



SZENT ISTVÁN UNIVERSITY

**The phenotype and genotype results of a wheat drought
tolerance mapping population**

Main points of the PhD thesis

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1. BACKGROUND AND AIMS

Among the abiotic stresses, causing yield losses, drought is the most damaging factor and drought tolerance is one of the most difficult traits to improve by breeding. Therefore, increasing crop yield, under drought conditions is one of the most important challenge faced by the breeders. Owing to the climate change, intensity and frequency of drought periods are expected to increase, and act as a difficulty for sustainable crop production.

Drought tolerance is a quantitative trait influenced by environmental factors and numerous genes. Improving drought tolerance in plants requires complex approach using genetical, biological and plant breeding knowledge.

Like in other wheat growing regions of the world, climate change emphasized the importance of drought stress in Hungary too. Despite the achievements in basic and applied research, the physiological and genetic background of drought tolerance has not yet been fully explored. The genotype response to the lack of water depends on many factors, so breeding of reliably tolerant genotypes requires complex approach. Phenotyping results of drought tolerance from glasshouse and field experiments provide data for breeders. Furthermore, identification of chromosomal regions responsible for quantitative traits, provide information of the genetical background of drought tolerance.

Drought tolerance studies in the Cereal Research Non-profit Ltd. of Szeged, has a decade-long history. My PhD fellowship provided me an opportunity to join this research work. During the three-year experimental period (2014 - 2016), 135 DH genotypes derived from the cross, Plainsman V/Cappelle Desprez and 29 varieties were tested for water withdrawal in glasshouse conditions. In addition, the DH population was tested in a field test for drought tolerance. Our goal - besides phenotyping - was to select tolerant and sensitive genotypes and the genetic mapping of the population with molecular markers.

The aims of the study

- Glasshouse phenotyping of the Plainsman V/Cappelle Desprez DH mapping population under optimal watering and water withdrawal conditions to detect the drought tolerance of the genotypes.
- Selection of drought tolerant and sensitive genotypes by the yield loss performance under stress conditions.

- Glasshouse phenotyping of wheat varieties of different origins to evaluate their drought tolerance.
- The field test of the Plainsman V/Cappelle Desprez DH mapping population under normal rainfall and rain out shelter to confirm the glasshouse experimental results.
- Detection of QTL regions in the mapping population based on the phenotype and genotype data.

2. MATERIALS AND METHODS

2.1. The Plainsman V/ Cappelle Desprez DH population

The winter wheat population used in this work consisted of 135 DH lines derived from a cross between the drought tolerant Plainsman V (Pl) and the drought sensitive Cappelle Desprez (CD) varieties. The DH population was developed by anther culture from the F1 generation. Plainsman V (year of release, 1974) is an American hard red winter wheat with high protein content and drought tolerance. Cappelle Desprez (year of release, 1946) a high yielding variety in intensive farming, but sensitive to drought, was bred in France.

2.2. The tested varieties

Twenty-one wheat varieties released by the Cereal Research Non-Profit Ltd Szeged, Hungary (CR Ltd) and eight varieties of different origin were studied in glasshouse. The drought tolerant Plainsman V and the sensitive Cappelle Desprez genotypes were set up as control varieties under well-watered and drought stress conditions.

2.3. The greenhouse phenotyping experiments

The phenotyping experiments were carried out in 2014-2015-2016 in the glasshouse in Szeged. The one-week-old seedlings were vernalized at 4°C in a cold chamber, under continuous dim light for 6 weeks. The pots contained soil mixture (526 g peat soil, and 1340 g dry sandy soil) and 4 g controlled release fertilizer (Osmocote Exact, Scotts Company, Marysville, Ohio; including N16+P9+K12+2,5MgO). Each genotype was planted in two pots/treatment and three plants/pot, so we had together six replicates/treatment. The phenotyping experiment was carried out in controlled conditions using the standard glasshouse wheat growing program.

2.4. Water management

At the time of potting, the wheat plants were watered with 100 ml water/pot to ensure the adaptation of seedling. We irrigated twice a week.

The well-watered (WW) plants were irrigated to the 60% of the soil water capacity. Five WW PI and five CD pots weight was measured by a computer-controlled scale (GSE model 350, 6.000±1 g), which gave the average irrigation norm. In the WW treatment each plant during the experiment got 1575 ml (2014);1025 ml (2015); 1070 ml (2016) water.

