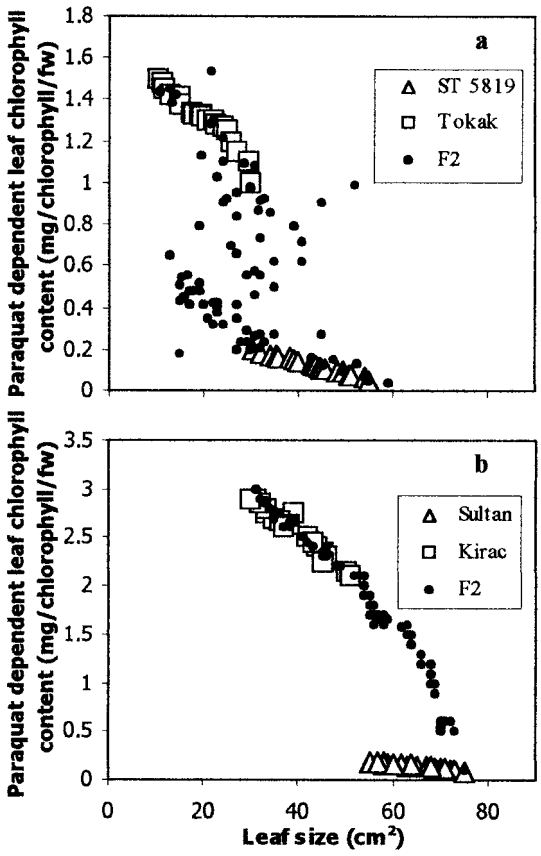

Figure 1. Association between PQ-dependent leaf chlorophyll content and leaf size in drought sensitive (ST 5819) and drought tolerant (cv. Tokak) barley parental genotypes, and their F_2 progeny (80 individuals) (**a**), and in drought sensitive (cv. Sultan), drought tolerant (cv. Kirac) wheat parental genotypes and their F_2 progeny (80 individuals) (**b**).

genotypes was tested by treating the leaves with PQ. Initially, several PQ concentrations (100 μM, 200 μM, 300 μM, 400 μM, 500 μM) and exposure times (12 h, 24 h, 36 h) were tested on parental plants to determine the optimum PQ concentration and exposure time. Consequently, 100 μM PQ concentration and 24 h exposure time under a light intensity of 12,000 lux were found to reliably distinguish tolerant barley and wheat parental plants from the sensitive ones. Level of PQ tolerance was estimated by measuring loss of chlorophyll after PQ treatment because reduction in the amount of chlorophyll after PQ application was reported to be a good indicator of PQ tolerance (Cakmak & Marschner, 1992). In addition, PQ damage on the treated leaves from tolerant individuals was substantially more significant than the ones on sensitive plants upon visual inspection of the treated leaves (data not shown). To ensure that the reduction observed in the amount of chlorophyll after PQ treatment was not due to leaf size (i.e. genotypes with larger leaves receiving higher amounts of PQ), PQ treatments were conducted by floating the leaves on a PQ solution. This ensured that the unit area of each leaf had received equal amount of PQ, irrespective of the size of the leaf. When homozygous plants of barley and wheat parental genotypes were treated with 100 μM PQ, reductions in chlorophyll content were observed to be different between the tolerant and sensitive parents (Figure 1a and 1b). The leaf chlorophyll contents of plants from PQ treated tolerant barley (cv. Tokak) and wheat (cv. Kirac) plants were found as 1–1.5 and 2.1–2.9 mg/chlorophyll/fresh weight, respectively, compared to those from untreated barley (1.9–2.1 mg/chlorophyll/fresh weight) and wheat (2.9–3 mg/chlorophyll/fresh weight) plants from the same genotypes. In contrast, in the drought sensitive parental barley line, ST 5819, reduction in chlorophyll content after PQ treatment was more dramatic as this value ranged between 0.05 and 0.2 mg/chlorophyll/fresh weight for treated and between 1.8 and 2.1 mg/chlorophyll/fresh weight for untreated plants, respectively. Similarly, this value ranged between 0.09 and 0.19 mg/chlorophyll/fresh weight and between 2.5 and 2.8 mg/chlorophyll/fresh weight in the drought sensitive wheat parent cv. Sultan.

These results suggested that PQ tolerance estimated as reduction in the leaf chlorophyll content may be associated with drought tolerance of the parental barley and wheat genotypes. To further examine this association, we generated F_2 progenies by making crosses between tolerant and sensitive barley and wheat genotypes and determined the leaf chlorophyll content of each F_2 individual derived from these crosses after PQ treatment. As expected, leaf chlorophyll content of the F_2 individuals in these populations showed variation with values ranging from 0.036 to 1.535 mg/chlorophyll/fresh weight for barley and 0.1 to 3 mg/chlorophyll/fresh weight for wheat (Figure 1a and 1b).

