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Pre-final Progress Report

QTL mapping of drought resistance derived in wild barley, *Hordeum spontaneum*

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EXECUTIVE SUMMARY:

Project objectives: To reveal the genetic basis of unique resistance to water stress in wild barley, *Hordeum spontaneum,* from Israeli desert populations, as a potential source for a radical improvement of cereals drought resistance. This includes identification of physiological mechanisms of drought resistance of desert wild barley and genetic dissection (QTL mapping) of the revealed differences between resistant and susceptible genotypes.

Project findings over the third year: The results of field and laboratory experiments obtained during the first and second years of the project based on a mapping population derived from a cross *Hordeum spontaneum* (desert) x *Hordeum vulgare* (mesic), were used for joint QTL mapping analysis. This allowed us to conduct a simultaneous mapping of laboratory physiological scores related to drought resistance and 20 field traits characterizing plant performance upon stressful conditions. During the third year we extended this analysis by using more advanced map and more sophisticated (hence, more powerful and precise) mapping tools developed by the PI's group. Likewise, during the third year we also finished the second mapping population, initiated earlier by crossing between a mesic and desert ecotypes of *Hordeum spontaneum* using laboratory traits as a target for QTL mapping. Several genomic regions with significant drought-related QTLs were detected using multiple trait mapping analysis. The results of this mapping were complemented by gene expression analysis (Northern and RT-PCR) under drought stress using several dehydrin candidate genes. After dehydration stress, the expression of some of these candidate genes in desert ecotype appeared earlier and is manifested stronger compared to mesic ecotype.

Contribution to the project objectives and international development: The results of the third year proved the efficiency of the chosen strategy of tagging unique drought resistance genes from Israeli desert barley. The final results will be of high theoretical and practical importance. The theoretical importance is due the need of understanding the molecular-genetic basis of plant survival at edge of life (extremely harsh desert environment: the used wild barley plants cope with as low as 100 mm year rainfall). The practical importance is in the fact that mapping of unique resistance to desert conditions will generate DNA markers flanking the target genes, enabling marker-assisted introgression of resistance from wild progenitor to modern cultivars and future map-based cloning. Coincidental mapping of the drought-resistance QTLs and candidate genes in the second mapping population illustrate this point.

Nature of collaboration: Fruitful relationships have been established between the partners based on complementation of the strong aspects of each group. This includes the Israeli experience of molecular marker analysis and genetic mapping, QTL mapping analysis, and drought resistance physiology at the cell and seedling levels. The Almaty group is good in field experimentation and plant physiology at the whole plant level. This complementation was already used to achieve some of the project objectives related to generation of new data. Likewise, members of the Almaty group participated during their training visits in molecular marker scoring. The equipment and chemicals purchased by the Almaty group within the framework of this project, combined with the experience obtained during the training visits, allowed them to conduct molecular marker analysis on their own mapping population derived from a cross between Israeli highly resistant desert ecotype of *H. spontaneum* and a local Kazakh barley cultivar (cross C). The PI of the project has visited the Almaty group (September 2001) and discussed in detail the results of the first two years. Likewise, the shared mapping population is now being evaluated under field conditions of Kazakh Institute, opening the possibility to address the important question of QTL-environment interaction. The Israeli group developed a special algorithm to analyze such data, and during the last visit of the PI to Almaty this subject was also discussed.

SECTION I

A) RESEARCH OBJECTIVES:

Wild relatives of many cultivated plants, including wild barley, *Hordeum spontaneum*, have long evolved in the Near East Fertile Crescent across a wide ecological range. This generated a rich spectrum of adaptive diversity to multiple diseases, pests and ecological stresses, including drought. The objective of our research is to reveal the genetic basis of unique resistance to water stress in wild barley, *Hordeum spontaneum,* from Israeli desert populations, as a potential source for a radical improvement of drought resistance. This includes identification of physiological mechanisms responsible for drought resistance of desert ecotypes of *H. spontaneum* and genetic dissection (QTL mapping) of the revealed differences between resistant and susceptible genotypes.

B.1) RESEARCH ACCOMPLISHMENTS OF THE ISRAELI AND KAZAKH RESEARCH GROUPS:

Over the past year, the research has been focused on (1) physiological phenotyping of the second mapping population in the laboratory, (2) establishing molecular map of the second mapping population and saturation of the first population by additional SSR markers, (3) expression analysis (Northern and RT-PCR) of dehydrin genes in desert and mesic ecotypes of *H. spontaneum,* (4) Genetic mapping effort for F2 Yuzhnokazahstanskii 43 x H.spontaneum segregating population is underway. Currently, DNA samples from 123 F2 plants were prepared for AFLP analysis and scored for 10 EcoRI/MseI primer combinations (**Fig. 8**).

RESULTS

1. Integrative mapping of the first mapping population (cross A)

Improving the methodology of multipoint marker mapping (Cross A. Fig. 1) A linkage map was first constructed for **cross A** using MAPMAKER, version 3.0. Due to the paucity of linkage information from dominant markers linked in repulsion phase, two map versions were constructed for each chromosome, according to the source of the dominant alleles at the marker loci, cultivar Mona (map version M) or wild barley (map version W). The Mona version (M) consisted of 48 SSRs, 66 AFLPs, 3 STSs, and 2 Dhn genes, spanning 1736.5 cM, with an average distance between markers of 15.0 cM. The wild barley version consisted of 51 SSRs, 60 AFLPs, 3 STSs, and 2 dehydrin *(Dhn)* genes, being 1841.8 cM in length with an average interval of 16.4 cM. Motivated by the projects challenges to map dominant markers, a new highly efficient and fast method for multipoint marker mapping was developed that may complement the popular tool MapMaker tools. Our new algorithms enable ordering many dozens and hundreds markers with subsequent verification of the established maps using computing intensive bootstrap and/or jackknife methods developed by Dr. Mester at the PI's lab. This approach proved to be rather robust with respect to various complications like missing data, marker miss-classification and negative interference. It is now being further developed to allow for synchronous ordering of two groups of markers, with dominant markers in linkage phase in each group and shared codominant markers, with subsequent integrating of the two maps into a combined map.

Single trait QTL analysis: For QTL detection, the threshold values of the test statistics (LOD) were calculated based on permutation analysis. Bootstrap technique was used to evaluate confidence intervals of the putative QTL location and effect. Single trait interval analysis revealed several QTLs

affecting drought-tolerance related scores, at both seedling and whole-plant levels (**Fig. 1**). These included drought tolerance traits scored in the lab and in the field, as well as traits related to changes in plant performance/productivity in the field caused by drought stress. The main effects revealed using single trait analysis included:

Chromosome 1: root length under stress, survival after dehydration, and, mainly, several field performance traits.

Chromosome 2: dry weight under stress and field performance traits (heading date and plant height under stress).

Chromosome 3: survival after dehydration and tillering after stress.

Chromosome 4: root number and length under stress, plant height and seed weight under field stress.

Chromosome 5: survival after rehydration, root number and length under stress, accumulation of ^{13}C and several field performance traits (heading date and seed weight under stress).

