



**Szent István University**

**Investigations on the effects of drought stress on the morphology, physiology and yield  
biology of durum wheat**

**Main points of the PhD thesis**

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## **1. BACKGROUND AND AIMS**

The increase in the frequency of extreme climatic events has resulted in greater variability between both ecological regions and years. The need to feed an ever greater population means that the efforts of biologists and plant breeders resemble a race to produce genotypes that have not only greater productivity but also better adaptability.

In regions with a continental climate prone to drought, adaptability, drought tolerance and yield potential are closely related traits. The maintenance and improvement of productivity therefore require the breeding of drought-tolerant cultivars with better water use efficiency and adaptability to a changing environment, and the more widespread introduction of water-saving agricultural systems. To achieve this, biological research and breeding programmes endeavour to gain a better understanding of the mechanism of drought tolerance, to identify genes responsible for drought tolerance and to incorporate them into modern cultivars. The aim is to breed drought-tolerant genotypes capable of producing satisfactory yields even in the case of water deficit due to their better water use efficiency and their better adaptation to water shortages and osmotic stress.

If more detailed information is to be acquired on the drought stress tolerance of individual genotypes in different years, it will be necessary to combine phenotyping methods with physiological and genetic knowledge.

The Green Revolution has now fulfilled its role, making a substantial contribution to an increase in plant productivity, in various ways in different periods and regions. However, future aims will not be attainable with conventional methods and technologies alone, but will require the use of new techniques. The application in breeding of results obtained in molecular biology could be an effective way of facing these new challenges. The identification of regions responsible for quantitative traits (QTLs), gene expression analysis, and the investigation of molecular and biochemical mechanisms could form the basis for the selection of drought-tolerant genotypes capable of producing satisfactory yields under water-deficient conditions.

In the course of four years of experimentation (2011–2014) the effect of drought stress was examined on 188 different spring durum wheat cultivars and on eight durum wheat near-isogenic lines under field conditions. A combination of four approaches was applied: plant responses to drought stress were examined under rain-fed and irrigated conditions, a record was made of weather data during the growing period, changes occurring in the soil were

monitored, and chromosome regions linked to drought tolerance were mapped. The aim of this joint plant–environment–soil–genetics analysis was as follows:

- to analyse the effect of the environment (treatment, genotype × treatment) on drought stress and to identify morphological and physiological traits that exhibit a close relationship with higher yields even in the case of insufficient water supplies
- to detect correlations between the chlorophyll content of the flag-leaf (SPAD index), the spectral reflectance of the whole plot (NDVI index) and drought tolerance
- to identify genotypes with both outstanding drought tolerance and high yield stability
- to identify loci responsible for drought tolerance by means of association mapping on durum wheat cultivars with broad genetic diversity
- to analyse the effect of the QYld.idw-3B QTL region on the yield by studying near-isogenic lines (NILs) of spring durum wheat with different parental alleles at the *QYld.idw* locus responsible for yielding ability on the 3B chromosome
- to determine the polyamine content and antioxidant enzyme activity of the flag-leaf during exposure to drought stress and to analyse the relationship between these defensive compounds and the yield in near-isogenic lines of spring durum wheat.

## 2. MATERIALS AND METHODS

### 2.1 Plant materials

The work described in the PhD thesis involved the analysis of the durum wheat experiments performed in the framework of the *EU FP7 DROPS (FP7-244374)* project. The 188 durum wheat genotypes (*T. durum* Desf.) were sown in spring in irrigated and rain-fed plots (GWA panel). The cultivars, landraces and lines included in the experiments originated from a wide range of countries (Italy, Mexico, Morocco, Spain, Syria, Tunisia, USA) and have frequently been used by breeders in Mediterranean countries over the last 40 years. They were selected on the basis of their closely similar flowering times, so that the treatments, scoring and measurements should be performed on plants in the same stage of development. This helped to make the genotypes comparable and facilitated a more accurate determination of their different or similar responses to stress. On the basis of their high yields, 20 of the 188 cultivars were selected (Core panel) for more detailed studies.

Eight near-isogenic lines of spring durum wheat, derived from four different recombinant inbred lines of a cross between the spring wheat durum wheat cultivars ‘Kofa’

and 'Svevo', were examined in the field in 2013 and 2014 and on a plot fitted with a removable polyethylene rain shelter in 2014.

