The main goal of the proposal was the better understanding of the osmotic adaptation mechanisms in wild wheat (Aegilops) species, wheat relatives (barley) and in their introgressed (additional, substitutional and translocation) lines developed in Martonvásár. Our aim was to reveal how they can contribute to improve the drought or salt tolerance of wheat. For this, complex molecular genetic, biochemical and physiological investigations are planned to be performed.

In the research grant we studied the drought or salt stress responses in genesources of wheat including wild wheat (Aegilops) species, wheat relatives (barley) and in their introgressed (additional, substitutional and translocation) lines developed in Martonvásár. Our aim was to reveal how they can contribute to improve the drought or salt tolerance of wheat.

The grant focused on 4 topics (1 topic /year):

1: Studies of drought stress response in wild (Aegilops) wheat.

2: Studies of drought tolerance in wheat/Aegilops introgression lines

3: Studies of salt tolerance mechanisms in wheat/barley additional lines,

4: Studies of the salt stress response in wild wheat introgression lines.

The effects of osmotic stress induced by drought or salt was followed by monitoring several physiological, metabolic processes together with gene expression studies

1: Studies of drought stress response in wild (Aegilops) wheat.

In 2015, the drought tolerance mechanisms were compared in wheat (6) and Aegilops accessions (28) and (in addition, in wheat-Aegilops amphiploids (containing all wheat and Aegilops chromosomes). The plants were grown in soil (in a glasshouse) and drought stress was applied before flowering (Zadok scale 41) for 2 weeks by withholding water to 40% of control (35% SMWC). Several physiological investigations were performed during the stress treatment and leaf samples were collected for metabolic analysis (see below). After 2 weeks the plants were rewaterred and grown to harvest. The drought-induced changes in growth and (plant height and weight) and yield production (tillering, seed number and weight) were also determined.

On the basis of growth parameters and yield production, the wheat genotype Capelle Deprez (CD) seemed the most drought sensitive wheat cultivar, the wheat cv. Mv9kr1 and cv. Chinese Springs, the wheat parents of several wheat-Aegilops introgression lines showed better drought tolerance than CD, but presented relative drought sensitivity as compared to most Aegilops accessions. The wheat cv. Plainsman presented relative drought tolerance within the wheat genotypes. Among the Aegilops accessions (28) investigated, all of them presented higher drought tolerance than CD, 66 % of them showed higher drought tolerance as compared to wheat Mv9kr1 and CS and 33% of them could continue better the growth and produced relatively (% of control) higher yield under adverse environmental condition than the drought-tolerant wheat cv Plainsman. Interestingly, the drought-induced decrease of harvest indexes were significantly lower for 85% of Aegilops accessions compared to wheat cultivars.

The differences between the wheat and Aegilops accessions in the physiological and metabolic processes were also studied. Those plants which presented better drought tolerance according to the growth and yield parameters (8 of 28 Aegilops accessions) could keep their CO_2 assimilation capacity more than drought sensitive accessions, especially CD. The lower decrease in CO_2 assimilation was accompanied by the lower stomata closure (gs) and by maintenance of high transpiration rate (E). For discrimination of the processes responsible for limitation of CO_2 assimilation under drought stress condition, the changes of A was measured between 0 –1000 umol / mol CO_2 at the light -saturated state of photosynthesis using 1000 umol / m^2 s actinic light intensity. The stomatal (Ls) and non-stomatal (Lns) limitation were determined on the basis of Ci v.A curves, as described by Lawlor (2002). On the bases of these investigation we could demonstrate that the drought-induced decrease of CO2 assimilation is limited mainly (70-90%) by the stomata closure (Ls) in drought tolerant plants, while in drought sensitive genotypes, especially in CD, the metabolic limitation (Lns) was also important, accounting for 40-55 % of the total limitation. These were also supported by the studies of the photosynthetic electron transport processes through the chlorophyll a fluorescence measurements. In spite of the fact that the maximal quantum yield of PSII photochemistry (Fv/Fm) did not modify significantly even in drought-sensitive plants (10% reduction of Fv/Fm could only be detected for CD), the utilization of absorbed light energy significantly reduced under drought stress condition in drought sensitive plants as indicated by the decrease of the (Y(II) parameter, the effective quantum yield of PS II. In parallel to the decrease of Y(II), the closure of PS II RCs (as indicated by the decrease of qP and qL) and the increase of dissipation of excitation energy, Y(NPQ), was also detected. Comparing the wheat and Aegilops accessions, while a 35% reduction of Y(II) was detected for wheat cv. Mv9kr1 and 10% reduction of Y(II) for wheat cv. Plainsman under drought stress condition, but no significant changes of Y(II) were detected for drought tolerant Aegilops accessions (8 of 28) and less than 15% was detected for the other Aegilops accessions.

