SZENT ISTVÁN UNIVERSITY

FACULTY of AGRICULTURE and ENVIRONMENTAL SCIENCES

POSSIBILITIES AND LIMITS OF BREEDING WHEAT *(Triticum aestivum* L.*)* FOR DROUGHT TOLERANCE

PhD Thesis

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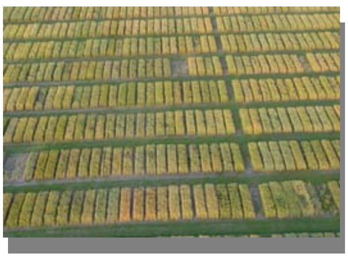

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1. PREVIOUS WORK, MAIN AIMS

The main factors limiting the world's plant production at present are environmental stresses. Drought, one of the most significant abiotic stress, makes plant production impossible on more than 26 % of the world' arable land, and also causes huge variations of the grain yield on the cultivated areas. Almost one-third of the land surface can be categorized as dry regions, but at the same time more than 10 % of the human population of the world lives and attempts agricultural production there. Water shortages (even in Hungary) are general crop-limiting phenomena. Winter wheat a long cropping-cycle, drought-tolerant culture, generally tolerates water shortages quite successfully, but its yield may fluctuate appreciably. Drought can cause extensive damage, as in 2003. Because of the extremely hot summer and low precipitation (476 mm in the whole year) in 2003, the wheat grain yield was 2640 kg ha^{-1}, while in the next rainy year (686 mm) it was 5120 kg ha^{-1}. Since the climate in Hungary is mostly influenced by continental climatic effects, the defense against water shortages has a long history.

Breeding for drought tolerance is one of the possible strategies against drought. Under Hungarian conditions, the main goal is the breeding of a variety which can adapt to drought at every stage in its life cycle. The Hungarian requirements for drought-tolerant varieties are different from those the continuosly drought-prone regions. A drought-tolerant plant is Understood here to be one whose relatively high grain yield will not decrease markedly due to drought stress. Accordingly our goal is not a genotype which survives extreme conditions but a high-yielding variety which will yield economically during a water shortage too. Among the difficulties in conventional breeding are the facts that drought resistance and yield capacity are mostly negatively correlated to each other, and in consequence of the complexity of drought resistance, the selection for certain characteristics will not prove successful, and there is no accepted method with which we could reliably test these characteristics.

On the other hand it is also desirable that its morphophysiological features should make the plant able to utilize water in the most efficient way under dry conditions, with minimization of water losses and simultaneous maximization of water uptake. Since the weather in Hungary varies considerably from year to year, it is a difficult task to determine the level of drought tolerance of the local wheat varieties involved in commercial production. The task is made even more complicated by the situation that most of the adaptation mechanisms are negatively correlated with productivity. Because of the large number of breeding materials featuring in a wheat-breeding system, we need special laboratory and field testing methods which are not affected by the weather conditions, which are fast and inexpensive, and which give reliable results.

Between 1989 and 1992, we carried out a survey to evaluate the feasibility of different testing methods in the selection of winter wheat for drought tolerance.
Among the fast and practically useful methods to be found in the international literature, we evaluated the germination tests, determination of the water-retention ability (WRA) of excised leaves, analyses of the proline content of live-wilted leaves, the use of anti-transpirants, plant density tests, chemical desiccation tests, and water potential measurements with a pressure chamber.
Between 2001 and 2004, we applied the two most effective tests, i.e. determination of WRA of excised leaves and the translocation test, and started a selection program based on these two testing methods.
The number of testing methods was increased in subsequent years by the inclusion of irrigation field tests, canopy temperature measurements and estimation of the chlorophyll content of the canopy.

We additionally evaluated the stability of the yield and the yield components of 70 old and new varieties originating in Szeged in yield trials in two successive years. We were looking for yield components that play a major role in stability and adaptation in response to changing environments.

The main aims of this thesis relate to:

- Tests of the feasibility of methods (based on physiological characteristics) for the selection of drought tolerance in winter wheat
- Evaluation of the selection work by these methods in a wheat-breeding program.
- Clarification of the roles of the major yield components of some old and new winter wheat genotypes originating in Szeged as concerns productivity and abiotic stress tolerance
- Determination of the correlation between the results of field and laboratory tests of drought tolerance,and the field data based on the results of multilocation field trials.
- Utiliziation of the selected testing methods in a winter wheat breeding program to improve drought resistance and general adaptability.

2. MATERIAL and METHODS

2.1. Testing methods for drought tolerance

2.1.1. Germination tests in osmoticum

In 1989, in 1991 and 1992 10, 46 and 74 genotypes of winter wheat (*Triticum aestivum* L.) and winter durum wheat (*T. turgidum var. durum* Desf.) were evaluated in germination tests in osmotic pressure media. Omotic stress was created with polyethylene-glycol (PEG) 6000 (PEG Karbowax 6000 Fluka AG.) solution. In the 1989 trial the germination rates of the varieties were tested under 3 conditions (control and 25% and 30% PEG 6000 solution), and in 1991 and in 1992 under 2 conditions (control and 25% PEG 6000 solution). The osmotic pressures of the 25% and 30% stress media were -0.82 MPa, and -1.0 MPa respectively. The surface of the tested seeds was disinfected prior to testing and, after a 24-hour cold (5˚C) treatment, they were maintained for 4 days at 24 ˚C. Germination was performed in Petri dishes, on filter paper wetted with 8 ml tap-water (control) or the mentioned stress solutions. During evaluation, a seed was considered to have germinated if its primery root length was 2 mm or more. The germination percentage was established from the data on 25 seeds in each of 4 repetitions.