Chromosome 6: only traits related to plant performance in normal field conditions were affected by this chromosome.

Chromosome 7: root length under stress, traits related to plant performance in normal field conditions and heading date and tillering under stress.

Noteworthy, the empirical values of significance were calculated using permutation test on chromosomal level for each trait. Therefore, in final analysis we will have to provide a correction of these values to get genome-wise significance. Moreover, the correction should also take into account the fact that the analysis included many quantitative traits. There are several approaches to deal with this problem, including those that control the false discovery rate (FDR) (e.g., Weller, J. et al., 1998; Genetics **150**: 1699-1706). This analysis is underway and will be conducted for QTL mapping results obtained separately by single-trait and multiple-trait analysis and provided in final report and in the two papers in preparation. An important conclusion that follows from the foregoing QTL mapping results is that the highest contribution for both laboratory and field scores of drought resistance is of the chromosomes known to carry dehydrin genes, especially chromosome 5 that harbors a cluster of *Dhn* genes.

Multiple trait analysis (Figures 2-5): In addition, preliminary multitrait QTL analysis was conducted using the novel QTL mapping methodology developed at the Institute of Evolution, **(Fig. 2)**. The analysis included simultaneously ten laboratory scores and about 30 field scores reflecting drought resistance and plant performance under normal and drought conditions, amounting altogether about 50 (!) traits. The analysis was carried out using MultiQTL package (**http://esti.haifa.ac.il~theorpop**) developed by PI, in which the maximum likelihood interval-mapping approach is used. The preliminary results represented in **Figures 2-5** and dealing with traits related to plant performance in field under drought stress and field scores of drought-tolerance traits (^{13}C) were obtained by single-QTL analysis. However, for some of the chromosomes, two-to-three QTLs can clearly be seen from the multitrait LOD graphs, calling for further analysis and tests using linked QTL models. Necessary models and tools for such analysis are now being developed and tested by the PI's group (Ronin et al., in preparation).

2. Genetic analysis of the second mapping population (cross B)

(*H. spontaneum* x *H. spontaneum*)

Target traits: For this mapping population twelve drought-resistance traits were analyzed:

1. Wilting time (WT), the number of days after the last irrigation when all leaves showed dehydration.
2. Relative water content (RWC), measured in last fully expanded leaf, RWC(%)=(FW-DW)100/(TW-DW), where FW is the leaf fresh weight, TW the turgid weight, and DW the dry weight.
3. Osmolarity (OSM), measured at full turgor of last fully expanded leaf, using rehydration method (5520 Vapor Pressure Osmometer, Wescor, USA).

4.& 5. First leaf length (l1l) and second leaf length (l2l), measured after they were fully expanded.
6. Relative prolonging rate (RPR), relative length growth rate of leaves, RPR (%) = (Lw – L_1)100 / (L1-L2), where Lw is total leaf length at wilting time, L1 - total leaf length one day after the last irrigation, and L2 - total leaf length at two leaves stage.
7. Senescence (SEN), the grade of yellowish of leaves after plant wilting.
8. Soil water content (SWC), representing plant transpiration, measured as weight percent at the wilting time.
9. Recover rate (RCR), total leaf length growth rate after 3 days of re-hydration.
10. Regrowth (REG). Plants were water withheld again after recovery. The wilted shoots were cut, and the plant was re-hydrated. Total length of new growing leaves were measured as REG.
11. & 12. Root length (RLE) and thickness (RTH), measured at 5-days-old seedlings with Delta-T scan system (Delta-T Devices Ltd, UK).

Molecular mapping: A linkage map was constructed with 46 SSRs and 51 AFLPs using MAPMAKER 3.0 with subsequent versification by using the new tool developed in the PI team (Mester et al, 2002a). The total map size exceeded 1880 cM and covered the entire genome. Due to paucity of linkage information from dominant markers linked in repulsion phase two map versions were constructed for each chromosome (**Fig. 6 map A and B**), except of chromosome 2.

QTL detection: All twelve traits were scanned for putative QTL on the whole genome level. One of the genetic models, including additive, dominant, recessive, heterotic (no additive) effect, was chosen for each of the putative QTL. Models of ***single-*** and ***two-linked-QTL per chromosome*** were employed for single- and two-trait analysis. We also conducted multiple-trait analysis assuming single QTL per chromosome. For QTL detection, the threshold values of the test statistics (LOD) were calculated based on permutation analysis. Bootstrap technique was used to evaluate confidence intervals of the putative QTL location and effect.

Phenotypic distribution of the measured quantitative traits (Fig. 7): The xeric genotype 23-38 showed significantly higher L1L, L2L, and RLE than the mesic genotype 10-30. Traits, such as WT, RWC, OSM, RPR, RCR, SWC, SEN, REG, were not significantly different between two parental lines. However, except of SEN and REG, all the other traits showed slightly higher drought resistance in xeric genotype 23-38 than in mesic genotype 10-30. Although the difference between parental lines, mesic genotype 10-30 and zeric genotype 23-38, were not great, obvious segregation of the F_4-family means was observed for all the 12 measured traits in the present F_2 mapping population with coefficient of variation (CV) ranging from 4.8 to 43.1%. Some of these traits segregations show strong transgressive segregations, suggesting that mesic genotype contains positive alleles for drought resistance traits.

QTL mapping: Single-trait analysis with ***single-QTL-per-chromosome*** model revealed fifteen putative QTLs. Eleven additive QTLs were found by two-trait analysis, and four new putative QTLs by multiple-trait analysis. Compared with one-trait analysis, two-trait and multiple-trait analysis improved the significance, precision and power of QTL detection. The number and location of QTLs found in the two map versions (**a** and **b**) were similar.

1. QTLs with high LOD scores ($p<0.001$) were revealed for RPR, L2L, REG, in chromosomes 2, 5, 4, 5, respectively. QTLs for RWC and OSM were located in chromosomes 1, 2, 5, 6, and 2, 3, 4, 5, 6, 7, respectively, corroborating the results of other authors.
2. Xeric genotype-derived alleles at ten QTLs affected positively six resistance-related traits (WT, RWC, SEN, L2L, REG, and RLE) whereas at four other QTLs xeric alleles had a negative impact on RWC, RPR, L1L, REG.

3. Our mapping results show that revealed QTLs are involved in drought resistance through mechanisms of osmotic adjustment (OA), deep rooting and small plants.
4. In total, drought resistant QTLs were located on chromosomes 2, 5, 6 and 7 (see map in **Fig. 6**). Some of the revealed chromosome regions with strong effects need to be further studied for fine mapping.

3. Expression analysis of candidate genes

Despite numerous studies aimed to characterize drought-inducible genes (including the dehydrin gene family), the molecular basis for plant tolerance to water stress remains far from being well understood. Moreover, it is clear that not each stress-inducible (stress-repressible) gene contributes to stress resistance, and *vice versa*. There might be resistance genes that do not change their level of transcription upon stress. Clear discrimination between "stress-induced" and "stress-resistance" genes will allow to reveal the major genetic components of plant cell resistance to water deficit, particularly genes related to protection and repair mechanisms.