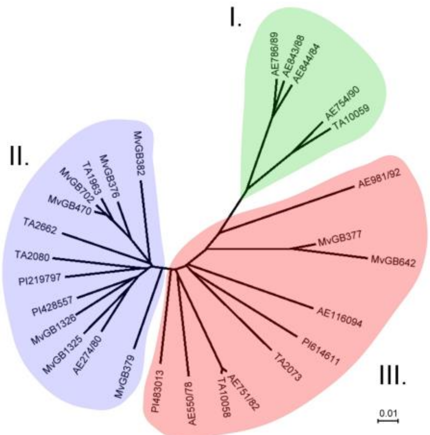
In spite of the lower stomatal closure (gs) and higher transpiration rate (E) of the drought tolerant plants, they could keep their water content, as demonstrated by the lower decrease of RWC of leaves, which suggested an efficient osmotic regulation. It was also supported, when the osmotic potential of leaves were determined (Girma and Krieg (1992, Plant Physiol 99, 577-). The investigations revealed that in drought sensitive wheat cv. CD, the increase of osmotic potential of leaf sap was mainly due to the water loss (passive osmotic adjustment), while in drought resistant Aegilops accessions, the proportion of active osmotic adjustment (e.g. accumulation of different osmolites) was significantly higher than that of drought-sensitive cultivars.

To reveal the metabolic background of the osmoregulation, several metabolic processes, such as prolin accumulation, changes of sugar and starch content were investigated on wheat (CD, Mv9kr1, CS and Plainsman) and on selected Aegilops accessions having different drought tolerance or cross partners of the interspecific hybridization.

The proline content increased significantly in leaves of drought treated plants, but no correlation was found between the proline content and the drought tolerance of genotypes. The starch and sucrose content (determined by special kits for assays, Megazyme) of leaves decreased significantly under drought stress condition, but the amount of glucose and fructose increased in drought treated leaves. The decrease of sucrose was more intensive in drought sensitive plants than in drought–tolerant wheat and Aegilops accessions, while higher increase of glucose and fructose was observed in drought tolerant wheat and Aegilops accessions. These results suggest that in drought sensitive accessions, the elevated glucose content is originated from sucrose degradation, while in drought tolerant plants the elevated glucose content may be due to the synthesis. To prove this, the activity of acid invertase responsible for sucrose hydrolysis to glucose and fructose, was also measured (as described in Majláth et al. 2015), and we found that the invertase activity increased only in drought sensitive plants, while lower invertase activity was measures in drought-tolerant plants.

Later these investigations were completed with genetic studies. The 28 Aegilops were further analysed by 14262 SilicoDART) molecular markers. On the bases of the results, a correlation between the drought tolerance and the origin of the Aegilops accessions were found. According to the results of the UPGMA cluster analysis, 3 exactly separated clades and some minor clades could be identified. The clade 1 contained 5 accessions and these accessions were mainly originated from Libya. The clade 2, containing accessions (6) originated from Iran, Jordan and Syria and from clade 3 containing accessions (5) from Azerbaijan. 12 of 35 genotypes with different origin didn't form separate groups. Strong correlation was found between the marker-based genetic map and the drought sensitivity of plants: the genotypes in Clade 1 showed the best drought tolerance, followed by Clade 2 and 3 accessions, while most of the ungrouped accessions showed drought sensitivity. 73 markers were found to be specific in clade 1, among them 55 were polymorphic to wheat.

3 clades were identified by 14262 SilicoDArT markers in NJ cluster analysis



73 molecular marker were identified specific for the most drought tolerant accessions, among them 55 were polymorphic to wheat