Identification of other traits associated with PQ tolerance

To determine whether enhanced PQ tolerance, a trait derived from the drought tolerant barley and wheat genotypes, is correlated with the other putative drought associated physiological and morphological traits, leaf size and RWC were measured both in the

parental genotypes and in the F_2 progenies. Previously, reduced leaf size was reported to be positively correlated with enhanced tolerance to drought possibly by minimizing water losses through transpiration (Hsiao, 1973). Therefore, we next examined whether the drought tolerant and sensitive parental genotypes used in this study differ by their leaf size. All the leaves in the drought tolerant parental barley genotype, cv. Tokak, were significantly smaller than those in the drought sensitive genotype ST5819. For comparison, we then measured the size of single selected leaves from several parental plants and their F_2 progenies. When measured at the pre-flowering stage, average size of the second leaf of the tolerant parent approximated 20 cm^2 whereas this value was 43 cm^2 for the sensitive barley parent. The leaf size of the F_2 progeny varied between 11 cm^2 and 59 cm^2. The correlation between PQ tolerance and the leaf size in the F_2 progeny showed that the reduced leaf size was associated with enhanced tolerance to PQ. The correlation coefficient calculated between these two traits was significant (r = –0.423, $p > 0.05$). A similar variation was also observed for leaf size of tolerant and sensitive wheat lines. Average size of the second leaf of cv. Kirac was around 39 cm^2 whereas this value was 65 cm^2 for the cv. Sultan. The leaf size of the F_2 progeny varied between 31 cm^2 and 75 cm^2. Estimation of correlation between PQ tolerance and the leaf size in the F_2 progeny showed that the reduced leaf size was also associated with enhanced tolerance to PQ. The correlation coefficient calculated between these two traits was highly significant (r = –0.977, $p > 0.01$). It is expected that the correlation between these two traits could be even more significant if the genotypes were compared under water stress conditions. Nevertheless, this result suggested that leaf size is an important morphological trait that possibly associates with the drought phenotype of the individuals in these particular crosses.

Next, we examined a physiological trait namely RWC, which was found to be positively correlated with water stress tolerance (Jamaux et al., 1997). To further estimate the drought tolerance phenotypes of parental and F_2 individuals based on their RWC, plants were exposed to water stress and the RWCs were measured after 2, 4, 6 and 8 days of water stress and finally at the end of 2 days of rehydration. These measurements demonstrated that the RWC of both the wheat and barley genotypes decreased following 2 to 4 days of water stress although no significant difference was detected in the RWC of the parental genotypes at

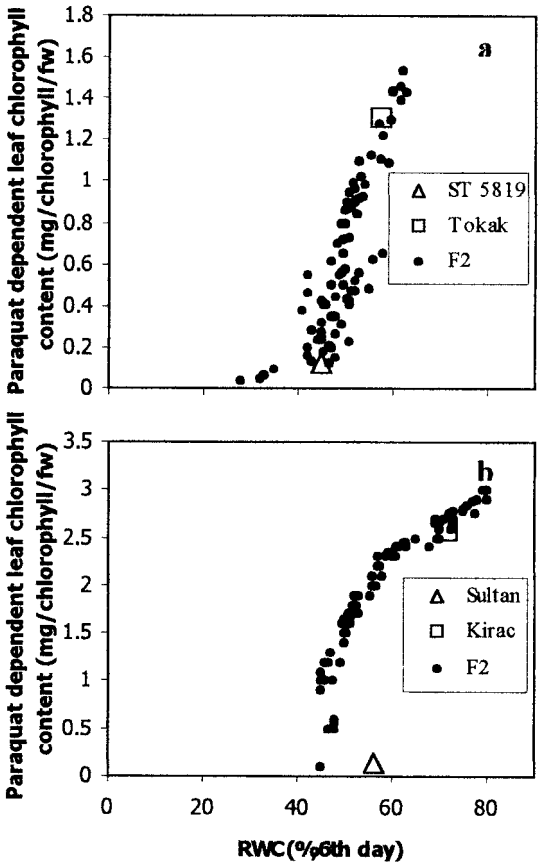

Figure 2. Association between PQ-dependent leaf chlorophyll content and RWC on the 6th day in drought sensitive (ST 5819) and drought tolerant (cv. Tokak) barley parental genotypes and their F_2 progeny (80 individuals) (**a**), in drought sensitive (cv. Sultan), drought tolerant (cv. Kirac) wheat parental genotypes and their F_2 progeny (80 individuals) (**b**).