The objective of the study presented here was to analyze the expression of dehydrin genes in the drought tolerant and drought susceptible genotypes of barley under experimental drought conditions. We focused on the dehydrin (*Dhn*) gene family, one of the most important genetic systems involved in response to water stress. We assumed that dehydrins may play a major role in naturally occurring tolerance to drought in Israeli desert populations of *H. spontaneum,* due to specific allele combination, and/or regulatory genetic elements affecting the expression of these genes. Two of the *Dhn* genes which were polymorphic between the parents were used for mapping in Cross A; no polymorphism was detected between the parents of cross B.

Induction and differential expression of dehydrin genes in wild barley (*H. spontaneum*) in response to drought stress: Seven days old seedlings were placed into an opaque aquarium, filled with Hogland medium (0.5) circulated by air pumps. After 10 days of growth (4^{th} leaf stage), the aquariums were drained, and plants remained in the greenhouse for 24 hours. Leaves were harvested in intervals of 0 (control), 3, 6 and 24 hours after draining. The plant material was frozen immediately in liquid nitrogen and stored at -80°C until used. The expression of dehydrin genes was scored using Northern blotting and RT-PCR analysis.

Northern analysis: It appeared that under normal growth conditions (time 0) five genes (*Dhn 1, 3, 4, 6, 9*) were not expressed, whereas *Dhn5* was expressed at a low level in the desert genotype (Appendix 1 Fig. 1). When plants were subjected to progressive drought by withholding irrigation, the level of the transcripts increased reaching a maximum after six hours of treatment. Remarkably higher intensities were observed in the resistant rather than in susceptible genotypes at 3 and 6 hours after dehydration. The difference in expression between the tested plants was less apparent after 24 hours (see **Appendix Fig. 1**).

RT-PCR: Although we used gene-specific oligonucleotide primers for generating *Dhn* nucleic acid probes, results of Northern-hybridization demonstrate simultaneously a detection of more than one *Dhn* transcript. The reason of this is clear: *Dhn* genes belong to a multi-gene family with a rather high level of homology of its members. Nevertheless, hybridization with *Dhn5* probe revealed a gene-specific band which could be identified, because *Dhn5* gene has a considerably higher molecular weight than all others members of this multi-gene family. The induced expression of *Dhn5* could be quantified by densitometric analysis. The histogram in Appendix 1 - Fig. 2 shows the normalized values of *Dhn5* expression (after standardization to *18S* ribosomal signal intensities) presented as percentage of the highest value. Dehydrine genes (*Dhn*) are associated with tolerance to dehydration, and most of the *Dhn* genes are up-regulated by dehydration in barley. Our objectives were to study the time course of induction and differential expression of eight *Dhn* genes (*Dhn 1,3,4,5,6,7,9,10*) in

response to dehydration, between drought-tolerant and drought-susceptible wild barley (*Hordeum spontaneum*) originating from xeric and mesic regions in Israel. Major differences in expression were observed in *Dhn1* (Chr. 5H) and *Dhn5* (Chr 6H), both in the time of induction and in the level of expression. Namely, earlier induction and higher levels of expressions were obtained in the desert genotype than in the mesic genotype.

These results corroborate the foregoing evidence on QTL mapping of regulatory factors on Chr. 5H in barley and 5B in wheat in vicinity of the *Dhn*. The allelic variation found in *Dhn3,* together with our previous results (Close et al., 2000) which showed allelic variation in *Dhn4* in wild barley from Israel, demonstrate that the naturally occurring variation in dehydrin genes have an important role in drought stress tolerance (**Appendix, Figures 1-4**).

B.2) REPORT OF THE KAZAKH RESEARCH GROUP:

Responsible researchers: Dr. Turuspekov, Dr. Abugalieva, Dr. Ryabushkina
Additional staff: Junior researcher Mrs. Dzhardemalieva.

Major objectives of the Year 2 were to study genetic diversity of wild and cultivated barley varieties from Kazakhstan and prepare the genetic material for localization of the major QTLs contributing to drought tolerance in barley.

1. Description of the wild barley plants *H. spontaneum* from Israel and Central Asia compared with varieties of cultivated barley *H. vulgare* from Kazakhstan.
2. Establishment of a mapping population derived from a cross between Israeli highly resistant desert ecotype of *H. spontaneum* and a local Kazakh barley cultivar (**cross C, Fig. 8**)

A. Field data:

According to the Work Program, the barley varieties from Kazakhstan (Donetskii 8, Bereke 54, Arna, SK 43) and populations of *H. spontaneum* from Israel (Arad, Sede Boker, Yavne, Jericho) along with samples from Central Asia were sown in the fall of 1998 and 1999 and spring of 1999 and 2000. All wild barley accessions were tested for following traits: heading date, height of plant, color of spike and grain, number of productive spikes, weight of grains per one plant. In general, the lines from population Jericho were most adaptive to the conditions of the South East of Kazakhstan. Therefore, the majority of hybrid lines between *H. vulgare* and *H. spontaneum* were developed involving individual plants from population of Jericho.

B. Physiological analyses:

The objective of this investigation was to analyze the stomatal control in different barley cultivars and lines of wild species of *Hordeum spontaneum K.*

C. Results and discussion:

Line 3 (Jericho) of *H. spontaneum* and Arna showed two important features for drought avoidance (presumably along with some other traits which were not under present investigation). The first, shorter period of vegetative stage, (the time of the beginning the flowering stage). The second, line 3 from Jericho of *H. spontaneum* and Arna had the higher level of stomatal conductance and transpiration compared to other lines and cultivars. The influence of precocity, osmotic adjustment and stomatal conductance (Field Crop Res. 62:23-34) showed significant linear correlation between grain yield and stomatal conductance ($r=0.77$, $p=0.05$) in stressed barley genotypes. We explained this observation by a smaller reduction in conductance, which may favor photosynthesis at critical stages in yield

determination, and consequently increase yield under drought. The next step of this investigation will be related to segregation analysis of F_2 hybrid populations Bereke 54 × Jericho of *H. spontaneum* and South Kazakstan 43 × Jericho of *H. spontaneum*, which differ in time of the beginning of flowering and in stomatal conductance level.

D. Genetic diversity analysis:

1. **Biochemical and molecular markers analysis:** Both wild and cultivated barley accessions were screened using several biochemical markers, such as isozymes and storage proteins. The isozyme analysis of 10 barley varieties from Kazakhstan and *Hordeum spontaneum K.* from Israel was done for Esterase, Malate dehydrogenase, Acid phosphatase, Peroxidase, 6-Phospho-glucanatedehydrogenase, Glutamate dehydrogenase. Comparative analysis of genetic diversity between Israeli and Central Asian population is underway. Population "Yavne" has been characterized by significant intrapopulational polymorphism on hordein components.
2. **DNA analysis:**
   a. *SSR (Simple sequence repetitions) analysis*: 96 wild plants *H. spontaneum* representing both Israel and Central Asia and Kazakh barley varieties were screened using 18 SSR primers
   b. RA*PD (random amplified polymorphisms of DNA) analysis*: Ten cultivated barley varieties from Kazakhstan were analyzed using 11 PARD primers. Both SSR and RAPD data will be extended in order to develop genetic catalogue of barley varieties in Kazakhstan.
3. **Preparation of plant material for genetic analysis:** According to the Work program research group from Kazakhstan has prepared hybrid material from crosses between cultivated barley varieties from Kazakhstan and wild barley from Israel. F1 plants from the first year of the project were sown in order to obtain F2 segregation populations. Currently, we concentrate on two different F2 hybrid lines; *H. spontaneum* x Bereke 54 and *H. spontaneum* x South Kazakstan 43.