Drought stress response *of Ae. biuncialis* accessions: Yield components

	Plant height	Plant weight	Tillering	Seed weight	Seed number
low value	Ae. biu PI219797	Ae. biu TA2073	Ae. biu 274/80	Ae. biu 274/80	Ae. biu 274/80
	CD	CD	Ae. biu PI614611	Ae. biu PI219797	Ae. biu PI614611
	Ae. biu 274/80	Ae. biu PI614611	Ae. biu 550/78	Ae. biu 550/78	Ae. biu 751/82
	Ae. biu TA2073	Ae. biu PI219797	Ae. biu 1160/94	Ae. biu 1160/94	Ae. biu 1160/94
	Ae. biu 428557	Ae. biu 379	Ae. biu PI483013	CD	CD
	Ae. biu PI614611	Ae. biu 550/78	Ae. biu 751/82	Ae. biu 10058	Ae. biu 550/78
	Ae. biu 379	Ae. biu 10058	Ae. biu 10058	Ae. biu 751/82	Ae. biu TA2073
	Ae. biu 550/78	Ae. biu 274/80	Ae. biu 377	Ae. biu PI614611	Ae. biu PI483013
	Ae. biu 10058	Ae. biu 1160/94	Ae. biu TA2073	Mv9kr1	Ae. biu 379
	Ae. biu 1160/94	Ae. biu 642	CD	Ae. biu 642	Ae. biu 377
	Mv9kr1	Mv9kr1	Ae. biu TA2080	Ae. biu TA2073	Ae. biu 10058
	Ch Spring	Ae. biu TA2080	Ae. biu PI219797	Ae. biu 379	Ae. biu 642
	Ae. biu 642	Ae. biu PI483013	Ae. biu 642	Ch Spring	Ae. biu PI219797
% of control	Ae. biu TA2080	Ae. biu TA1963	Ae. biu 1326	Ae. biu PI483013	Mv9kr1
	Ae. biu 470	Ae. biu 1326	Mv9kr1	Ae. biu 702	Ae. biu 1326
	Ae. biu 376	Ae. biu 377	Ae. biu TA1963	Ae. biu TA1963	Ch Spring
	Ae. biu PI483013	Ae. biu 382	Ae. biu 379	Ae. biu 470	Ae. biu 376
	Ae. biu 377	Ae. biu 428557	Ae. biu 376	Ae. biu 377	Ae. biu 382
	Ae. biu 702	Ae. biu 376	Ae. biu 702	Plainsman	Ae. biu 981/92
	Ae. biu TA1963	Ae. biu 751/82	Ae. biu TA2662	Ae. biu TA2080	Ae. biu TA2662
	Ae. biu 751/82	Ae. biu 702	Ae. biu 382	Ae. biu 376	Ae. biu 702
	Ae. biu 382	Ae. biu 981/92	Ae. biu 428557	Ae. biu 382	Ae. biu TA1963
	Ae. biu TA2662	Ae. biu TA2662	Ch Spring	Ae. biu TA2662	Ae. biu 470
	Ae. biu 981/92	Ch Spring	Ae. biu 981/92	Ae. biu 1326	Plainsman
	Ae. biu 754/90	Ae. biu 470	Ae. biu 754/90	Ae. biu 428557	Ae. biu TA2080
	Plainsman	Plainsman	Ae. biu 470	Ae. biu 981/92	Ae. biu 428557
high value	Ae. biu 10059	Ae. biu 754/90	Plainsman	Ae. biu 754/90	Ae. biu 843/88
	Ae. biu 1326	Ae. biu 10059	Ae. biu 1325	Ae. biu 786/89	Ae. biu 1325
	Ae. biu 786/89	Ae. biu 786/89	Ae. biu 10059	Ae. biu 10059	Ae. biu 754/90
	Ae. biu 844/84	Ae. biu 1325	Ae. biu 844/84	Ae. biu 843/88	Ae. biu 10059
	Ae. biu 1325	Ae. biu 843/88	Ae. biu 786/89	Ae. biu 1325	Ae. biu 786/89
	Ae. biu 843/88	Ae. biu 844/84	Ae. biu 843/88	Ae. biu 844/84	Ae. biu 844/84
	(55-95%)	(30-80%)	(10-85%)	(10-75%)	(10-80%)

The drought tolerance mechanisms of different clades were also related. The Aegipops accessions of clade 1 showed high photosynthetic activity and WUE, accumulation of sugars, especially fructose and glucose under drought stress conditions. The genotypes found in clades 2 retained their photosynththetic activities in spite of moderately water loss and low prolin accumulation. In case of clade 3 and ungrouped accessions no correlation was found between the metabolite (prolin and glycine-betaine (GB) and sugars) contents of plant and the drought sensitivity of genotypes. Altogether these results showed (we could prove) that many Aegilops biuncialis accessions could be a potential gene sources for improving drought tolerance of wheat. A part of these investigations were presented in a conference (in Barcelona) and as publications, however, further papers are still plant to publish.

2. Studies of drought tolerance in wheat/Aegilops introgression lines

The first stable step in the transfer of agronomically useful traits from an alien species to wheat is the production of amphiploids through interspecific crossing. The amphipolids contain all of both parental chromosome sets. Later several addition, translocation and substitution lines was produced in Martovásár which were used for the investigations.

First, we studied the drought stress response (similarly as described above) of 3 different wheat-*Ae biuncialis* amphipolids. These amphiploids contained all chromosomes (AABBDDUUMM) of wheat cv. Mv9kr1 and Aegilops biuncialis accessions cv. MVGB642, MVGB470 and MVGB382 (as male parents) with different drought sensitivity. In this way, the effects of all Aegilops chromosomes could also be investigated. On the bases of the results, the drought-induced decrease of growth (plant weight) and yield production was lower in amphiploids than those in wheat Mv9kr1, but the total amount of seeds (seed number and weight) were significantly lower in amphiploids than in wheat parent Mv9kr1, due to the genetic instability of the 70 chromosomes under the meiotic process. In spite of this, lower decrease of RWC content, higher photosynthetic activity (A, gs and E) and osmotic adjustment capacity (see above) were also detected for wheat-Aegilops amphiploids than for wheat parent Mv9kr1.