2.1.2. Hydroponic test

Ten winter wheat varieties were tested in a hydroponic trial in 3 treatments and 3 replications in 1989. Osmotic stress was generated with PEG 6000 solution. The osmotic pressures in the 3 treatments were 0 MPa (control), -0.6 MPa and -0.82 MPa. Plants were kept in 600-cm^3 plastic containers in hydroponics. 15 plants were grown in each treatment and each replication. Loss due to evaporation was replaced with distilled water. In the first 11 days the plants were grown in KNOP solution (0.2 g l^{-1} KNO_3, 0.8 g l^{-1} $Ca(NO_3)_2$, 0.2 g l^{-1}KH_2PO_4, 0.2 g l^{-1} $MgSO_4x7H_2O$, 0,1 g l^{-1} $FePO_4$) in all the containers. At the 2-leaf stage, control plants were transplanted into fresh KNOP solution, while the plants subjected to treatments 2 and 3 were transplanted into KNOP + 20% PEG 6000 solution. At the same time, 5 plants from each treatment and repetition were harvested and evaluated. After 7 days, the control plants were transplanted into fresh KNOP solution, the treatment 2 plants were transplanted into KNOP + 20% PEG 6000 solution, and the treatment 3 plants were transplanted into KNOP + 25% PEG 6000 solution. Again, samples were harvested and evaluated. After 7 days, all the remaining plants were evaluated. During the evaluation, we measured the root and shoot lengths, the root and shoot weights, and (after drying at 60 °C for 4 days) the dry weights of the roots and shoots. Water contents were calculated and ANOVA was used for statistical evaluation.

2.1.3. WRA of excised leaves

Early in the morning, 5 healthy, and similar-sized flag leaves (from each replication) were harvested from a 4-replicated field trial which involved 46 winter wheat varieties. Leaves were excised under the leaf sheet, wrapped in polyethylene bags and transported to the laboratory, where the fresh mass (FM_1) of the leaves was measured and they were placed into Petri dishes filled with tap-water for 24 h. After the 24 h saturation, their turgid mass (*TM*) was measured. The leaves were then kept in a controlled environment (24 $\pm$ 2 ˚C, 65% RH) for 8 h, after which the desiccated mass (FM_2) was determined. The dry mass (*DM*) was measured after a 24 h drying at 70 ˚C. The following formula was applied to calculated the fresh (RWC_1) and the desiccated relative water content (RWC_2):

$$RWC = \frac{FM - DM}{TM - DM} * 100(\%)$$

The WRA is the ratio of the initial and desiccated relative water contents. The WRA data were evaluated by ANOVA. Measurements were repeated twice from late booting until heading. This test has been applied as a selection method since 2001.

2.1.4. Proline analyses of live-wilted leaves

With the testing method of Pálfi et al. (1988), proline accumulation can be compared when excised wheat leaves undergo lethal wilting in a controlled environment, for a fixed period. In 1988, the proline accumulation of 11 wheat genotypes' was studied in 2 variety groups. 24 Fresh shoots of each variety were harvested from the nursery of the Cereal Research Institute early in the morning and transported to the laboratory. The leaves were excised and taped on filter paper. These excised leaves were then kept under high relative humidity (RH) (90%), at constant illumination (5000 m^{-2} cd s) and temperature (26-28 ˚C) for 60 h. The RH was next decreased to 60% and maintained for a further 12 h. With this method the excised wheat leaves lost their moisture content in the same way, and reached their lethal water deficit at the end of the third day. The live-wilted leaves were cut into small pieces, dried at 90 ˚C, and powdered. The powder was dried at 105 ˚C and stored in glass containers. The very same procedure was applied to the excised spikes. Proline contents were determined by paper chromatography.

2.1.5. Water potential measurements

The weight and water potential data on excised flag leaves were measured for 15 bread wheat and 1 triticale genotypes. These genotypes originated from different parts of the world, both from arid regions and from western Europe. For the measurements, whole plants were harvested from the nursery and transported in polyethylene bags to the laboratory early in the morning. Three leaves of each genotype were excised and the fresh mass was weighed. Following this, the turgor pressure was determined. Both measurements were repeated at least 7-8 times in order to have enough data-pairs to characterize the dynamics of the water loss. The water potential (turgor pressure) was measured with a P.M.S pressure chamber (PMSI Co., Corvallis, Oregon). Following these measurements, the leaves were kept between wet filter papers for 24 h and the turgid mass was then determined. After complete drying the dry weight of the leaves was determined. Calculation of the relative water content data, allowed pressure-volume curves to be drawn, and a regression line was fitted so as to determine osmotic potential of the samples at full turgor, and the water potential at the point of turgor loss. The water saturation deficit (*WSD*) at zero turgor, and the ratio of the turgid mass and the dry mass of the flag leaves were established. The data were evaluated by ANOVA.