C) SCIENTIFIC IMPACT OF COLLABORATION

The first two years demonstrated a fruitful collaboration between the partner teams. In particular, this was manifested in a complementation of the strong aspects of each group. The Israeli team has accumulated a good experience of molecular marker analysis and marker mapping, QTL mapping analysis and drought resistance physiology at the cell and seedling levels. The Almaty group is strong in field experimentation and plant physiology at the whole plant level. Currently, the first Israeli mapping population (cross A) is being scored under the field conditions at Almaty. These results will be used to address the QTL-environmental interaction problem.

D) DESCRIPTION OF PROJECT IMPACT

The final results of the project will be of high theoretical and practical importance. The theoretical importance is due primarily to the unique drought resistance manifested by the used Israeli desert genotypes of *H. spontaneum*. The genetic dissection of this resistance and testing for coincidence with already identified dehydrin genes will open the possibility to clone new unique alleles of drought resistance genes. We have already included several dehydrin genes into the mapping program aimed to look for their coincidental location with drought resistance and/or plant performance under stress. Once such coincidences are found, the next step will be testing the corresponding candidate genes for differential response to stress (assayed by expression analysis) between the parental genotypes as well as the alternative QTL groups of the mapping population defined by the flanking markers. Such an analysis could be considered as a genuine bridging between structural and functional genomics. The immediate practical importance of the obtained results is in the fact, that direct mapping of the unique resistance will result in defining molecular markers flanking the key resistance genes, hence allowing for marker-assisted introgression of the resistance genes for the wild progenitor to modern cultivars.

E) STRENGTHENING OF DEVELOPING COUNTRY INSTITUTIONS

This project has manifold positive consequences for the Kazakh Institute, in particular, and for Biological and Agricultural Science of Kazakhstan, in general. These include:

(1) **Purchasing modern equipment** to conduct Molecular Biology studies. They have got the money for equipment and chemicals that allowed them to initiate PCR analysis for genotyping their mapping population at Almaty.

(2) **Training the personnel** was considered an important aspect of our collaboration. During the first year, two members of the Almaty team (Mr. Sersenbaev and Dr. Ryabushkina) visited Haifa, with total duration of the visits of about 5 months. We planned additional visits for the second year. The target of the planned visits was to continue training in molecular biology techniques and, in addition to experimental techniques, to learn how to use new software (MultiQTL package, see website (**http://esti.haifa.ac.il~theorpop**) for QTL mapping developed by the Israeli PI. Due to technical problems these visits were postponed for the next year.

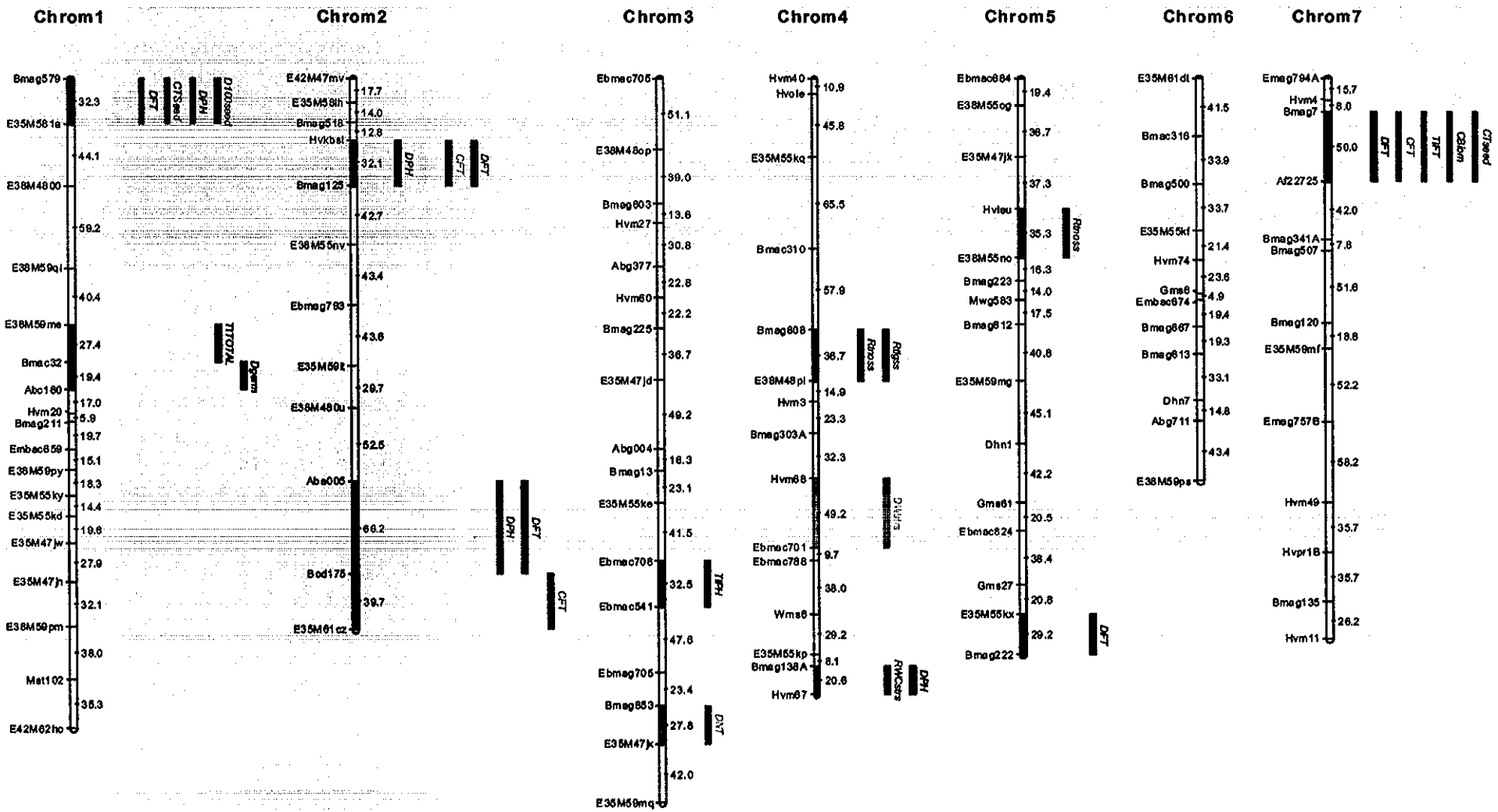
(3) **Bridging between modern quantitative genetics and breeding** for crop resistance is one of the consequences of projects aimed to dissect genetically complex traits related to plant adaptation. Barley is a very important crop for Kazakhstan and resistance to drought stress is a limiting factor in productivity and yield stability of barley in this country. Therefore, the possibility to facilitate selection for drought resistance based on unique desert alleles of Israeli wild barley and developed molecular markers is a major contribution of this project to their agriculture. Moreover, the facilities, stuff and know-how, that is being established in Almaty as a result of this project, will serve a basis for future development for other crops and other limiting factors of agriculture productivity in this region.