Besides these investigations, the drought-induced changes of polyamine content were also studied on wheat and Aegilops parents and on their amphiploids. The investigation presented that the putrescine and cadaverine contents increase significantly in wheat genotypes under drought stress condition, but slightly or not at all in amphiploids and Aegilops parent. Slight changes were observed for spermin, and no significant modification was found in case of spermidine. These results are found very interesting, however, later (see below) further investigation were performed to study the role of polyamines in drought stress response.

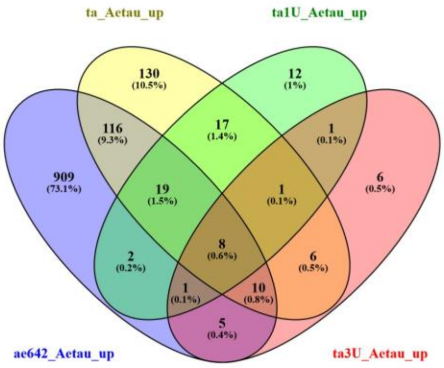
Later, the investigations were continued with studying the drought stress response of wheat/Aegilops addition lines together with their wheat cv. Mv9kr1 and Aegilops biuncialis (cv. AE642) parents. The drought stress response of wheat/Aegilops addition lines (1U-7M, 18 genotypes) was investigated similarly as in previous experiments for Aegilops accessions (in 2015). Growth and yield production, physiological and metabolic responses (prolin, GB, sugar and polyamine) were studied.

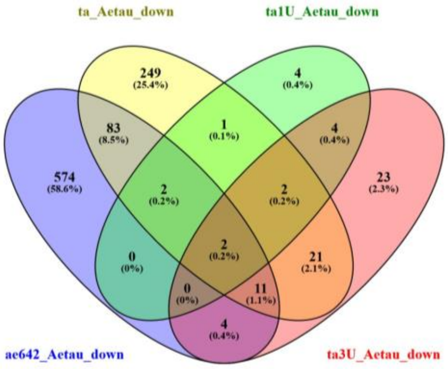
On the bases of the growth (plant height and weight) and yield production (tillering, seed number and weight) the wheat/Aegilops biuncialis addition lines 1U, 3U , 1M, 3M and 2M showed better growth under drought stress condition than the wheat parent Mv9kr1. The higher growth and yield production of these addition lines manifested in the retained photosynthetic activity under adverse (drought) condition. The addition 3M showed the highest Pn values. In drought tolerant plants the slight decrease in Pn was accompanied by the stomatal limitation (Lgs), while in drought sensitive genotypes, including the wheat parent, the intense stomatal closure was accompanied by limited metabolic processes (Lm), in consistent with previous results. When the proline, Gb and sugar metabolism were compared in the addition lines, intense proline, GB and sugar (especially fructose, glucose and maltose) accumulation was found in the leaves under drought. The proline accumulation was high in wheat parent, while the sugar content increased more intensively in addition lines, especially in 3M addition line than in wheat parents.

The polyamine metabolism showed similar tendencies as was found previously (for amphyploids), namely, neither the spermidine (SPD) nor the spermine (SPN) contents changed significantly in drought-treated plants as compared to well-watered plants, while significant increase in putrescine (PUT) content was detected in all genotypes. It seems that the putrescine accumulation plays role in the drought stress response, however, since the PUT, SPD and SPN contents did not differ among the genotypes, it is unlikely that the polyamine metabolism plays important role on the drought tolerance of plants.

To get further information on the metabolic changes in plants, samples were collected for RNAseq analyses from 5 genotypes (Wheat 'Mv9kr1', Ae. biuncialis 642 and 382 having different drought sensitivity, wheat-Ae. biuncialis addition lines 1U and 3U, having elevated drought tolerance compared to wheat parent) grown under well-watered and drought stress conditions. Total RNAs were isolated from leaves of each genotype and treatment in 3 replicates and the RNA samples (30 pc.) were sequenced on an Illumina HiSeq2500 lane. After transcript assemblies and the abundance estimation of control and drought treated samples, differential expression (DE) analysis was performed. Before running the differential expression analysis a quality check comprising the overall similarity between the samples has to be run. The mean and standard deviations for the pooled transcripts of the investigated genotypes were similar, while the principal component analysis showed that control and drought treated samples were clearly discriminated in each genotype.

For each experiment the DESeq2 ‘results’ function has been applied with the parameters alpha=0.1 (False Discovery Rate) and lfcThreshold = 2 (log fold change). According to the transcript analysis, several differentially (up or down) expressed genes were identified (see figure at the end of the document). After annotation of the differentially expressed transcripts, almost thirty annotated genes were selected for the qPCR validation of the abundance data. These genes were expressed in most genotypes and their expression change folder (log2 values) cover a wide range (between -7 and +11) representing both the down(-) and up-regulated (+) genes together with 4 reference genes (Actin, RLIa, CDCa, Ta30797). These primer pairs were tested by gel electrophoretic technique and melting curve analyses of the amplification. Since the expression of Ta30797 gene seemed to be the most stable both in control and drought-treated plants, therefore it was used as reference for the validation. The results showed that fifteen gene-specific reliably operating primers were suitable for the validation. After RT-PCR reaction, the log2 of ΔΔCt values were calculated according to Livak and Schmittgen (2001; Methods. 25, 402–408) and correlation analysis were performed between the RNA seq and RT-PCR data. We determined a strong correlation (R2 = 0.834) between the two types of measurements. It should however be noted that the bioinformatics analysis has been performed slowly, because our bioinformatics colleague, who would have been responsible for performing the tasks, left the institute before the sequencing was completed. It was difficult to find a new bioinformatics specialist, thus this work delayed. Therefore, the publication of these results is still pending, although some papers has already been published in this topic too.