2.1.6. The use of an anti-transpirant

The effects of an anti-transpirant (Phytowax MAFKI, Veszprém) on the thousand-kernel mass (TKM) and on the morphophysiological characteristics, were studied in a field trial. In this trial, 19 winter wheat and 3 winter durum wheat genotypes were planted in a 4 replicated trial. The 6.5 m^2 plots were planted by an 8-row Wintersteiger Øjyord Plot Drill and divided into 2 equal parts. Phytowax was applied at the recommended concentration (10-fold dilution) by spraying the treated half of each plot, 5 times during the vegetation period. Our goal was to decrease the effects of a presumed drought stress, and to find some measurable advantageous influence of the decreased transpiration. During the evaluation, we compared the plant height, the TKM, the grain yield, and the heading time. Data were expressed as percentage of the control to make them comparable. ANOVA was performed with these control percentage data.

2.1.7. Sowing density test

In 1990, 12 winter wheat varieties were tested in a 4-replicated field performance trial; they were planted at 2 sowing densities at the Kiszombor nursery. The sowing density of the control plots was 550 germs m^{-2}, whereas it was 1650 germs m^{-2} in the stress treatment. The plots were planted by an 8-row Øjyord sowing machine. Our aim was to evaluate the high-density stress tolerance of the genotypes, which has a significant drought stress component. In spring, we checked the plant densities of both treatments. During the vegetation period, we scored the changes in the main morphophysiological parameters (heading date, plant height and lodging) due to the extreme density. Before combining, we took samples to determine the TKM. The data were also expressed as percentages of the control.

2.1.8. Chemical desiccation in the field

The field trial was carried out in 1990, 1991 and 1992 in the breeding nursery of the Cereal Research Institute. In the first 2 years, plots were planted by a Seedmatic (Wintersteiger), 6-row self-propelled sowing machine, while in 1992 all the varieties were grown in a 4-replicated 1-row plot (sown by a Wintersteiger Plotspider precision planter). The number of varieties in the desiccation test was increased continuously: in 1990, 46; in 1991 74; and in 1992 110 varieties were tested. The plots were divided into 2 equal parts. The heading time and anthesis were determined exactly and desiccation treatment was applied in the very same phenological phase, 14 days after the anthesis of each genotype. As desiccant 2% sodium chlorate ($NaClO_3$, Fluka A.G.) was applied, by a sprayer until full wetting of the shoots, (this chemical is a contact one). The yields of half plots were harvested by a Wintersteiger Hydrostatic plot combine at the time of full ripening of the control plots. Before harvesting, small samples were taken to evaluate the difference in TKM. The decrease in TKM was expressed as a percentage of the control. This test has been used as a selection method in wheat breeding since 2001.

2.2. Drought tolerance nursery

Our field tests were carried out in a 3-replicated completely randomized block design selection nursery. These tests comprise functional parts of our breeding work, and are incorporated in our selection procedure. The tested genotypes are the advanced lines and variety candidates of our breeding program with check varieties. In 2001, 2002, 2003 and 2004 55, 70, 100 and 100 genotypes, were tested. In this trial, 2-row plots were planted in 3 replications (size 0.5 m^2) in a Seedmatic system. One week before heading and 1 week after heading, flag leaves were harvested in order to determine the WRA of the excised leaves. The WRA was determined according to the protocol detailed in section 2.1.3. The translocation abilities of the stem reserves were determined by the desiccant test described in the protocol in section 2.1.7. We have applied irrigation tests since 2002. Irrigation was started before heading and continued until ripening. Depending on the rain conditions in 2002, 2003 and 2004, 50, 140 and 150 mm water was applied for irrigation. We measured the changes in the different morphophysiological characteristics (plant height, heading date, and TKM), the canopy temperature and the chlorophyll content. The temperature of the canopy surface was measured with a hand-held infrared thermometer (Crop-Track, Spectrum Inc.), while the chlorophyll content was measured with a CM-1000 chlorophyll meter.

2.3. Multilocation trials

In our multilocation yield test network, our 4-replicated trials are planted at 9-11 locations yearly, to acquire information about the adaptation abilities of our advanced lines. Each year 40-60 genotypes participate in these types of trials. On the basis of the meteorological data (precipitation, cumulated heat hours of sunshine), we can calculate the stress susceptibility index (S) for comparisons between stressed and stress-free locations and between the average grain yields, and the results of the drought tolerance tests at the 9 locations.