F) FUTURE WORK (remained for finalizing the project)

Linkage map: we still have to improve the density of markers in a few intervals where strong effects on drought-related traits have been detected in both Israeli mapping populations, cross A and cross B. Likewise, we will have to establish the genetic map based on the molecular markers being developed currently on the Kazakh mapping population (**Fig.7**).

Candidate genes: After successful testing of the parents for differential expression of the candidate genes in response to drought stress, the dehydrin genes manifested differential expression are now being mapped on our mapping population in order to relate the positions of the detected QTLs with those of candidate genes related to drought resistance (in particular, osmotic adjustment) and plant performance in the field upon drought stress.

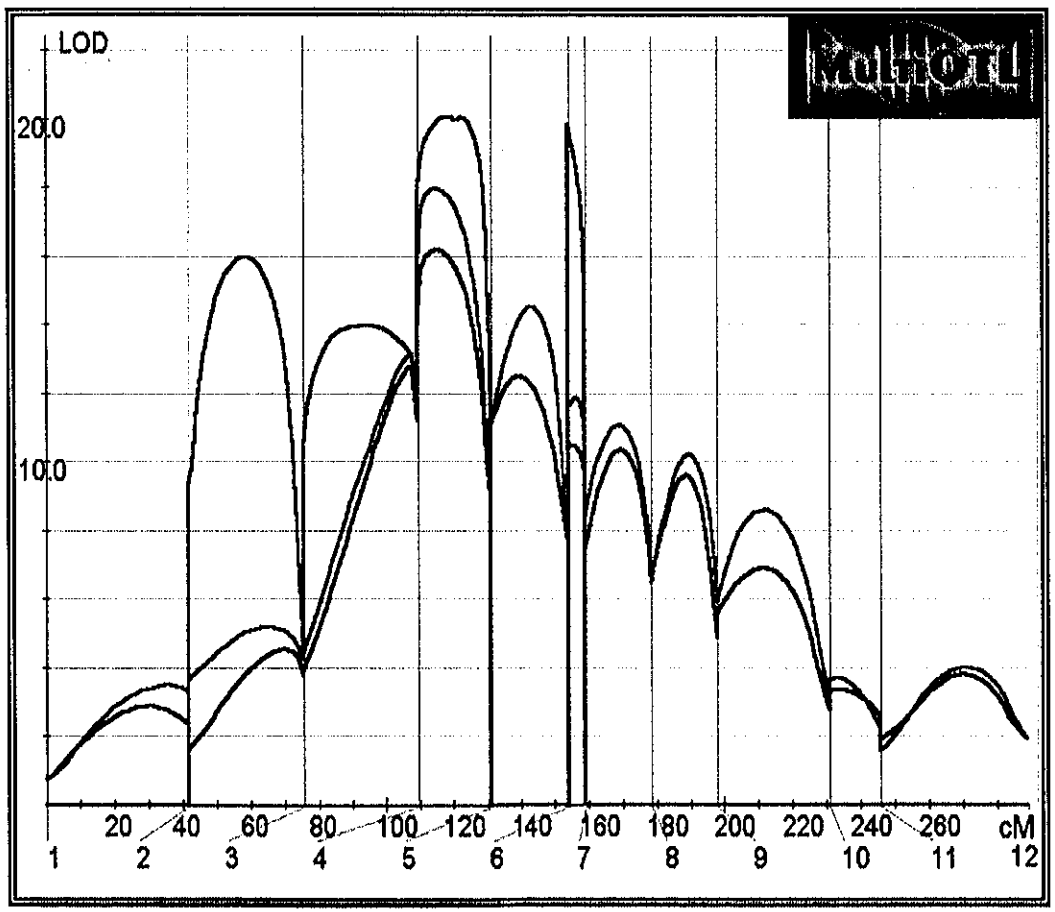
Drought resistance QTL analysis: A detailed multiple trait analysis will be conducted using a revised map with added co-dominant markers and additional scores of resistance traits on the cross B population. Special attention will be paid to multiple trait analysis as a more powerful and precise technique of QTL mapping, in order to integrate the entire data accumulated during the project.



Cross A. Figure 1. Drought resistance QTL map of *H.spontaneum* from Wadi Qilt x *H. vulgare* - Cross A

Genetic map was constructed by Mapmaker/Exp (version 3.0) with 63 SSR and 34 AFLP markers, Single QTLs were detected by MultiQTL, maps were drawn by MapChart (version 2.0). Designition of physiological traits: CPH: plant height of control, DPH: plant height under stress, CFT: flowering time of control, DFT: flowering time under stress, Dgerm: seeds germination rate under stress, DNT: number of tillers under stress, Ctseed: total seed weight of control, DWstrss: dry weight under stress, Rtnoss: root number under stress, Rtlgss: root length under stress, RWCstrs: relative water content under stress, D100seed: 100 seeds weight under stress, TIPH: tolerance index of plant height, TITOTAL: tolerance index of grain yield, TIFT: tolerance index of flowering time, Cbiom: biomass of control

Fig. 2 (Cross A) Example of increased QTL detection power and mapping precision owing to joint analysis of multiple traits related to barley drought resistance



Consecutive steps of analysis of a set of drought related traits in F_4 (*H. vulgare* x *H. spontaneum*) by using the ***MultiQTL*** mapping software **(http:/esti.haifa.ac.il/~poptheor).** Data on chromosome six were used in the example presented here. Starting from 34 traits (red), we reached an optimized 10-trait complex (blue) with QTL detection power of 98.6% at significance level 0.001. Consequently, the confidence interval for the QTL position is **2.4-fold narrower** compared to that for the most significant effect obtained by using standard single-trait interval QTL mapping analysis.