Water consumption (WC) treatment: plants were weighed by a computer-controlled scale, and amount of used water was resupplied to maintain the required soil water status. WC was calculated by summarising the water regime after harvest.

In the water stressed (WS) treatment, we irrigated the plants to the 20% of the soil water capacity in the same way as in the WW treatment. During the growing season the stressed plants got 575 ml (2014); 275 ml (2015), 259 ml (2016) water.

2.5. Recorded traits

Days to heading, plant height, the aboveground dry biomass, spike length, non-threshed spike weight, number of grains/mean ear, and total grain yield/plant were measured. At the end of the experiment each root from the pots were carefully washed out and dried to measure root dry mass. Harvest Index - the ratio between harvestable yield and aboveground biomass – and stress Tolerance Index (STI) was calculated using the formula below, $STI = (Y_{si} \times Y_{pi}) / (Y_p)^2$

In 2015 water use efficiency (WUE= grain yield/ water use during growing period) of the DH lines was also calculated.

2.6. Field drought test in 2015

The DH population was sown under rain out shelter in the nursery of the Cereal Research Ltd. (Szeged, Kecskés), in October 2014.

The control experiment received 274 mm of water in the form of rain during the growing season. The rain out shelter was in use from March (excluding natural rain until harvest), so the drought stress experiment reached 141 mm of water in the form of rain from October to February. The plant height, heading time, grain yield and thousand grain yield was measured.

2.7. Statistical analysis

Analyses of the collected data were made by IBM SPSS Statistics 22 software ‘Descriptive statistics’ function to calculate means, minimum and maximum values, percentiles, standard deviation, to test the normal distribution of the phenotyping data (heading time, plant height, grain yield, aboveground biomass, non-threshed spike weight and root dry mass).

The 'General Linear Model' (GLM) function was used to perform the two-way ANOVA, investigating the genotype-by-treatment interactions.

Correlation analysis was performed for each treatment separately (WW, DS) on the yield parameters and root dry mass of the individual lines (mean value of six plants) of the population using IBM SPSS Statistics 22 software 'Pearson Correlation' function.

Principal Component Analysis was carried out by Minitab Statistical Software 18, 'Multivariate', 'Principal Components1' function.

2.8. Molecular mapping and QTL analysis

DNA was isolated from seedling leaf tissue according to the CTAB method. The genetic mapping of the PI/CD population was carried out using 897 DArT (Diversity Arrays Technology) markers. Linkage groups were constructed by using JoinMap[®] 3.0 software, and interval mapping was carried out with MapQTL[®] 5 software. Interval mapping (IM) was carried out with the average of each trait. The minimum LOD score was set to 2.0 during the mapping.

3. RESULTS

3.1. Glasshouse drought tolerance phenotyping experiments

The water withdrawal treatment resulted in significant decrease compared to optimal watering in nearly all the evaluated traits. Plants in the stress treatment showed a major reduction in growth, as shown by the decreased mean aboveground biomass, plant height. Also, the DH lines of the population showed a diverse distribution in the case of heading time.

In the glasshouse experiments Plainsman V variety showed 35% yield loss under water withdrawal compared to optimal watering while the yield decrease for the Cappelle Desprez was the highest 78%.

In the 2015 glasshouse experiment the water need of the genotypes was measured. The DH genotypes water need showed large distribution (899-1870 ml). At the time of heading increased water need was detected in the population. The water use efficiency of the DH population was between 0,27 kg/m⁻³-1,41 kg/m⁻³. The mean of the population was 0,77 kg/m⁻³.

Strong positive correlation was calculated between the water need and the grain yield, also with the aboveground biomass. The kernel weight, number of spikelets, root dry mass root length correlated with water need. The water use efficiency correlated negatively with the water need in our experiment. The correlation coefficient was positive between WUE and nearly all traits except the aboveground biomass, grain number, and root traits.

3.2. Field drought tolerance experiment

In the field trial the plants grown under the rain out shelter responded to the drought with decreased growing compared to optimal conditions. The average yield decrease of the population was 38,4% in the drought treatment. The grain yield correlated positively with heading time in the field experiment. The stress tolerance index showed positive correlation with all traits except of heading time.

3.3. Drought tolerant and sensitive genotypes

The sensitive plant group - in the optimal watering treatment - showed better aboveground biomass production than the tolerant DH lines. The aboveground biomass depression was higher in the sensitive group than in the tolerant.