2.4. Performance trial in the field of wheat genotypes originating in Szeged

The yield performances and the changing of yield components of 70 old and young bread wheat and durum wheat genotypes were tested in 2 successive years (2002/2003 and 2003/2004) in a four replicated field trial with two treatments (control and high input). The control plots received only basic fertilizing (70+70+70 kg NPK active ingredient) and insecticide (Enduro, Fendona, Sumi Alfa) treatment. For the high input treatment 120 kg more N fertilizer and 2 additional fungicide treatments were given. In both cases, harvesting was performed at full ripening at the optimal time with a Wintersteiger plot harvester. Before harvesting, whole plant samples were taken to determine the yield components, the biological yield and the harvest index (*HI*).

Besides the grain yield, we evaluated the grain yield per head, the grain number per head, the number of spikes at unit area, plant height, the TKM, the biological yield and *HI*. The above-mentioned characteristics were based on the data on 50 shoots. By using near infrared (NIR) fast method, we determined the protein content, the wet gluten content and grain hardness data, too.

2.5. Breeding system

Our breeding system is based on a classical pedigree method; genetic variance is generated by crossing, while uniformity is achieved by multiple selection of individuals and the head-line system. Crossings are done by hand. The F_1 generation is mostly planted in the greenhouse, so from the crossings the F_2 generation can be planted to the nursery within 1 year. We sow the F_2 generation in spaced planting and harvest the mean spikes of those individual plants which fit the basic morphological and phytopathological demands.

The grain yield of the mean heads will be planted to head rows. Uniform head rows will be harvested, and bulk seed will be planted in preliminary information yield trial plots (6.5 m^2) without replication, but in parallel genetic purity will be maintained in head rows. Best lines will be tested in 4-replicated yield trials. At this stage, all the lines have 1 seed multiplication plot and 48 head rows. Those lines which prove best in this trial will take part in a multilocation yield test. The 4-replicated yield trials will involve planting at 8-11 locations of the most important wheat-growing areas in Hungary to test the yield stability and adaptability. Only the very best ones will take part in the official state trials of the Registration Office. Selection work is facilitated by pathology tests, frost and drought tolerance tests, and detailed evaluations of technological quality.

3. RESULTS

3.1. Survey on the feasibility of application of the drought tolerance testing methods on winter wheat genotypes

3.1.1. Effects of genotype, year and osmotic stress on the germination of wheat grains

In the 1989 test, germination rate decreased by 15.8% and 46.4% in the 25% and 30% PEG 6000 solutions respectively. The results show that, by change of the germination media, the rank of the varieties was changed completely. In the 1991 trial, the germination percentage was lower by 0–44% in the osmoticum (25% PEG 6000 solution), than in the tap-water control. The treatment decreased the germination rate by 15% on average for the varieties ($LSD_{5\%}$=9.04).

The trial was repeated in 1992. This trial had 2 treatments (a tap-water control and 25% PEG 6000 solution) and we found significant differences between the results of the 2 trials. When we compared the results on the same varieties in both years, we did not find a correlation between the 2 data sets (r = 0.119). The reason was that the weather was different before the harvesting in 1989 and in 1992, and, due to the genotype x environment interaction, the varieties reacted differently.

Germination tests are fast, simple and easy-to-perform, but the information value of the results is very poor. By using these tests, we can screen the water-absorption ability of the caryopses of the varieties under osmotic stress, although this characteristic is not related directly to the drought tolerance of the plants. The germination percentage is also affected by different heritable and non-heritable effects, such as the health conditions of the seeds, dormancy, or special year effects. The results of germination experimentsindicate, that these tests are not suitable for a reliable selection for drought resistance.

3.1.2. Genotype dependece of shoot and root growth in a hydroponic test.

This trial gave us an opportunity to compare the responses of 10 varieties to osmotic stress as regards the dynamics of root and shoot growing. This test allowed observation of the development of the shoots and roots of the control plants and those subjected to stress treatments. The varieties reacted in significantly different ways. The stress media containing 20% and 25% PEG 6000 solutions decreased the shoot growth by 24.4% and 30.6%, and the root growth by 30% and 34% respectively. The extents of shoot growth depression were similar among the tested varieties, whereas the root growth depressions differed more appreciably. The root growth increase in the case of Jubilejnaja-50 was probably a reflection of an adaptation mechanism to lower moisture conditions. However the responses of plants to drought in the field and in hydroponics are different; they even involve differencies in morphology. Thus the results from these tests cannot be used directly as selection information; the correlations between the results must be validated. We did not find any correlation between the results of the germination tests and the changes due to the osmotic stress in hydroponics. The test furnishes reliable information concerning the early adaptability of genotypes to osmotic stress, but again we could not find any links between this trait and the adaptability of a mature plant from the aspect of its tolerance to drought. For the reasons discussed above, the test is not suitable for widespread application in the selection for water stress tolerance.