Fig. 3 Joint analysis of multiple traits (18 traits) related to barley drought tolerance in field conditions (cross A)

Chromosome 1: reduced to 8 traits
P<<0.0005; $ß_{0.001}$=99.9%

Chromosome 2: reduced to 12 traits
P<<0.0005; $ß_{0.001}$ =100%

(where P is the chromosome-wise significance level, and ß is the power of QTL detection)

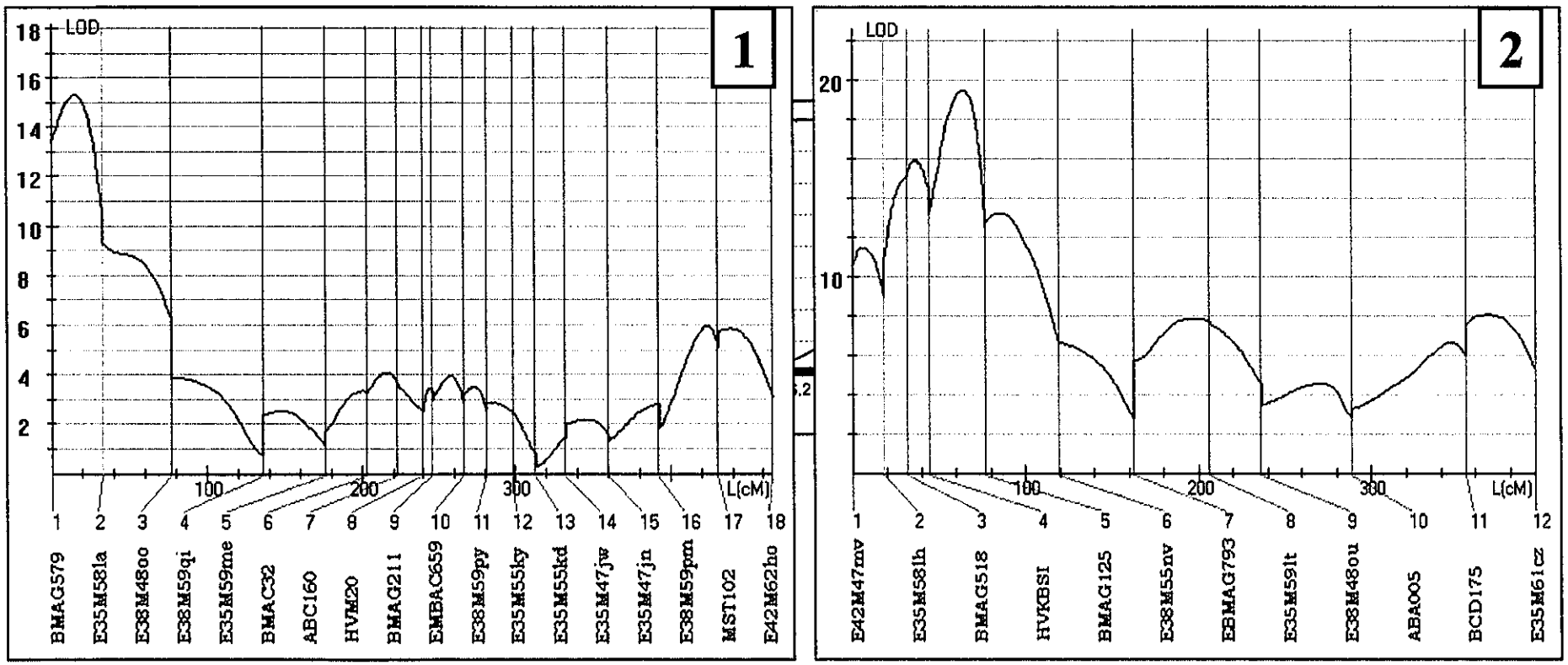
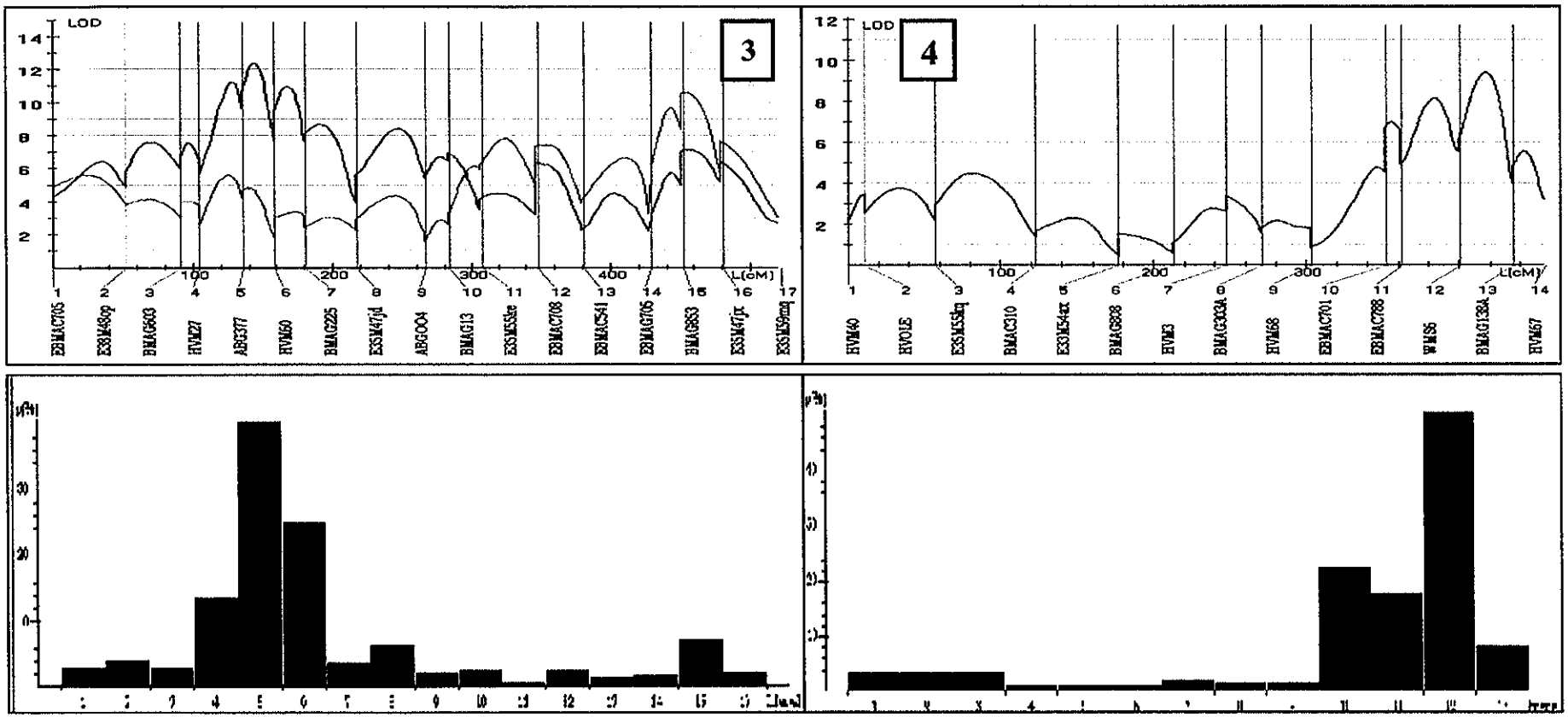


Fig. 4. Cross A. Joint analysis of multiple traits (18 traits) related to barley drought tolerance in field conditions