Under optimal conditions the sensitive DH lines had better grain yield performance than the tolerant ones. Drought affected to a higher extent the sensitive DH lines, so under stressed conditions the tolerant DH lines had better performance.

Sensitive DH lines under optimal conditions had significantly higher root dry mass than the tolerant DH lines. The root dry mass loss was also higher in the sensitive group than in the tolerant. Each sensitive DH line root dry mass reduced significantly, while the reduction in the tolerant DH lines root dry mass was not significant.

The DH lines in the tolerant group had less water consumption than in the sensitive group. The difference between the group means was more than 300 ml. In the case of the selected DH lines, there was a moderate positive correlation between the tolerant DH line group 's grain yield and root dry mass, and a weak correlation between the sensitive DH line's grain yield and root dry mass.

Under optimal watering conditions the grain yield of the tolerant DH lines showed a strong positive correlation with the water consumption. The sensitive DH lines showed moderate positive correlation between grain yield and water consumption under optimal watering conditions.

3.4. Glasshouse drought tolerance experiment with varieties

In the experiment average grain yield of a well-watered single plant was 1.48g while it is decreased to 0.41g under drought stress. The highest effect of water withdrawal was detected in the case of the varieties which lost all their yield. The variety GK Berény had good performance under drought stress, 59,4% decrease compared to optimal conditions. Under well-watered conditions both plant height, aboveground biomass, yield and root dry mass were correlated significantly with heading time. Root dry mass was also correlated under well-watered conditions with plant height and aboveground biomass. The correlation was also strong under drought between root dry mass and heading time. But no significant correlation was found between root dry mass and above ground biomass or plant height. The correlation between root dry mass and yield was negative under drought conditions.

Three-dimensional plots were created to present the relations between varieties and traits based on the stress tolerance index, harvest index, thousand grain weight in both conditions. Varieties GK Vitorlás, GK Piacos, GK Verecke, Plainsman V, GK Berény, GK Körös, GK Garaboly, GK Csillag, GK Petur, GK Angéla, GK Aranka, Sumai3 were assigned to group A, which had relatively high yield potential under both

conditions. Based on the same traits Cappelle Desprez, Midas, GK Szala. were assigned to group D, with dropped yield potential under DS conditions.

3.5. Identification of QTLs under optimal watering and water withdrawal conditions.

897 polymorphic molecular markers were available to create the linkage map. During the analysis, 810 markers were mapped to 36 linkage groups, and the linkage groups were identified by chromosome.

Under optimal watering conditions, 33 QTLs have been identified on chromosomes 1A, 1B, 2A, 3A, 3B, 4B, 5B, 6A, 6B, 7A, which were related to the following phenotypic traits; grain yield, grain number, plant height, kernel weight, heading time, harvest index, root dry mass, aboveground biomass, thousand grain weight.

QTL mapped on chromosomes 4B and 6B related to plant height were identified each year under optimum conditions, explaining 9,3-23,9% of the phenotypic variance.

On chromosomes 3B and 6A QTLs were detected every year related with kernel length. The QTL associated with kernel length explained 7.5-8.5% of the phenotypic variance. The harvest index-linked region on chromosome 1A was also detected in all years.

The QTL on chromosome 1A was identified in all glasshouse experiment, explained 8% of the phenotypic variance. QTL on chromosome 3A was mapped in the 2014 experiment, determined 10,6% of the phenotypic variance. The QTL linked to grain yield on 3B chromosome was identified in 2014, 2016 glasshouse experiment, explained 8.8% of phenotypic variance. Another QTL, on chromosome 5B in 2014, related to grain yield explained 12.4% of phenotypic variance. QTL mapped on chromosome 6B in 2014 and 2015 was responsible for 12.2% of phenotypic variance.

In the water withdrawal experiments, 35 QTL regions were detected related to 12 agronomic traits. The traits were, grain yield, grain number, stress tolerance index, plant height, kernel length, kernel weight, harvest index, root-dry weight, above-ground biomass, thousands grain weight. The QTLs were identified on the following chromosomes, 1A, 1B, 2A, 2B, 3A, 3B, 4B, 5B, 6A, 6B, 7A, 7D.

QTLs identified on chromosomes 6B and 4B linked to plant height were found in each experiment. Under drought condition the 7.6-13.2% of phenotypic variance was explained by them.