## **2.2 Experiments in the field and under the rain shelter**

A durum wheat experiment consisting of 100 genotypes in 2011 and 188 in 2012 and 2013 (GWA panel) was sown in five replications, two of which were irrigated (W) and three rain-fed (RF). The eight durum wheat NILs examined in 2013 and 2014 were sown with two sowing densities (low: 320 and normal: 480 seeds/m<sup>2</sup>) in eight irrigated and eight rain-fed replications. Due to their location over a wide area, they were sown each year in an unbalanced incomplete alpha-lattice block design. Irrigation was performed using the Micro-sprinkler technology, using an ECRN-100 rainfall meter with a sensitivity of 0.2 mm for the accurate measurement of the quantity of water applied.

Two treatments were used on the plot with a rain shelter: (i) non-irrigated and (ii) irrigated throughout the growing season. Each row was sown with 50 seeds of a single line, with plant and row spacings of 3 × 15 cm, so the randomly placed NILs were replicated 12 times in each treatment. The six experimental plots (3.4 × 5 m) situated under the rain shelter could be irrigated separately, the amount of water applied to the area being regulated automatically by the drip irrigation system.

Capacitive soil moisture meters (5TE-Decagon Devices, USA) were placed at depths of 30 and 60 cm on five points in the GWA panel plots and eight points in the NIL plots. The sensors recorded the moisture content (%), temperature (°C) and specific electrical conductivity (mS/cm) of the soil every hour from emergence to harvesting. The exact irrigation time was determined using an MPS-2 tensiometer.

## **2.3. Phenotypic observations**

The characteristics scored in the field GWA panel, Core panel and NIL plots were as follows: early development, evaluated visually before first node appearance (Z29–31) and at third node appearance (Z33); heading, flowering and ripening date; plant height, measured to the flag-leaf, the base and apex of the spike, and spike length; chlorophyll content of the flag-leaf in five phenophases; NDVI (normalised difference vegetation index) in three phenophases; flag-leaf area; relative water content of the flag-leaf; biomass and number of fertile tillers; antioxidant enzyme activity (APX: ascorbate peroxidase, GPX: guaiacol peroxidase) and free polyamine content (PUT: putrescine, SPD: spermidine, SPN: spermine) of the flag-leaf.

The plants were raised to full maturity and numerous yield components were scored, including the grain weight per hectare, thousand-kernel weight, grain number in the main spike, grain weight in the main spike, and the number of sterile spikelets at the base and apex of the main spike.

#### **2.4 Meteorological data in the experimental years and water supplies to the plots**

In 2011 there was 236.8 mm rain in Martonvásár, which was only 42% of the 30-year mean (559 mm). Only 102.2 mm fell from March to the end of June, i.e. until the end of grain filling. Despite the sporadic rainfall, the soil moisture content quickly dropped to 21–22 vol%, so the spring-sown plants struggled with water deficit from emergence to harvest. In the irrigated plots 167 mm irrigation water ensured good water supplies, with a tension of –10 to –35 kPa in the root zone. In 2012 the soil was already dry at sowing, as both the growing period in the previous year and the winter months had very little rain. The moisture content recorded at a depth of 30 cm averaged 16–17 vol%, so the plants did not have sufficient water for optimum development and yield formation. In the irrigated plots a total of 221.6 mm irrigation water was applied, resulting in a mean soil moisture value of 27 vol% at a depth of 30 cm and 25 vol% at 60 cm. Apart from a lack of rain in April, the weather in 2013 was favourable for cereals. The weather in 2014 was characterised by showers and rainstorms from late April until the end of May, but the soil moisture content began to decrease after flowering, accompanied by high temperatures after May 20<sup>th</sup>. In the rain-fed plots values lower than 22 vol% were recorded during grain filling, showing that little water was available to the plants. In the irrigated plots the soil moisture content averaged 24 vol% after five irrigations, while the specific electrical conductivity was 0.54 mS/cm.