3: Studies of salt tolerance mechanisms in wheat/barley additional lines,

Salt tolerance was also screened in a set of wheat/barley introgression lines and in their parental wheat and barley genotypes. Previous studies demonstrated that the addition line 7H could contribute to improving the salt tolerance of wheat. However the exact mechanisms in the contribution of barley chromosomes for wheat salt tolerance has not known before.

In the present proposal detailed studies were performed to reveal what kind of biochemical and molecular processes are involved in the elevated salt tolerance of introgesssion lines. Since salt stress causes ionic (especially Na+ toxicity), osmotic and oxidative stresses, these processes were investigated in details together with monitoring of the growth, photosynthesis, RWC and changes of osmotic potential in leaves and roots. On the basis of the results, we proved evidently that the salt tolerance of barley cv. Manas is higher than the wheat parent Asakaze and both the Na accumulation and transport and the osmotic adjustment potential differed between the two parents. Using a set of wheat/barley addition lines (2H-7H) we proved that the barley chromosome 7H affected positively to the salt tolerance in wheat background. Then the separate effects of the short or long arms of chromosome 7H on the salinity-induced metabolic changes were studied in both the roots and shoots. Growth, photosynthetic activity, chlorophyll degradation, proline, glycine betaine accumulation, sugar metabolism, Na+ and K+ uptake and transport processes and the role of polyamines and antioxidants were monitored in young plants grown in hydroponic culture with or without salt treatment.

Changes in growth and photosynthetic activity of plants demonstrated that the salt tolerance of the addition lines 7H and 7HL was similar to that of barley parent cv. Manas, while the sensitivity of the addition line 7HS was similar to that of the wheat parent cv. Asakaze. Salt treatment induced a complex metabolic rearrangement in both the roots and shoots of all the genotypes. In the roots, Na uptake was accompanied by proline and GB accumulation and an increase in catalase activity, especially in salt-tolerant cultivars, while the amount of soluble sugars and starch decreased intensively. In contrast, the sugar metabolism was the dominant process in the shoot. Higher quantities of sugars such as fructose, glucose, galactose and raffinose were found even in the control plants, and these sugars accumulated more intensively under salt stress in barley cv. Manas and addition lines 7H and 7HL than in addition line 7HS and wheat cv. Asakaze. In wheat cv. Asakaze and the 7HS addition line the polyamine metabolism was activated. Interestingly, under salt stress condition, the SPD content significantly decrease and the amount of spermine increased meanwhile the PUT content changed slightly. These results indicated that different polyamine responses occur under salt and drought stress. The role of several genes responsible for the Na+ uptake and transport processes, such as SOS1, SOS2, HKT1, NHX, HVP) were also studied. We found that many genes (SOS1, SOS2, NHX2 (only in leaves) and HVP1) were up-regulated under salt stress conditions, but significant difference was not found between the genotypes. The results suggest that osmotic adjustment is a more important process in the improvement of the salt tolerance in 7H addition lines than the modification of the ion metabolism or antioxidant defence. These results have already been published in many papers.

4: Studies of the salt stress response in wild wheat/wheat introgression lines.

Aegilops species are wild relative of wheat and they are often exposed to extreme living conditions such as drought and salt stress in their natural habitats. These wild species preserved their wide genetic diversity and serves as potential gene sources for increasing salt (and drought) tolerance of wheat. In the 4th year, the salt stress response of wheat/Aegilops additional lines was studied. The aim was to reveal which chromosomes are suitable for improving salt tolerance of wheat and what kind of mechanisms operate in these lines.