The QTL related to thousand grain weight on the chromosome 4B was identified in all experiment, responsible for 10.1% of the phenotypic variance.

Yield related QTL on the 1A chromosome was identified in the 2015 glasshouse experiment, explained 24% of the phenotypic variance. QTL identified on chromosome 6B defined 9.1% of phenotypic variance in the same experiment.

3.6. New scientific results

1. The importance of water need as selection criteria was emphasised in the experiments. The sensitive DH genotypes water need negatively correlated with grain yield under optimum watering conditions. In the case of the tolerant genotypes the opposite was found. These results revealed that the selected genotypes are useful to drought tolerance breeding.
2. The mapping results of the Plainsman V/Cappelle Desprez DH population increased the genetic knowledge of drought tolerance (33 QTLs optimal watering; 35 QTLs water withdrawal). Many of the previously described QTL regions have been localized to confirm their earlier published place. The plant height related QTL on chromosome 4B, 6B were identified in all experiments under both treatments. On chromosome 4B the QTL linked to root dry mass was published earlier in relation to root density under dry conditions. It is most likely that this region is related with root traits. The QTL on 4B chromosome related to thousand grain weight was identified in all water withdrawal experiments.
3. QTLs related with grain yield were identified on chromosome 1A, 3A, 3B, 5B, 6B under optimal watering conditions in the Plainsman V/Cappelle Desprez DH population. These results agreed with published scientific papers.
4. On chromosome 1A – in the Plainsman V/Cappelle Desprez DH population- thousand grain yield related QTL region was identified, which is not included in the wheat drought tolerance references, we processed.

4. CONCLUSIONS AND RECOMENDATIONS

The aim of our study was to phenotype the Plainsman V/Cappelle Desprez (PI/CD) DH population under drought stress and optimum water supply conditions, to carry out QTL analysis and to select tolerant and sensitive lines. For this purpose, glasshouse water withdrawal experiments were carried out during 2014-2016. In 2015 the PI/CD mapping population was phenotyped in field under rain shelter and irrigated conditions. Heading time, plant height, yield, thousand grain yield, harvest index, stress tolerance index, were evaluated in the experiments.

Heading time under drought stress conditions was two days earlier compared to optimal conditions in all our glasshouse experiments. While under field conditions the difference was only one day. The decrease in plant height was 15-19% under drought stress in the glasshouse experiments, while under field conditions the difference was only 5 %. Furthermore, in the glasshouse experiments more than 50 % yield loss was measured under drought conditions compared to optimal irrigation.

In 2015 the water uptake of the DH population was evaluated. The difference in water uptake was two times between the genotypes with highest and lowest water uptake. The highest water uptake was measured at the time of heading and the next four weeks. In the experiments the higher water uptake was not always related to higher yield. The water uptake with grain yield could be good selection criteria for drought tolerance, because genotypes using the water to increase biomass could be selected.

The selected tolerant and sensitive genotypes can be used in drought related breeding programs and will be the fundamental part of our root research project. The tolerant genotypes had less water uptake compared to sensitive ones.

In the second experiment the earlier wheat varieties had better performance under drought conditions. In addition, the excellent drought tolerance of GK Berény was demonstrated in the experiment.

Further aim of the work was to identify drought tolerance related loci in the PI/CD population by interval mapping. Under optimum conditions 33 QTLs were mapped for ten agronomic traits. Most of the identified QTLs appeared only in one of the experiments. The plant height related QTLs were mapped on chromosomes 4B, 6B significantly appeared in all our experiments. The QTLs on chromosomes 1A, 3B, 6A

related to ear length were detected in all cases under optimal conditions. A harvest index related QTL was located on chromosome 1A. Yield related QTLs were detected on chromosomes 1A, 3A, 3B, 5B, 6B.

In total 35 QTLs were detected under drought stress conditions related to twelve agronomic traits. Some of the mapped QTLs appeared in only one experiment. Plant height related QTLs detected on chromosome 4B, 6B appeared in all experiments. Moreover, the thousand-kernel weight related QTLs were identified on chromosomes 1A, 4B, 6B, while yield related QTLs were identified on chromosomes 1A, 6B.

The results of our work revealed, that the complex stress diagnostic system provides additional information on the drought tolerance of wheat genotypes. It could also be a complementary technique to field trials in a breeding program. Furthermore, the importance of measuring the water uptake and root traits in drought tolerance experiment were enhanced in our experiment. However, due to the complex agronomic nature of drought tolerance, further field trials are needed in the forthcoming years.