#### **2.5 Data analysis**

Analysis of variance on the phenotypic data was performed for each year and for all the years combined using the linear random/mixed effect model in the Genstat 18 software package (REML-Linear Mixed Models). Interactions and correlations between the traits and treatments were determined using principal component analysis, while the best-performing genotype was selected by means of principal component biplot analysis. Relationships between the scored parameters were analysed using General Linear Model (GLM) regression, Pearson's correlation and Spearman's rank correlation analysis with the help of the Statistica6 software package. Duncan's test was used to detect significant differences between the genotypes. A modified equation was used to calculate the area under the SPAD value decline

curve (AUSDC) and the area under the vegetation index curve (AUVIC), which allowed genotypes that stayed green for a longer period to be selected. Instead of additive variance, the variance components were used directly for the calculation of the heritability/repeatability index. A dendrogram was constructed based on the kinship matrix in the Tassel5 program to determine the population structure of the GWA panel. A total of 25 146 SNP markers were available for the analysis of kinship between the genotypes.

### **3. RESULTS AND DISCUSSION**

#### **3.1 Effect of drought stress on the plant development, morphology, physiology and yield biology of durum wheat**

In the field experiment a detailed study was made of changes in the plant development, morphology, physiology and yield biology of spring durum wheat cultivars in three consecutive years. The aim was to find traits exhibiting a close relationship with higher yield even when water supplies were low. Statistical analysis was used to determine the effects of genotype, year and treatment (irrigation) on the groups of traits investigated, and to analyse correlations between the groups of traits. Genotypes were selected that had both good yield stability and high yields.

In 2011 and 2012, in response to high temperatures and insufficient rainfall, significantly lower values were recorded for morphological, physiological and yield traits in the rain-fed plots. In addition to rainfall deficit there was an increase in the number of days with a maximum temperature of over 30°C, so even in plots with good water supplies, where the soil moisture content was optimised by irrigation, heat stress led to earlier senescence.

The variability in the heading date for each genotype during drought stress and in its correlation with the yield led to the conclusion that the heading date is indicative mainly of the adaptation mechanism of the cultivars, while its correlation with the yield is less absolute and depends on the environment. During the dry season of 2011 cultivars were found which headed late but produced higher yields, but this was not confirmed in later years. The main reason for this could be the genotype × environment interaction, which results in diverse genotype responses due to the variability of climatic components (temperature, rainfall) and soil structure. The plasticity of the heading date is also shown by the fact that in the present work 41.4% of the phenotypic variance was explained by the year. In environments where the grain-filling period is shortened by early senescence arising due to drought, early heading and the completion of the life cycle before the occurrence of terminal heat and drought stress can

be regarded as a yield-positive adaptation mechanism. Genotypes with later heading, flowering and maturing times, however, are able to accumulate greater biomass and store more nutrients under optimum conditions. Taller genotypes that mature later have more time and capacity to accumulate assimilates, manifested as higher yields, as proved by the fact that the yield was in close correlation with the height and plant development parameters in these experiments.

In the present work the response of high-yielding cultivars to drought stress in various phenophases was manifested as later heading, higher chlorophyll content at the early dough stage (SPAD83) and longer stems. Interestingly enough, the tall cultivars ‘Cham-1’, ‘Quadalete’ and ‘Karim’ had higher yields under both optimum and stressed conditions, which could have been promoted by a number of indirect traits (deeper, more extensive root system, longer duration of photosynthetically active plant area). It also proved possible to identify high-yielding, tall genotypes (‘Marjana’, ‘Miki-1’, ‘Svevo’, line ‘1807’) that produced significantly lower yields in both dry years due to greater evapotranspiration, confirming the complex nature of the stress response and the diverse regulatory mechanisms involved.

In the case of optimum water supplies the high-yielding genotypes had greater thousand-kernel weight, associated with plumper grains. As the yield is influenced to the greatest extent by the various yield components, selection for any of the parameters could contribute to an increase in yield. The significant positive correlations revealed by principal component analysis between yield and the grain-filling period, the grain number in the main spike, the grain weight and the thousand-kernel weight made it clear that these traits also contributed to the yield in the case of drought stress, so it is essential to take them into account during selection. These results provided further confirmation of the fact that selection for yield leads to the simultaneous selection of genes responsible for yield and abiotic stress adaptation. By contrast, if selection concentrates on a few major tolerance genes influencing physiological processes related to drought tolerance, there may be an increase in drought tolerance, but this may spoil yield performance.