The salt response was studied during germination and in young plants of wheat–Aegilops disomic addition lines 1U, 3U, 1U/6U, 2M, 3M and 7M and the translocation line 3M.4BS developed from a wheat Mv9kr1 and Aegilops biuncilis 642 (Ae. biuncialis Vis. MvGB642) together with their parental genotypes. In the germination test, the sterilized seeds were exposed to 0, 100, 200 or 300 mM NaCl for 5 days, then the germination % and the length and weight of root and coleoptiles were determined. Salt tolerance mechanisms were also studied in young plants. The plants were grown in modified Hoagland solution in a phytotron growth chamber for 28 days and the salt stress was imposed by applying NaCl in 7-day cycles at increasing concentration of 0, 100mM, 200 mM and 300 mM. The solutions were renewed twice a week. The control plants were grown in Hoagland solution without NaCl treatment till the end of the experiment. All experiments were repeated 3 times. Without salt treatment (0 mM NaCl) the germination % varied between 94 and 100 % among the genotypes. The 100 mM NaCl treatment reduced the germination % slightly in the case of Mv9kr1 and Mv9kr1/Aegilops addition line 1U, 3U but not in Aegilops MvGB 642 and addition lines 2M, 3M and 7M and translocation line 3M4BS. At 200 mM NaCl concentration the germination % was inhibited in all the genotypes with the smallest reduction of 3M4BS translocation line, followed by Aegilops parent 642 and 3M and 2M addition lines, while the lowest germination % was observed in Mv9kr1. Similar conclusion was obtained when the length and weight of root and coleoptiles were compared. These parameters did not differ significantly among the wheat Mv9kr1 and addition lines 1U 1U/6U and 7M. When 300 mM NaCl was applied, the NaCl-induced reduction in the germination % was more intense, but the differences among the genotypes were less pronounced.

When the plants were grown in hydroponic solutions with and without NaCl, the growth and morphological parameters such as root and shoot length and weight showed similar results as was found in germination test. Namely, the root and especially the shoot growth reduction were more intense in wheat parent than those in Aegilops parent and in the addition line 2M, 3M and 3M4BS. In addition, the photosynthetic activity of leaves was also determined by gas exchange and chlorophyll a fluorescence measurements. In wheat parent the CO2 assimilation rate (Pn), the stomatal conductance (gs) related to the stomata closure and the transpiration rate (E) decreased with increasing concentration of NaCl with the highest rate in case of wheat parent. For example, at 200 mM NaCl the Pn reduction was more than 70% in wheat Mv9kr1, while it was less than 30% in Aegilops parent. Among the introgression lines, the Pn, gs and E values of 2M and 3M4BS are closed to Aegilops parent, while those of 3M was between the two parents'. Salt stress induced also the chlorosis of leaves as indicated by the decrease of the chlorophyll content of leaves. 40% of chlorophylls were lost in wheat parent (Mv9kr1) at the end of the experiments. In 2M, 3M and 3M4BS lines, the decrease of chlorophyll content was no more than 25%. With these experiments we could demonstrate that elevated salt tolerance of Aegilops parent could manifest in addition lines having additional chromosomes of 2M and 3M and also in the 3M4BS translocation line.

Further question was what kind of mechanisms changed in these plants. Since the main reasons of salt tolerance are generally related to the changes of Na and K transport mechanisms, and/or the changes of osmotic adjustment, the accumulation of Na and of several compatible osmolites was studied in root and leaves.

The salt stress induced the Na accumulation and the inhibition of K, Mg and Ca transports processes in all genotypes. In spite of the fact that lower Na was transported to leaves in Aegilops parent as compared to wheat parent, the amount of accumulated Na did not differ significantly among the wheat genotypes and addition lines. These results indicated that the Na transport and the Na exclusion mechanisms are not the main reason of the improved salt tolerance. As compatible osmolytes, the amount of proline, glycine betaine, and sugar metabolites were determined in these plants. Intense proline accumulation was found in leaves and moderate in roots in all genotypes. Proline content differed among the genotypes: higher proline content was detected in the leaves of Aegilops biuncial and 2M addition lines and in the roots of 3M and 3M4BS lines than in wheat parent. These results suggest that proline may have role in the salt response of genotypes. In contrast, the accumulation of glycine-betaine was not observed in the roots, was small in the leaves, and there were not differences between genotypes. The total amount of sugar metabolites determined by HPLC analyses, changed slightly in the root, but increased extremely in the leaves of salt-treated plants as compared to control plants. However, the sugar composition changed significantly both in the root and leaves. In general, the salt stress induced a decrease in fructose and sucrose contents and an increase in the amount of glucose, maltose and raffinose in the root. In leaves, the amount of fructose, glucose and sucrose slightly changed, while intense accumulation of galactose and raffinose was observed. In addition, a new unidentified sugar also appeared between the maltose and raffinose both in the root and leaves. Further investigations are necessary to identified this sugar compound. Comparing the genotypes, elevated amount of glucose and unidentified sugar were found in Aegilops parent, 3M and 3M4BS addition lines as compared to wheat parent in the root both under control and salt stress conditions. In leaves raffinose content was significantly higher in Aegilops parent and in the 2M addition line. These results suggest that the osmotic adjustment including mainly the sugar metabolic pathway plays important role in the salt stress response of these genotypes. These are related to the changes in glucose pathway in case of addition lines 3M and translocation line 3M4Bs, while to the raffinose pathway in the addition line 2M. Since till now, there is no any information which genes are localized on the Aegilops chromosomes* 3M and 2M, these investigations are also important in the revealing the genetic background of these metabolic changes.

* The Aegilops umbellulate (having UU genome) is sequesced during this period.