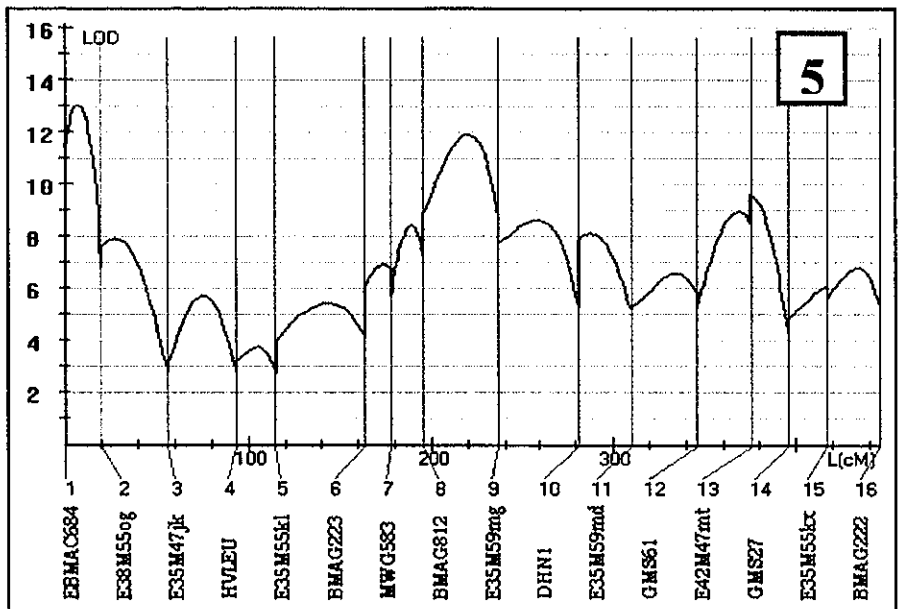
Chromosome 3: **red** - reduced to 12 traits
P=0.005; $ß_{0.001}$=90.5%
blue - reduced to 11 traits
P=0.007; $ß_{0.001}$ =93.4%

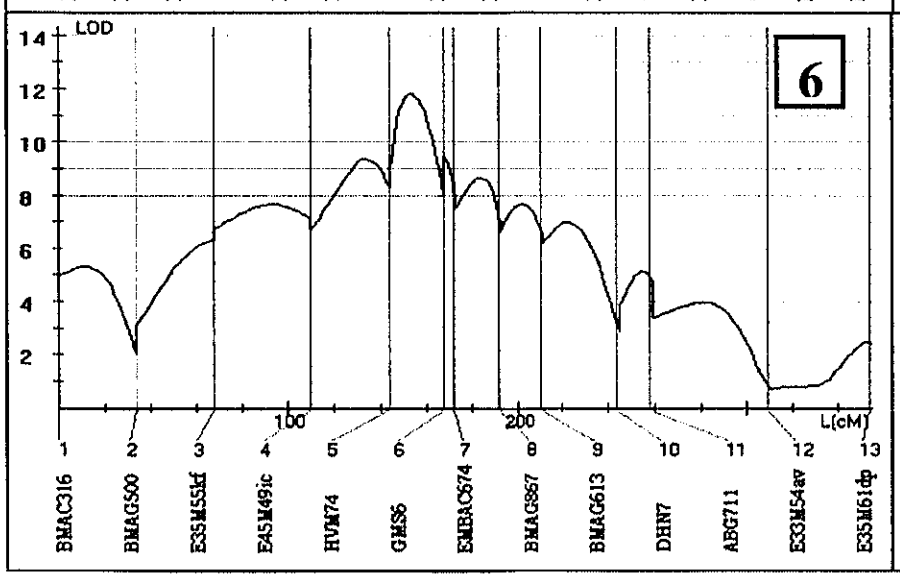
Chromosome 4: reduced to 9 traits
P=0.007; $ß_{0.001}$=83.4%

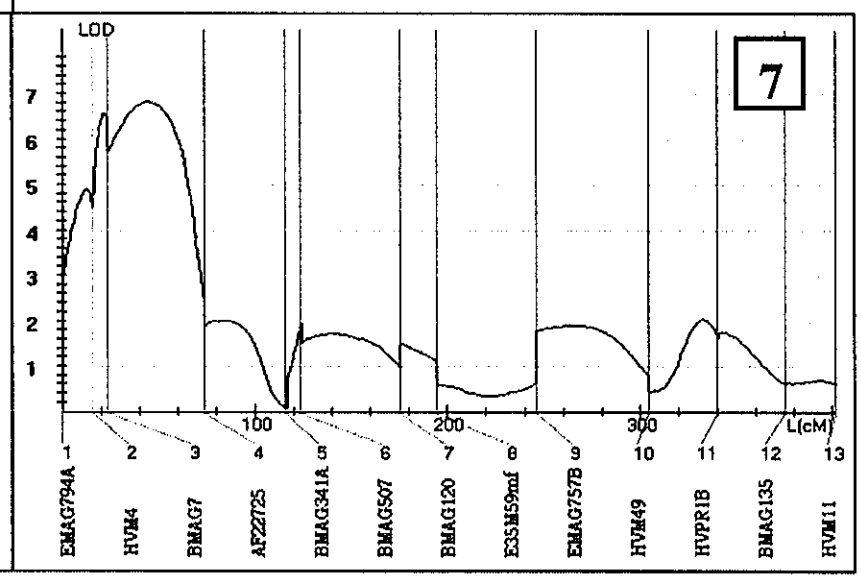


The upper graphs represent the LOD score distributions along the chromosomes, whereas the lower graphs are the results of 1000 bootstraps for the same chromosomes.

Fig. 5. Cross A. Joint analysis of multiple traits (<u>18 traits</u>) related to barley drought tolerance in field conditions