3.1.3. The grouping of genotypes based on the WRA of the excised leaves

In 1990, after a 24-hour desiccation, we detected considerable differences in the degree of water loss among the examined 46 varieties. The average water loss was 74%. The experiment was repeated in 1991 and in 1992. On average, the degree of water loss in 1991 was 64.6%, and in 1992 was 62.7%. The correlation calculation indicated a close connection between the 1990 and 1991 (r = 0.7223**) the 1990 and 1992 (r = 0.6276**) and the 1991 and 1992 data (r = 0.3885*). The WRA of the excised leaves is an important characteristic in drought tolerance, because it relates to an important mechanism for decreasing the water loss of the plant. This trait is independent of the number of stomata, which can be found mainly on the leaves, as they close because of the loss of turgor after the leaves are cut off. This method tests the water-permeability of the epidermis. In this case, morphological traits such as leaf pubescence or the presence of epicuticular wax are of special importance (the water loss can decrease by 10%). With this method, which is fast, very simple and non-laborious we can compare hundreds of lines within a short period of time. Measurements can be made at any phenophase and the results are quickly obtained and informative. Before heading, the results are more informative; the differences between the genotypes more numerous and articulated.

3.1.4. Genotype dependence of accumulation of proline of live-wilted leaves

The live-wilting method was carried out on 2 variety groups (early and medium-early groups) during anthesis. Despite the high RH, the excised shoots lost a considerable amount of water after even on the first day. Due to the drought stress the leaves and the spikes accumulated proline which slowed down additional water loss. We found a more marked difference as regards the proline accumulation in the spikes than that in the leaves, in spite of the fact that the leaves accumulated 3-4 times more proline: for GK Szőke 3.2%, for Jubilejnaja-50 3.1% and for GK Örzse 2.3%. As an osmoticum, proline plays an important role in protecting enzymes and cell membranes. This test can be used very well in the selection process. However the large-scale application of the test is limited because of the laborious live-wilting procedure and the preparation for analyses. Proline levels can be measured much more precisely by means of HPLC.

3.1.5. Genotype dependence of osmotic potential

We observed considerable differences between the selected genotypes in the examined characteristics. The varieties originating from drought-prone countries and the drought tolerant GK Tiszatáj m. from Hungary proved to have higher osmotic potentials. The calculated osmotic potential was lowest for the Kobomugi, Mv-8 and NE 83/T varieties. Plainsman V, Pitic 62, T 79 and Tiszatáj m. had the highest level at full turgor.

Besides the water potential, one of the most important features is the degree of water saturation deficit at which the leaves lose their turgor due to the water shortage. The results partly justify the results of field trials and the water retention and desiccation tests. The results of these tests, (according to the analysis of pressure-volume curves) demonstrated that the sensitive genotypes proved to exhibit less effective osmoregulation. Tiszatáj m. was tolerant in most tests. The osmoregulation makes production possible in the event of drought stress. As opposed to the previous tests, the analysis of the pressure-volume curves indicated that OK 84343 was the only genotype which was susceptible.

3.1.6. Benefits and disadvantages of the field application of anti-transpirants

The differences in the examined characteristics between the varieties involved in the experiment were irrelevant. On the treated half of the plot, the plant height increased by 0.57 cm, which was practically negligible ($LSD_{5\%} = 4.65$). The calculated control percentage revealed that the treatment affected the varieties in different ways. The plant height of some of the varieties increased, whereas that of others decreased. We obtained the same results during consideration of the 2 examined crop characteristics, the TKM and the grain yield on the plot halves. The results permit the conclusion that the applicability of this method is limited, because it depends strongly on the season. Under stress-free conditions, the anti-transpirant reduces the degrees of vapor/water transport and photosynthesis relative to the control plants. The grain yield and the TKM may decrease because of this.

3.1.7. Testing the effect of high plant density in the field

Of the examined morphological characteristics, the dense sowing affected only the plant height, increasing it slightly. Of the yield components, the dense sowing decreased the TKM, by 6.3% on average in the field experiment. Undoubtedly, besides drought stress, many other stress factors can exert an effect in this experiment; nevertheless, this treatment had the least effect on the examined properties. This testing method is strongly influenced by the weather, which can restrict its applicability.

3.1.8. Effect of chemical desiccation on the TKM

The TKM measured on the untreated plot half in 1990 was between 32.7 g and 50.6 g with an average of 42.2 g. On the plot half treated with sodium-chlorate solution, the average grain mass was 29 g, i.e. the desiccant treatment caused an average decrease of 32.2% in the TKM ($LSD_{5\%}$ = 5.23%). Desiccant treatment decreased TKM by 29% in 1991, and by 31% in 1992. The order of the varieties was similar in the trials in the 3 individual years. The correlation analysis of the data indicated a strong correlation of the identical varieties in the 3 years. Between 1990 and 1991 r = 0.8151***; between 1990 and 1992 r = 0.7512**; and between 1991 and 1992 r=0,7834**.
Our results, did not prove a correlation between the TKM and the extent of the decrease. There were genotypes with high and low TKM among both the tolerant and the sensitive varieties.
The test is designed to compare the translocation abilities of the stem reserves in different cultivars. In wheat growing, the most frequent type of drought stress is the post-anthesis stress at the end of June and the beginning of July. By this time, all the yield components are formed except for the TKM. A water deficiency may cause grain yield depression due to seed shriveling as high as 20-50%. With the help of this highly reproducible test, we are able to demonstrate significant differences between certain wheat genotypes in terms of the translocation capacity of the stem reserves. In practice, this test can be performed independently of the weather conditions and in different years. It allows selection of the best and poorest individuals as concerns translocation ability. This trait is extremely important during a post-anthesis water deficiency stress, since in the absence of photosynthesis, plants can rely only on their translocated stem reserves during seed filling. By this time, all the yield components have already apart from the TKM.