Comparative biplot analysis of the irrigated plots, well supplied with water, showed that the cultivar ‘Cham-1’ and the line ‘CIMMYT-222’ came closest to the ‘ideal’ genotype. At the same time, the cultivars and lines identified as having high average yield and low annual variability in rain-fed plots (‘CIMMYT-23’, ‘CIMMYT-73’, ‘Aghrass-1’, ‘Awali-1’, ‘BLK-2’, ‘Ouaserl-1’) were also found on the positive side of the abscissa. In response to drought stress the factor variable correlation patterns of the scored parameters indicated that the yield

was also correlated with the number of fertile tillers, while a positive association with the chlorophyll content was also detected in later phenophases (Z83 and Z85). In 2011 the high number of productive tillers was accompanied by high grain number, thus compensating for losses due to lower grain weight. Genotypes able to retain completely or partially the usual number of fertile tillers during drought stress probably have favourable root properties (root weight, thickness and length, root/shoot ratio, number of lateral roots and root tips). The significant correlation between the variables also proved that the grain number and grain weight in the main ears were significantly influenced by the ratio of sterile spikelets at the base and apex. The number of sterile spikelets rose in dry years, especially in the basal part. Environmental factors (temperature, light and the agronomic properties of the soil) have a substantial influence on fertility conditions within the spike, causing annual variability in the ratio of sterile spikelets. The fertilisation of the young spikes and thus the number of grains per spike can be influenced until the flowers are set, as shown by the significant effect of the year and the treatment on the number of sterile spikelets in the rain-fed plots.

The significant positive correlation between the SPAD and NDVI values recorded in later phenophases (Z83, Z85) and the yield shows that under dry conditions the greatest variability between the genotypes can be observed in these phenophases. The SPAD83 and SPAD85 values and the NDVI values recorded at flowering and in the early dough stage were the main determinants of the AUSDC and AUVIC parameters characteristic of the 'stay green' trait. The stability of annual vegetation activity was variable in genotypes which had a high NDVI value at the Z83 stage of development and thus a longer vegetation period ('Ouaserl-1', 'Quadalete-1', 'Kofa', 'Meridiano'), as revealed by the low genotype effect and high year effect in the analysis of variance. Nevertheless, these genotypes usually had higher values in most environments.

It was possible to identify one cultivar ('Cham-1') that had stable vegetation activity from year to year even during drought stress and was thus able to produce higher yields. This could be attributed to various morpho-physiological traits (roots able to reach moisture in deeper soil layers, efficient photosynthesising and transpiration systems), which form the basis of the 'stay green' trait, and which can be pyramided to achieve further improvements in the agronomical performance of the genotypes.

### **3.2 Association analysis**

The SNP map compiled for the 188 cultivars was used to analyse the population structure of the association panel and to identify QTLs (quantitative trait loci) linked to yield,



heading date and morphological and physiological traits at various water supply levels. The analysis aimed to discover whether there was any QTL region linked to both the SPAD index associated with the chlorophyll content and to phenotypic values characteristic of the heading date, morphological traits and yield components. For this purpose phenotypic analysis was performed on 10 agronomical traits and on traits characteristic of the chlorophyll content recorded in 5 phenophases for plants grown in rain-fed and irrigated plots. A total of 25 146 SNP markers were used to analyse kinship. The analysis identified five subgroups, each of which contained at least three cultivars from the core panel of 20 genotypes. In the course of association analysis based on a significant threshold of  $-\log_{10}p \geq 2$  ( $p=0.01$ ) a total of 642 significant marker group–trait associations were detected, with an average of 42 for each trait. These groups comprised significant SNP markers separated from each other on the chromosome at a maximum distance of 20 cM. Among the marker groups 470 (73.32%) only appeared in a single environment, i.e. in one treatment in one year, while 27 marker groups (4.21%) were identified in three or more environments. Most of these groups were associated with various height parameters, yield components and chlorophyll contents (SPAD45, SPAD77). During the analysis of 188 genotypes, 68 marker group regions linked to yield were demonstrated, 48 of which only appeared in a single environment. Six regions were only found to be associated with yield in either the rain-fed or the irrigated plots. The 82.5 cM region on the 3B chromosome was linked with the yield at the LOD2.5 significance level in both two years and at the LOD3 threshold in 2011. The 74–90 cM region of this chromosome was associated with the chlorophyll content in the Z83 phenophase (SPAD83) at the LOD2.5 significance level and to the plant height measured to the spike apex in the case of good water supplies in 2013. The yield in the rain-fed plots mapped to the 192–200 cM region on the 5A chromosome, which was also associated with the heading date and the chlorophyll content at the soft dough stage (Z85). The thousand-kernel weight was associated with 36 marker groups, but none of these regions was found to be treatment-dependent. In most cases the marker group region characteristic of thousand-kernel weight was only linked with a single environment or with both treatments in a single year, and in many cases the grain width was also associated with this region. The heading date was associated with 68 marker group regions, 49 of them in a single environment. Eight of these regions were detected either in the rain-fed or the irrigation treatment in both years. Both heading date and the chlorophyll content recorded at flowering mapped to the 81–95 cM region on chromosome 1B in rain-fed plots, while heading date and yield mapped to the 47–56 cM (PPD-B1) region on chromosome 2B in both treatments in 2013. A total of 24 marker groups were discovered