these earlier stages of water stress. Detectable differences, however, were observed in the RWC of parental genotypes on day 6 of water stress. The RWC was 45% in ST 5819 and 58% in cv. Tokak. RWC of the F_2 individuals was in the range of 28–63% on day 6. The RWC was 72% in cv. Kirac and 56% in cv. Sultan. RWC of the F_2 individuals was in the range of 45–80% on day 6. As expected, the RWCs of both barley and wheat plants that are not subjected to water stress remained unchanged (90–100%) during this period. Rewatering of water-stressed plants on day 6 resulted in a rapid increase of the leaf RWC to the levels of unstressed control plants. We then examined whether there is any correlation between PQ tolerance and the RWC using the values obtained from the F_2 plants (Figure 2a and 2b). This analysis showed that

Table 1. Correlation matrix between paraquat dependent leaf chlorophyll content, leaf size and relative water content in barley and wheat crosses (the first and second figure, respectively)

	Paraquat dependent leaf chlorophyll content	Leaf size
Paraquat dependent leaf chlorophyll content	–	–
Leaf size	–0.423*, –0.977**	–
Relative water content	0.830*, 0.909**	–0.593*, 0.956**

* and ** significant at $p > 0.05$ and $p > 0.01$, respectively.

F_2 plants with higher tolerance to PQ also had higher RWC values, suggesting that these two traits were positively associated. The correlation coefficient calculated between these two traits was highly significant for barley (r = 0.830, $p > 0.05$) and wheat (r = 0.909, $p > 0.01$).

Finally, the association between RWC and leaf size estimated for both barley and wheat crosses exhibit significant correlation as shown in the matrices given in Table 1.

Discussion

Selection in breeding programs based on the secondary traits associated with tolerance to water stress is known to be useful (Ludlow & Muchow, 1990). It is clear that an ideal secondary trait should be heritable, inexpensive and rapid to measure. Unfortunately, the measurement of many putative drought-adaptive traits is too slow and/or costly for routine use in a conventional breeding program. In this study, we examined the association between drought associated traits in segregating populations of both barley and wheat. The drought tolerant barley (*H. v.* cv. Tokak) and wheat (*T. a.* cv. Kirac) genotypes used here are highly adapted to the regions of Central Anatolia where the average annual precipitation is only 300 mm and show significant superiority to drought sensitive parental lines *H. v.* ST5819 and *T. a.* Sultan under field conditions (M. Keser, pers. comm.). Although exact physiological mechanism(s) of drought tolerance in these lines is not known, they have been extensively used by plant breeders as reliable sources of drought tolerance genes.

In the study reported here the leaf size and the RWC (two putative secondary traits) were found to be associated with PQ tolerance, which in turn correlated with the overall stress tolerance phenotypes. However, leaf size seems to behave as a quantitatively inherited trait and selections based only on this trait may not be possible especially when the parental genotypes used do not differ in leaf size. On the other hand, although it may be a reliable marker for estimating water stress, RWC is not easy to measure because of the need to expose the plants to water stress before doing the RWC measurements. The significant genetic association observed between PQ tolerance and the other parameters affecting water stress tolerance, however, suggests that tolerance to PQ, which is rather convenient to test could be used as a secondary trait to select plants with higher water stress tolerance in segregating breeding populations. Our initial experiments also suggested that a simple visual test based on observation of PQ induced damage on the leaves may be developed to facilitate rapid screening of the plants in segregating populations for drought tolerance.

The correlation we demonstrated between PQ and drought tolerance was based on measurements of certain drought-associated morphological and physiological traits in segregating populations. Importantly, more evidence supporting such correlation is now accumulating from molecular studies. It appears that the effect of PQ mimicking the effect of water stress response is due to generation of active oxygen species (AOS) by both PQ treatment and water stress. Expression of a single gene for catalase in transgenic tobacco provided significant protection against photooxidative stress caused by drought or PQ application, possibly through more effective removal of AOS accumulating under these stresses (Shikanai et al., 1998). The photosynthetic activities measured as chlorophyll fluorescence were much less affected by the PQ treatment in transgenic lines expressing this gene (Miyagawa et al., 2000). The inhibition of enzymes functioning in removal of AOS generated by drought or PQ treatment is proposed to be the reason for oxidative damage caused by these stresses. Further supporting this hypothesis, Iturbe-Ormaetxe et al. (1998) recently reported that total ascorbate peroxidase activity and ascorbic acid

content showed an identical reduction in pea plants under stress conditions induced by severe water deficit or PQ. Additionally, pea cultivars that were more tolerant to water deficit had less reduction in catalase activity than the less tolerant ones following treatment with PQ (Iturbe-Ormaetxe et al., 1998). Although no attempts were made to measure the level of anti-oxidant enzymes in parental genotypes used in this study, it is tempting to speculate that the levels of such enzymes might also be different in genotypes with different drought tolerance phenotypes.

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