3.1.9. Correlations between the results of the applied selection methods

In most cases, we did not find a correlation between the results of the tests, or at most only a very slight correlation. The results of the different tests were expressed as percenteges of the control and the correlation calculations were carried out in this way. The closest correlation was that between the TKM change caused by the antitranspirant treatment and the decrease caused by plant density test (r = 0.7122***). However, in this case both factors exerted only a slight effect on TKM. The correlation coefficients revealed a weak positive connection even between the results of WRA and the application of the anti-transpirants, and between the WRA and the results of the chemical desiccation tests (r = 0.3541* and r = 0.2382).
The water saturation ability of the grains, the WRA of the leaves and the tolerance to stresses of dense sowing and high translocation ability are in all probability independent hereditary characteristics. Accordingly it is not surprising that, in a series of experiments in which numerous varieties are tested (the strength of these characteristics may appear in the most diverse variations), these characteristics do not show a close correlation.
So from among the methods tested by us the results of the water-retention ability of the excised leaves and the results of desiccation tests modeling the late water shortage stress used in the nursery gave the most useful results.

3.2. Drought tolerance tests in the field

We detected considerable differences between the tested genotypes as concerns examined characteristics in all years in the period 2001-2004 in the drought tolerance selection nursery.

3.2.1. Survey on WRA of excised flag leaves

The largest differences between the relative water content of the excised flag leaves were seen in 2001. The late varieties generally had the highest water content. In 2002, we examined 70 advanced lines and 26 varieties. Relative to the initial water content, the genotypes were able to absorb on average nearly 20% water, and they retained 55% of their water content due to the treatment. In consequence of the dessiccation, the flag leaves of the tolerant genotypes preserved 80%, while the worst ones preserves only 21 % of their water content ($LSD_{5\%}$ = 19.95).
In 2003 we tested 100 genotypes. Compared to the initial water content the genotypes were able to absorb on average only 7% water, and they retained 57% of their water content due to the treatment.
In 2004 similarly as in the previous year we all experienced considerable differences between the examined 100 genotypes both in the initial water content, and in water loss. Depending on the year and the time of the test, relative to the initial water content, the genotypes were able to absorb on average 7–20% water, and they preserved 40–50% of their relative water content due to the treatment. The flag leaves of the tolerant genotypes preserved 77% of their water content, whereas the worst ones retained only 21% due to the desiccation. We used the results of these tests during our selection work.

3.2.2. Results of desiccation tests

We carried out these selection tests on the same varieties as in the previous experiments each year between 2001 and 2004.
In 2001, the desiccation treatment caused a 27.0% decrease in TKM on average for the varieties ($LSD_{5\%}$= 9,5).
In 2002, the TKM decreased by 34.6% on average due to the desiccation treatment. 13 advanced lines proved to give better results then the positive control.
In 2003, as a result of the desiccation of the leaves, the TKM decreased on average by 38.8%. The best varieties were the CY-45 and Kharchia, the TKM of which decreased by only some 12.5%, while for the most sensitive line the TKM decreased by more than 42.5% due to the stress.
In 2004, the difference between the results of the desiccant treatment and the control treatment was much greater. The TKM decreased on average by 34.1%. In the control treatment, the highest TKM was 53.2 g, and the lowest one was 32.7 g. On the treated plots, the highest value was 40.35 g, the lowest one was 18.96 g.
A comparison of the examined 4 years, indicates that, despite the differentcies in the genotype combination and the weather, the average TKM decreases were similar (27; 34; 38 and 34 %). The tolerant genotypes exhibited a low measure of TKM depression in all the 4 years. These results helped our selection decisions.

3.2.3. Irrigation trials in the field

From 2002, irrigation tests were carried out. From 2003, for selection purposes at noon on the hottest days we measured the canopy temperature of the plots. In that year, temperature of the stress-free (irrigated) plots was 22.8 ˚C on average, while that of the control plots was 26.3 ˚C. For the irrigation treatment the interval between the temperature extremes was narrower, than that for the control plots. Drought elevates the average temperature by 3.6 ˚C.
Irrigation trials were also carried out in the year 2004 but because of the rainy season we could not detect any irrigation effect either in plant height, or in in grain yield.

To summerize our results, in dry years the irrigation led to increases in: plant height, grain yield and TKM, delayed heading and ripening, resulted in a higher chlorophyll content and, under heat and drought stress the plant canopy had a lower temperature. In only one of the 3 examined years were we able to measure appreciable differences in the studied characteristics.