where the heading date and the yield were linked to the same region for the same treatment in a given year. No treatment-dependent marker group regions were identified for the chlorophyll contents recorded when the boot was just swollen (Z45) or in the early dough stage (Z83). The chlorophyll content in the Z65, Z77 and Z85 phenophases, however, mapped to several chromosomes (1B, 2A, 3A, 4A, 5A, 6A, 6B), depending on the treatment. In the rain-fed treatment, complex traits mapped to the same marker group region on two chromosomes (to the 81–95 cM region on chromosome 1B and to the 192–200 cM region on chromosome 5A) in both years. The 198–211 cM region of chromosome 2A was associated with the SPAD85 trait under rain-fed conditions, and with the thousand-kernel weight and grain width regardless of the treatment.

### **3.3 Effect of drought stress on morphological, physiological, biochemical and yield biology traits in near-isogenic lines of durum wheat**

A detailed study was made of changes in the plant development, morphology, physiology and yield biology of near-isogenic lines of spring durum wheat sown in the field with different plant densities in two consecutive years (2013–2014) and under a rain shelter in one season (2014). Comparisons were made of the traits of the isogenic pairs *QYld.idw-3B++* and *QYld.idw-3--* both within and between the pairs. An analysis was made of the drought tolerance of isogenic lines selected for yield QTLs, and the presence of QTL effects was examined in spring sowings. Changes in the polyamine content and antioxidant enzyme activity of the flag-leaf were monitored in response to drought stress, and the relationship between these defensive compounds and the yield was analysed.

In the field experiments low values of soil moisture and high temperatures were recorded from the beginning of milky ripeness. The non-irrigated plots under the rain shelter were exposed to intense drought stress throughout the growing season, the effect of which was manifested in the formation and fertilisation of the flowers and in the grain yield. The leaf yellowing caused by intense stress could be observed in the whole stand soon after flowering, indicating early senescence, but the chlorophyll content decreased to different extents in the individual NILs. A significantly higher mean chlorophyll content was detected in the *QYld.idw-3B++* lines in phenophases Z77, Z83 and Z85, while the *QYld.idw-3B--* lines had lower chlorophyll content in both the irrigated and non-irrigated plots. In the case of moderate stress (in the field experiment) differences between the NIL++ and NIL-- pairs only appeared in the early dough stage, indicating that the *QYld.idw-3B++* QTL region was able to maintain photosynthetic activity for a longer period, resulting in greater grain number and grain weight

at the end of the growing period. The chlorophyll content of the flag-leaf in phenophases Z45, Z65, Z77 and Z83 was significantly correlated with the grain number and grain weight of the main spike, and that recorded in the Z85 phenophase with the grain number and grain weight of the side-tillers, thus confirming the above conclusion. The dry biomass was found to be in significant positive correlation with the yield and yield components and the height parameters, so the genetic variability could be utilised in breeding for drought tolerance. The grain yield was greatly influenced by the treatment, while the genotype had a significant effect on the thousand-kernel mass of the main spike and the grain number and grain weight of the side-tillers. When the lines were compared in the non-irrigated treatment, significantly more grains and significantly higher grain weight were observed in the main spike in lines NIL1++ and NIL2++, while in lines NIL1++ and NIL3++ the grain number and grain weight were significantly higher in the side-tillers. This confirms the theory that the higher yields of the *QYld.idw-3B*++ lines when sown in spring and exposed to drought stress could be attributed to the positive effect of the 'Kofa' QTL on chromosome 3B. Lower plant density, which resulted in a larger growing area per plant, clearly had a significant positive effect on the grain yield, the grain weight per spike and the grain width. This may have been promoted by a number of indirect traits (higher chlorophyll content and NDVI value, longer stems, larger root system) and shows that genotypes with good tillering ability perform better at lower plant density. This is probably also aided by the better utilisation of water, solar irradiation and nitrogen.