MAIN PUBLICATIONS OF THE AUTHOR

Scientific publications:

Publications in international scientific journals:

- Nagy É.**, Lehoczki-Krsjak S., Lantos C., Pauk J. (2018): Phenotyping for testing drought tolerance on wheat varieties of different origins. *South African Journal of Botany*, 116, 216–221. p. IF:1,424
- Lantos C., Bóna L., **Nagy É.**, Békés F., Pauk J. (2018): Induction of in vitro androgenesis in anther and isolated microspore culture of different spelt wheat (*Triticum spelta* L.) genotypes. *Plant Cell, Tissue and Organ Culture*, 133(3), 385-393. p. IF:2.002
- Nagy É.**, Lantos C., Pauk J. (2017): Selection of drought tolerant and sensitive genotypes from wheat DH population. *Acta Physiologiae Plantarum*, 39, 261, 1-9. p. IF:1,364

Publications in Hungarian scientific journals:

- Nagy É.**, Kenny P., Kondic-Spika A., Grausgruber H., Allahverdiyev T., Sass L., Vass I., Pauk J. (2017): A szárazság- és sóstressz hatásának vizsgálata búza fajtákon üvegházi fenotipizálási kísérletben. *Növénytermelés*, 66(2), 69-88. p.
- Nagy É.**, Pauk J. (2014) A 'Plainsman V./Cappelle Desprez' őszi búza szárazságtűrési DH térképezési populáció fenológiai vizsgálata és méretének szűkítése. *Növénytermelés*, 63(3), 57-72. p.

Other scientific publications:

Confernece Proceedings:

- Pauk J., **Nagy É.**, Lantos C., Kiss E. (2014): Néhány fontos fenotipizálási eredmény a 'Plainsman V./Cappelle Desprez' búza DH szárazságtűrési térképezési populáció vizsgálatából. In: Veisz Ottó (szerk.) *Növénytermelés a megújuló mezőgazdaságban: XX. Növénytermelési Tudományos Nap, 2014.03.18.* Budapest: MTA Agrártudományok Osztályának Növénytermelési Bizottsága, 349-353.

Conference abstracts:

- Nagy É.**, Kenny P., Kondic-Spika A., Sass L., Grausgruber H., Lantos C., Vass I., Pauk J. (2016): Wheat phenotyping for abiotic stresses via complex stress diagnostic system. In: Haesaert, G., Merbach, W., Kern, M. (eds.): *7th International IFSDAA Seminar on Sustainable Resource Management Towards Food, Energy, Environment and Livelihood, 2016.11.27-29.* Goettingen, Németország, p. 30.
- Nagy É.**, Varga M., Lantos C., Pauk J. (2016): Szárazságtűrési fenotipizálás őszi búzával. In: Veisz O., Polgár Zs. (szerk.): *XXII. Növénytermelési Tudományos Nap Összefoglalók. 2016. 03.10.* Budapest, MTA Agrártudományok Osztályának Növénytermelési Bizottsága p. 60.
- Lantos C., Jenes B., Mesterházy Á., Bóna L., Jancsó M., **Nagy É.**, Purnhauser L., Pauk J. (2015): DH technology in cereal science and breeding. In: *Pannonian Plant Biotechnology Workshop "Integration fundamental research into the practical agriculture".* Ljubljana, Szlovénia, 2015.06.08-10.: p. 11-12.

- Nagy É.**, Kondic-Spika A., Lantos C., Cseuz L., Sass L., Dudits D., Vass I., Pauk J. (2015): Wheat phenotyping for abiotic stresses via complex stress diagnostic system. In: Pannonian Plant Biotechnology Workshop "Integration fundamental research into the practical agriculture". Ljubljana, Szlovénia, 2015.06.08-10.: p. 29.
- Pauk J., Lantos C., **Nagy É.**, Bóna L., Cseuz L., Somogyi N., Somogyi Gy., Heszky L., Jancsó M., Bráj R., Simonné Kiss. (2015): In vitro androgenézis-kutatási eredmények nemesítési céllal. In: Veisz Ottó (szerk.) XXI. Növénynevelési Tudományos Napok: Összefoglalók. 155 p. Martonvásár, Magyarország, 2015.03.11-12. Martonvásár: MTA ATK, p. 29.