3.3. Correlations between the productivity and stress-tolerance of wheat genotypes

In 4 years (2001-2004), we tested our advanced lines in our multilocation (9-10 locations) field trial network. On the basis of the meteorological data, we chose 4 different locations. From the aspect of the average yield records at these 4 locations, from among the four years, 2004 was mostly free of drought stress, while 2003 was hit by the most serious water shortage. The highest grain yields were observed in 2004, when the average at the 4 locations was 7.58 t ha^{-1}. The lowest yield was found in 2003, with an average grain yield of 4.61 t ha^{-1} i.e. hardly more than 61% of the 2004 level. Significant differences in the grain yields of the genotypes were found in all the 4 years at the 4 locations. In each year we calculated the stress index and superiority index (S values and P_i values) from the grain yield data at the 4 locations.

In 2001, correlation analysis did not indicate any connection between the yield, the S value and the results of the most important drought tolerance testing methods.

We obtained similar results in the irrigation test carried out in 2002. There was no close correlation between the results of the different drought tolerance trials. However, in the same experiment, we found a slight (5%) significant correlation between the increase in grain yield due to irrigation and the yield depression caused by chemical desiccant treatment (r = 0.2468*). The WRA of the excised flag leaves and the translocation ability of the stem reserves in the desiccant experiments did not exert a considerable effect on the development of the yield and we did not find any correlation with the calculated indices.

2003 was a drought stress year in Hungary. This is probably the reason why we found the closest correspondence between the results of the physiological tests and the results of the multilocation yield tests in 2003. In that year there was a close (medium-level) connection between the S value calculated according to the yield of the multilocation yield tests and P_i (r = 0.6247***). The tests revealed a close correlation in the case of the TKM and the yield change of the plots in the dessication tests and irrigation tests (r = 0.6278*** and r = 0.8363***). A medium-close correspondence was also found between the P_i values measured in the multilocation tests, the yield depression of the desiccant test, the irrigation effect, and the calculated S value in Szeged. In 2003, the WRA of the excised leaves did not affect the tolerance to drought; the depression of the different measured characteristics was mostly caused by the post-anthesis drought stress.

2004 was one of the rainiest years of recent times and it resulted in a record grain yield throughout the country. The main reason for the differences between the yield results in the multilocation yield tests was not the different levels of rainfall but the quality of the agrotechnology and the quality of the soil. Therefore we found practically no correlation between S value, the P_i and the yield results at the 9 locations, nor between the WRA of the leaves and the translocation ability. We found a slighter than medium-level connection between the translocation ability (a TKM depression) in the dessicant test and the WRA of the excised flag leaves. We found a medium-level connection between the thousand kernel mass and the yield depression measured in the same desiccant test (r = 0.6278***), and also a weak correlation between the WRA of the flag leaves and the temperature of the canopy surface measured under stress (r = 0.2863**).

3.4. Study of the roles of certain yield components in the productivity and stress tolerance of wheat

3.4.1. Effects of the year on the grain yield and yield components

Because of the cold winter and dry summer in 2003, the additional input was not returned. We did not observe any yield increase, and even a non-significant yield depression occurred in the treated plots. In contrast with this, in 2004, the high-input treatment led to an 860 kg ha^{-1} (12.6%) yield increase in the average for the 70 varieties. The year (the ample precipitation) affected the grain yield much more than did the agrotechnological treatments. The grain yield of the bread wheats increased by 127%, and that of the durum wheats by 300% because of the thinning of the frost-sensitive varieties. Among the yield components, not only the number of heads, but also the number of seeds per spike (33% increase), and the TKM (a 21 % increase) changed significantly. The grain yield per spike increased by 26%. Of course not merely the grain yield and its components changed in consequence of the different weather conditions. The plant height (a 66,5 % increase), and the green mass (the total dry mass of the above-ground parts of the plant) (172% increase) changed markedly too. The increase in the green mass was the largest among the 70 genotypes tested. Since the increase in this trait exceeded that in the grain yield, the *HI* decreased slightly in 2004. The average *HI* for bread and durum wheats was 50.4% in 2003 and 49.8% in 2004.

3.4.2. Changes in yield and yield components in varieties from the last 60 years

We formed 5 groups of varieties on the basis of the date of their registration and the starting time of their commercial growing. We could conclude that the grain yields and yield components changed in time as compared with the average data for the different groups. The comparison demonstrated that the grain yield increased significantly due to the breeding. For durum whet, the very same results were found. While the average grain yield of the wheat cultivars registered before 1970 was 3.8 t ha^{-1}, that of the cultivars after the 1990s was 5.6 t ha^{-1}. Similarly, the biological yield also increased, but since the grain yield rose, the *HI* increased. The survey of the yield components indicated, that the most significant development was observed for the number of productive heads per square meter and the number of seeds per spike. The TKM was the only yield component which changed negatively during the genetic advance in the 60 years.

3.5. Using the testing methods in the breeding of winter wheat

The main methodological steps in breeding for drought tolerance are the same as those for variety production: selection according to the most important agronomic parameters.