The reduction in the APX activity of the flag-leaf in the non-irrigated treatment was of a similar magnitude in all the isogenic lines. By contrast, higher GPX activity was recorded in the flag-leaf of all the lines in the non-irrigated treatment compared with plots with good water supplies. In parallel to this, the APX activity exhibited a significant negative correlation with the grain weight of the main and side spikes, the flag-leaf area and the spermidine content under dry conditions. The GPX activity was also negatively correlated with numerous yield components, thus confirming the role of these enzymes in the stress response.

The polyamine accumulation was found to increase in the flag-leaf of the isogenic lines during drought stress, to the greatest extent for putrescine, spermidine and spermine. In addition a significant positive correlation was detected between the contents of the three polyamines. A further significant positive correlation was found between the polyamine content and the grain number and grain weight in the main spike in the non-irrigated treatment.

### 3.4 New scientific results

1. A complex field phenotyping system was elaborated which, by the joint analysis of plant–environment–soil–genetics, gave an accurate picture of the magnitude of the changes occurring in the individual parameters and on the changes caused by drought stress in the morphology, physiology and yield biology of the plants. This system will be suitable for use in the analysis of field experiments carried out on a large area.
2. The year and the water supply level were shown to have the greatest effect on the morphology, physiology and yield biology of spring durum wheat genotypes, even in the case of parameters with high heritability ( $h^2$ ) values. The only exception was the thousand-kernel weight, where the results of three years of drought stress proved the great stability of this parameter.
3. The SPAD and NDVI values recorded in the early dough stage (Z83) were shown to be in significant positive correlation with the yield under dry conditions. It proved possible to identify genotypes with higher chlorophyll content in the flag-leaf in this phenophase; these had delayed senescence, manifested in higher yields.
4. The population structure of the durum wheat GWA panel was determined, and an LD map was constructed using 5879 SNP markers from the 90K SNP Infinium marker platform.
5. Whole-genome association analysis led to the identification of chromosome regions with a role in the regulation of morphological, physiological and yield biology traits in single environments, as a function of year and treatment. Two marker group regions associated with QTLs for both the chlorophyll content of the flag-leaf and the heading date were identified on chromosomes 1B and 5A. The latter region was also associated with the yield QTL.
6. Photosynthetic activity was proved to be maintained for a longer period in the flag-leaf of *QYld.idw-3B++* spring durum wheat isogenic lines, with the result that the *QYld.idw-3B* QTL region had a significant effect on the yield in the two-year field experiment.
7. Significant changes in the quantity and activity of defensive compounds were detected in the near-isogenic spring durum wheat lines in response to deficient water supplies, thus confirming the role of these compounds in the stress response under field conditions. Numerous yield components were proved to be in negative correlation with the activity of the ascorbate peroxidase and guaiacol peroxidase enzymes and in significant positive correlation with the polyamine content.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

Satisfactory temperature and water supplies are essential if cultivated plants are to develop optimally and produce high yields. This is particularly important during the main phenophases in the life-cycle of cereals. Satisfactory soil moisture content will result in uniform emergence, growth, pollen production, seed setting and grain filling. The quantity of yield, however, is determined not only by overall differences in the years, but also by the occurrence of diverse environmental effects during the individual phenophases. In addition, as yield is a quantitative trait influenced by multiple genes, the genotype  $\times$  environment interaction also exerts a substantial effect on the yield. This interaction can be detected by changes in the relative order of genotypes compared in different years and environments or by changes in the performance of a given genotype compared with a standard genotype, and also draws attention to the limitations of the growing site. If breeding programmes are to be successful, it is essential to identify a target environment providing an adequate representation of typical growing conditions, which must be sufficiently large to make the breeding programme economically justified. The analysis of the target environment pinpoints the periods when the crop is most sensitive to drought stress, thus making it easier to select for the desired trait. In ideal cases, the order of the genotypes according to performance is the same in all the growing environments, i.e. there is no genotype  $\times$  environment (G $\times$ E) interaction. This is important because the complex G $\times$ E interaction reduces the heritability of the yield, especially in environments prone to drought, thus complicating the selection of cultivars with good yield performance. If the target growing sites have similar soil moisture profiles and almost identical biotic and abiotic stress factors, the cultivars can be expected to perform consistently well. It must not be forgotten, however, that the genotypes exhibit considerable variability for numerous traits, making it difficult to predict the response of any genotype to a given environmental factor from that of another. This is partly because the sensitivity of the genotypes to each individual change, and thus their response to the change, may be very different. What is more, these responses and their interactions may also differ in different stages of plant development, making the stress response even more complex. This complexity has a negative influence on the prediction and modelling of yields in diverse growing environments.