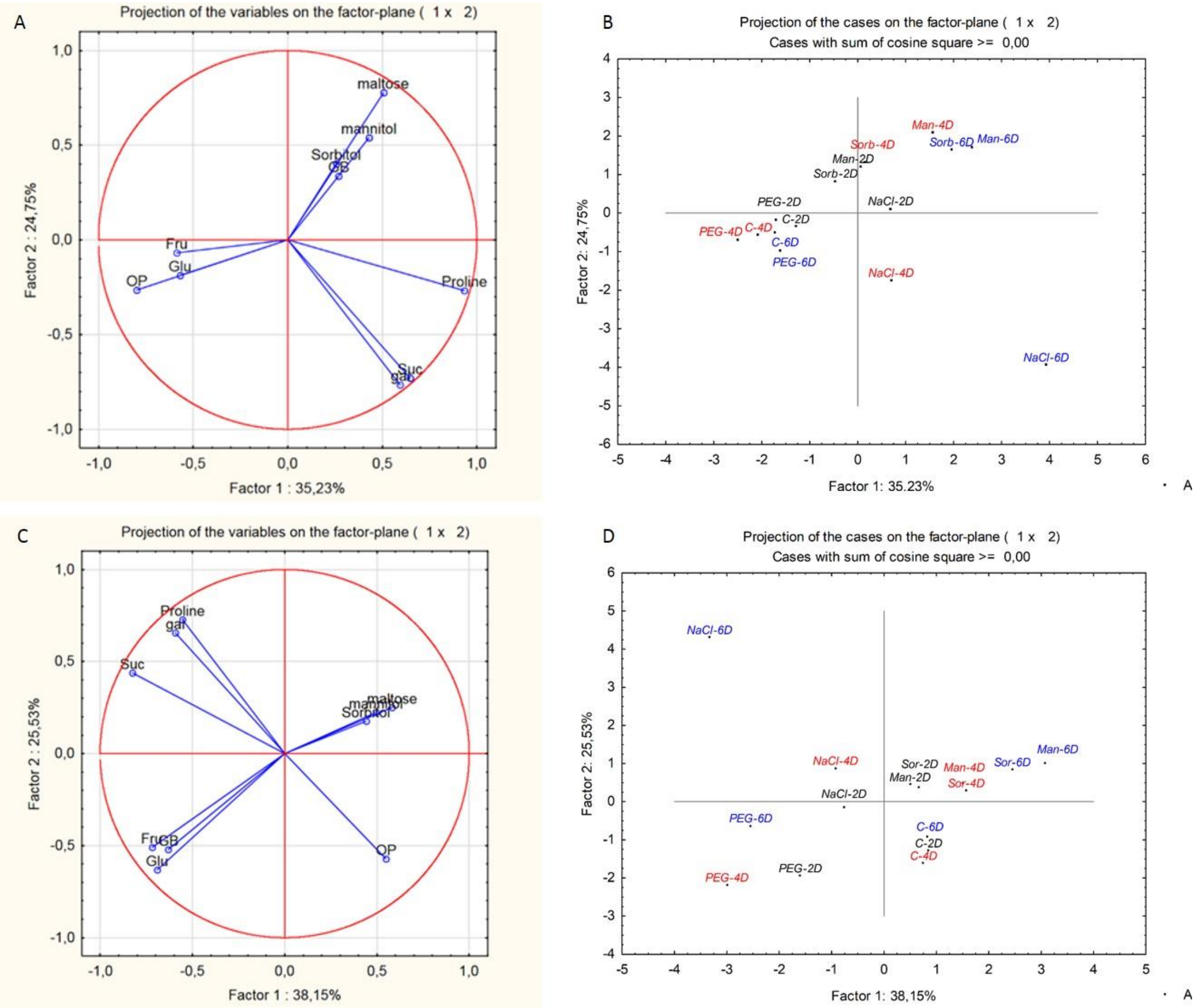
5. Additional task:

The previous studies revealed many differences in the metabolic rearrangement. These investigations showed that several metabolic changes differed between wheat and Ae. biuncialis accessions and in their introgression lines under drought stress, and between wheat/barley addition lines under salt stress conditions, while some of them were common in all genotypes. For instance, both proline, sugar and polyamine metabolism differed significantly in the previous experiments. It was possible, since both the genotypes and many environmental factors (such as the type and mode of the stress treatment, growth conditions) were different during the investigations (according to the work plan). Therefore, an additional (new) experiments were also designed and performed to reveal the osmotic stress-induced metabolic changes independently of the genotypes and environmental factors. For this, only one wheat genotype was treated with different kind of chemicals often used to induce osmotic stress in plants under similar environmental conditions. Thus, in this type of experiment, the effects of PEG 6000, mannitol, sorbitol (osmolytes often used to simulate drought stress under artificial environmental conditions) and salt (by adding NaCl to the nutrient solution) were studied on the growth, photosynthetic activity, chlorophyll content and metabolic processes including proline, glycine betaine, sugar and polyamine metabolism. The concentrations of different osmolites were chosen to reduce the osmotic potential of the culture media to the same extent (-0.8Mpa). In addition, we also tested here the possibility of determining the amount of each osmotic component directly from tissue sap.