We must look for crossing partners which have good adaptability, and types which are close to our ideotype. Via recurrent selection polygenic advantageous traits can be combined in segregating populations, while the important characteristics which are inherited monogenically can be translocated by back-crossing. In the F_2 generation, besides the basic agronomic parameters, we look for morphological traits which are important in adaptation to drought. We prefer awns on spikes, healthy filled seed, erect leaves, epicuticular wax and a hairy epidermis. We discard individuals with heavy stress symptoms such as leaf firing, serious turgor loss and leaf rolling, fast senescence and seed shriveling. In the segregating head-line selection (F_3-F_5) we always discard those lines which are susceptible to drought according to the visual scoring (leaf firing, leaf rolling, drying, shriveled grain etc.).

In later generations (F_5-) in parallel with the yield performance tests, field tests of drought tolerance (chemical desiccation, WRA of excised leaves, and the thermometry of the canopy) and visual scoring of the mentioned characteristics and stress symptoms are carried out. In this way, besides the main agronomic parameters (yield capacity, resistance, and technological quality), ample

information will be available concerning the stress tolerance of the candidate advanced lines until their registration in state trials.
Among the recently registered winter wheat genotypes, the adaptability and drought tolerance of the varieties GK Hunyad (*GK Mura/GK Kende*), GK Békés (*GK Kalász/GK Garaboly*) and GK Csillag (*GK Véka/GK Kalász*) are above the average. GK Hunyad proved to be drouht-tolerant according to the WRA and the canopy temperature under severe drought and heat stress. GK Békés and GK Csillag have excellent yield potentials and yield stabilities and are highly drought-tolerant.

3.6. New results

a) We have surveyed the efficiency and applicability of several testing methods in selecting for drought tolerance. According to the criteria, we have found 3 methods that are useful for these purposes: the water retention test, the translocation test by chemical desiccation and canopy temperature measurements.

b) We have elaborated the adaptation and introduction of the chosen selection methods in our pedigree breeding system. We apply these selection methods in the selection of the parental lines and the advanced lines.

c) We have analyzed the strengths of correlations between the results of laboratory tests and the multilocation yield tests. We have established that the drought test results correlate more closely with the results of the multilocation yield tests in drier years.

d) We have assessed the roles of the most significant yield components in the productivity and drought stress tolerance of 70 old and modern wheat cultivars. The results of our survey have revealed that the superiority of the modern varieties in grain yield is due to the higher number of spikes and the higher number of seeds per spike.

e) Based on these testing methods, we have developed a novel breeding system with which we can select for drought resistance as a routine. The methods applied can easily be incorporated into our working pedigree breeding system. The success of our research work is reflected in the new drought-tolerant wheat varieties released. GK Hunyad, GK Békés and GK Csillag varieties were registered in 2005 and have been grown successfully both in Hungary and in foreign countries.

Practical results and patents:

I started my activity in wheat breeding in 1987. So far I have participated in the breeding of 31 registered varieties and 96 variety candidates. In the case of durum wheat (*Triticum turgidum* L. *var. durum* Desf.), I am the co-breeder of 1 registered variety and 6 candidates. I am also the co-breeder of 2 registered triticale (*Triticosecale sp*.) varieties.
All the registered cultivars have been patented, or are currently under the patenting process. I am the major breeder of 3 registered bread wheat genotypes: *GK Hattyas, GK Verecke* and *GK Hunyad.*

4. CONCLUSIONS AND PROPOSALS

In our program, a genotype is considered to be drought-resistant if it yields fairly well under dry conditions, and its yield does not decrease, or not significantly, in drought-stress years. Our goal is not a survivor extensive genotype, but an intensive cultivar with a high yield potential and an economic, stable grain yield under even unfavorable conditions. The improvement of the yield

under stress conditions must therefore combine the high yield potential and specific factors able to protect the crop against reductions due to different stresses.
Breeding for drought tolerance begins with the selection of the parental lines of new crossing combinations. In younger, segregating generations (after the F2 generation, in the head-rows of the F3-F5 generations), we select on the basis of the visual scoring of morphological and phenological characteristics that are advantageous under dry conditions, for in these generations physiological tests cannot be carried out because of the large number of accessions. Field selection methods are used in our advanced line selection. We select in the breeding material by indirect testing methods for drought resistance or for characteristics which could be adventegous under dry conditions.
Late drought stress is modeled by desiccant treatment. In the irrigation trials, we measure the changes in different agronomic characteristics, and the temperature of the canopy surface. Under laboratory conditions, we can determine the WRA of excised flag leaves. All the results of these testing methods contribute considerably to making our selection work more effective.
Genotypes found to be tolerant to water shortages will be registered as new varieties only if they prove their stability in yield and technological quality as well.

Field testing methods were expanded with an automatic rain shelter, built to our design and installed in 2006. Since this equipment has rain sensors, shading is limited to the time of raining. In this way, this *in situ* trial can be conducted mostly independently of the weather conditions and we can study the effects of a real water shortage. The size and inner height of this tent allow machine cultivation and sowing of the trials. Side wetting is prevented by a circular drainage ditch around the tent. Automatic meteorological stations in both treatments measure and record the air temperature, the air humidity, the dew point, the solar radiation and the temperature and the moisture content of the soil every hour. The tested genotypes are the check varieties and the advanced lines which are undergoing selection.

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