The measurement of most agronomical and physiological traits is technically difficult or very expensive when a large number of genotypes are to be tested. This is further complicated

by the fact that selection criteria based on indirect traits exhibit significant changes in diverse populations, necessitating analysis on more diversified genetic material at multiple locations.

Over the last two decades various high-throughput techniques have been elaborated for use in studying the drought and heat tolerance of wheat for the purposes of breeding, genetic analysis and model construction. High-throughput phenotyping systems make it possible to perform experiments where the control environment has optimum water supplies from sowing to harvest. Due to the complex nature of drought tolerance, this means that the data can be compared in an exact manner.

The chlorophyll content of the leaves is one of the indirect traits that can be used as an indicator of the photosynthetic capacity of the plant tissues. It was demonstrated in the present experiments that the senescence rate, the genetic variability of which has been reported by many authors, is particularly sensitive to drought and heat stress. In some genotypes the maintenance of photosynthetic activity in the flag-leaf during drought stress is manifested as higher yield at the end of the growing season, primarily as a consequence of the storage of a larger quantity of carbohydrates in the grain. The results show that the 'stay green' trait could be an efficient way of achieving drought tolerance during the post-flowering period, so genotypes in which the leaves remain green during the grain-filling period despite water deficit could be potential candidates for breeding. This does not mean the selection of late-maturing genotypes, but that of genotypes capable of accumulating assimilates during the grain-filling period even in the case of drought, due to their active leaf area. The use of spectral reflectance data to select for genotypes that stay green for a longer period could be a component in high-throughput phenotyping systems. Techniques for measuring the indirect traits chlorophyll content and reflectance allow large numbers of genotypes to be uniformly analysed in multiple location trials, resulting in detailed phenotypic data sets. This is important, because molecular breeding and genomic selection put great emphasis on genetic information, and large quantities of reliable phenotypic data are essential for genetic analysis. Efforts to breed for drought tolerance therefore need to give consideration to the identification of yield-enhancing alleles of these indirect properties, so that they can be transferred into other genotypes. In the case of cultivars with outstanding drought tolerance it would also be worth analysing the effect of minor alleles occurring at a frequency of less than 5%, since unique allele combinations or allele types may be related with good stress tolerance. This could help genetic mapping to make a significant contribution to the identification of markers located close to genes responsible for traits with agronomical importance. It is vital to elaborate an optimum GWAS model that takes into consideration the distorting factors arising

in the course of analysis. The aim of experiments set up over several years at multiple locations is to identify marker–trait links and QTL regions, but so far little success has been achieved in the analysis of complex traits. This is partly due to the fact that no single GWAS model can be equally effective in identifying real relationships for different traits, since the population structure may not have the same effect on all the agronomical and physiological parameters studied. In the present work most of the chromosome regions identified for various traits were only valid for a single environment. It could well be, however, that the given region has a real effect in other environments, which could become detectable if the experiments were set up in a larger number of environments and if the statistical analysis were refined. The identification of significant SNP–trait associations will require the detailed analysis of QTL × environment and QTL × QTL interactions. It can be concluded from the results of the present work that QTL regions correlated with drought and heat stress on chromosomes 1B, 3B and 5A contain several major genes, or genes with a pleiotropic effect. The positional cloning of QTL regions responsible for yield and the annotation of the genes in the 3B chromosome segment (<http://www.wheatgenome.org/content/view/full/407>) could provide more accurate information on the role of genes in stress tolerance and on their sequence. The better yield stability of lines with the KK<sub>2BL</sub>KK<sub>3BS</sub> allele combination also indicates that it is worth giving further attention to the role of the QYld.idw-2B and QYld.idw-3B QTL regions in yield enhancement. More detailed knowledge on the physiology and gene expression of the flag-leaf could lead to a better understanding of the significant positive correlation between polyamines and yield components.

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