As expected the decrease of the osmotic potential of culture media resulted in a quick inhibition of the growth in case of all osmolytes, which manifested in the reduction of length and weight of roots and shoots. All osmolytes caused a reduction in the photosynthetic activity of leaves (decrease of Pn, gs, E), decreased the chlorophyll and water content of leaves. However, less injury was observed in leaves when PEG 6000 was applied (mainly due to the fact that PEG 6000 cannot transport to the shoot). Intense stomatal closure and decrease in Pn and RWC was detected in mannitol and sorbitol-treated leaves (equally). They caused typical drought stress symptoms in leaves, due to the fact that they transported to the leaves. In some cases, crystal formation in gluttated sap was also observed. Salt stress also inhibited the Pn, induced stomatal closure, and also resulted in chlorophyll degradation similarly as found by mannitol or sorbitol treatments. When the osmotic potential of tissues sap were determined, we found that the decrease of osmotic potential was the most pronounced in root of NaCl and PEG-treated plants follows by mannitol and sorbitol-treated plants. In case of leaves the changes of osmotic potential was opposite: more intense decrease were found in mannitol and sorbitol treated leaves, less in NaCl-treated plants, but no changes was found under PEG treatment as compared to control leaves. Similarly, significant differences were found when the concentration of different osmolytes was determined from tissue sap. Intense proline accumulation was observed in the root and leaves of NaCl-treated plants, and in the leaves of mannitol and sorbitol-treated plants. Significant proline accumulation was not detected in PEG-treated plants either in the root and leaves, and the amount of proline did not increase also in the root of mannitol and sorbitol-treated plants. The glycine-betaine (GB) content did not change at all in the root after any kind of treatment as compared to controls. However, slight and temporary increase (after 2days) of GB was found in leaves of NaCl, mannitol and sorbitol-treated plants. Quick and intense sugar accumulation was observed in the root and leaves of plants treated with mannitol and sorbitol. The sugar accumulation was slower in the root of plants treated with PEG and NaCl reaching as high values as was found for mannitol and sorbitol only at 6th days of treatments. In leaves however, less sugar accumulation was found in the leaves of PEG and NaCl-treated plants than was found in mannitol and sorbitol-treated leaves. In addition, not only the amount, but also the sugar composition (determined by HPLC) differred among the treatments. The salt (NaCl) treatement enhanced the glucose, fructose galactose and maltose accumulation both in the root and leaves. PEG treatment increased only the glucose and sacharose accumulation In mannitol and sorbitol-treated plants, mainly the mannitol and sorbitol was detected in root and leaves, while the synthesis of sugars was reduced, especially in root.

The most interesting differences were found in polyamine metabolism: The amount of polyamines increased only in the root of PEG-treated plants and in the leaves of mannitol and sorbitol-treated leaves. However, the relative amount of polyamins changed and these changes depended on the type of treatments. In salt treated plants, the amount of putrescine and spermidine decreased, while the amont of spermine increased, similarly as it was found previously in wheat/barley addition lines. The Put content increased, while the amount of spermin decreased in mannitol, sorbitol and PEG 6000-treated plants and slight changes were found for sperimidne. The intense accumulation of putrescine was similar as was found in drought-treated Aegilops accessions and introgression lines. These results suggests that the different metabolic changes observed under drought and salt stress was mainly caused by the different mechanisms operated under different stress conditions rather than the genetic variance of the genotypes or environmental factors modified by the experimental conditions. These investigation also proved that the osmolytes of proline, glycine betaine and sugar components could be able to be determined from tissued sap directly. In addition, these results demonstrated that different osmolytes were responsible for decreasing of the osmotic potential of tissue saps and they were strongly depended on the type of treatment.

The PCA analysis of metabolites summarized the most significant relationship in the osmotic stress-induced metabolic changes: it showed that glucose and fructose were the dominant factors in the leaves and roots of PEG-treated plants, while high amounts of GB also contributed to the decrease in OP in the roots. Salt treatment mainly induced the synthesis of sucrose and galactose, but there was also intense proline accumulation in both roots and leaves. Mannitol and sorbitol penetrated and transported in large amount to the root and leaf cells causing changes in the maltose and polyamine metabolisms.



We believed that these information are important for discussing of all results, we have already published it.

Summary,

The main objective of the research grant

The main objective of the research grant has been achieved and the research plan have been performed. Moreover, additional experiments have been done based according to the additional scientific questions raised during the experiments. However, we had a few unexpected difficulties in bioinformatics analysis, but we could solve this task. The results of the research grant has been presented in several national and international conferences and published in 10 scientific papers in high ranked international journals journals. However, further papers are still waiting for publication (they are under preparation).