<u>Chromosome 5</u>: reduced to 10 traits
P<0.0005; $ß_{0.001}$=100%

<u>Chromosome 6</u>: reduced to 8 traits
P<0.0005; $ß_{0.001}$=93.7%

<u>Chromosome 7</u>: reduced to 11 traits
P<0.002; $ß_{0.001}$=74.2%

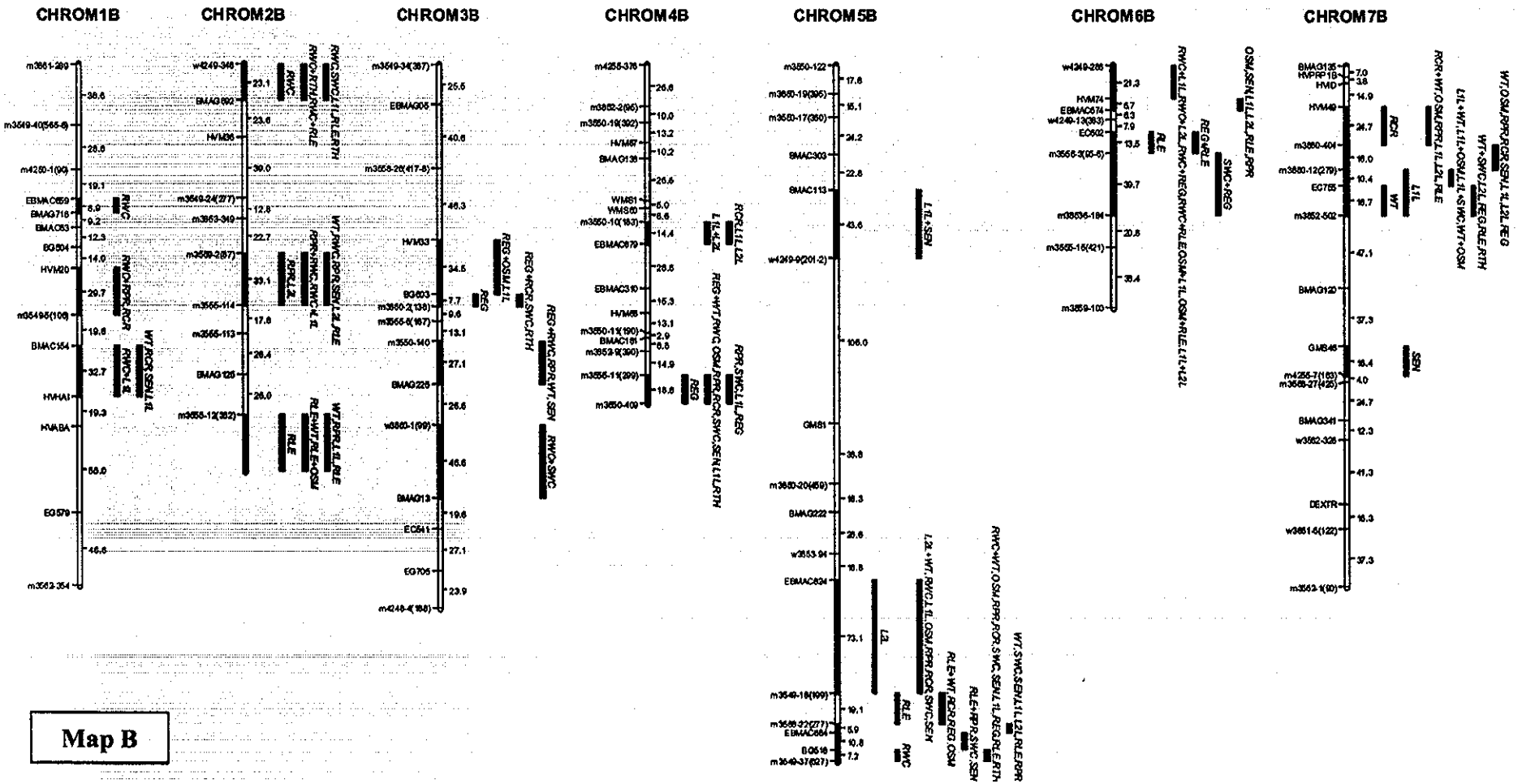


Figure 6. Cross B. Drought resistance QTL map of *H.spontaneum* from Wadi Qlt x Malot

Map A is for mesic genotype 10-30, Map B is for xeric genotype 23-38. Genetic maps were constructed by Mapmaker/Exp (version 3.0) with 46 SSR and 51 AFLP markers. Single QTLs were scanned by MultiQTL; maps were drawn by MapChart (version 2.0). Physiological traits: wilt time (WT), relative water content (RWC), osmolarity (OSM), leaf relative prolong rate (RPR), recover rate (RCR), soil water content (SWC), senescence (SEN), regrowth rate (REG). Morphological traits: first leaf length (L1L), second leaf length (L2L), root length (RLE), and root thickness (RTH).

▬▬: Single QTLs detected by *single trait to single QTLs* model; ▬▬: Single QTLs detected by *two traits to single QTLs* model; ▬▬: Single QTLs detected by *multi-traits to single QTLs* model.

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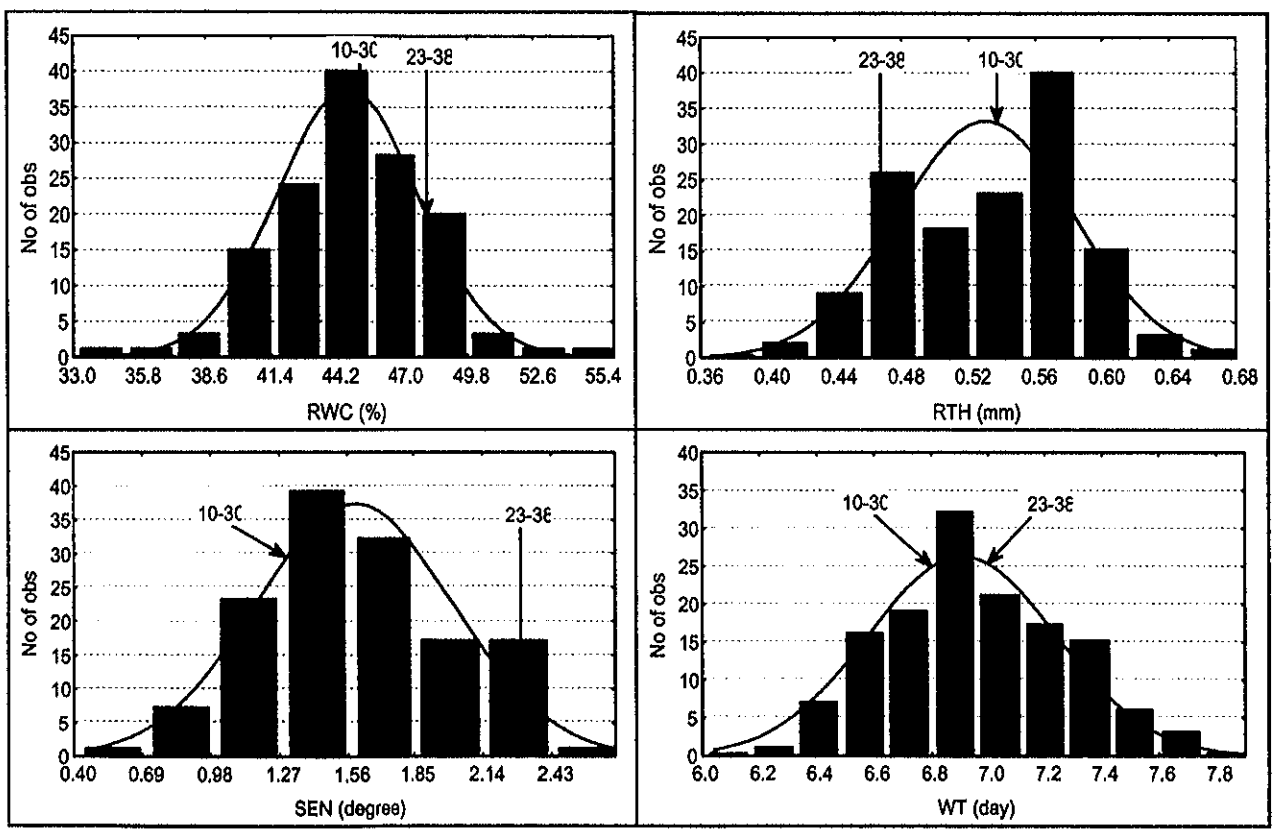
Map A

Fig. 7 – cross B. Distribution of "tolerance traits" of F4 means in cross B of mesic x xeric ecotypes of *H.spontaneum*. (the transgression mode is noteworthy)



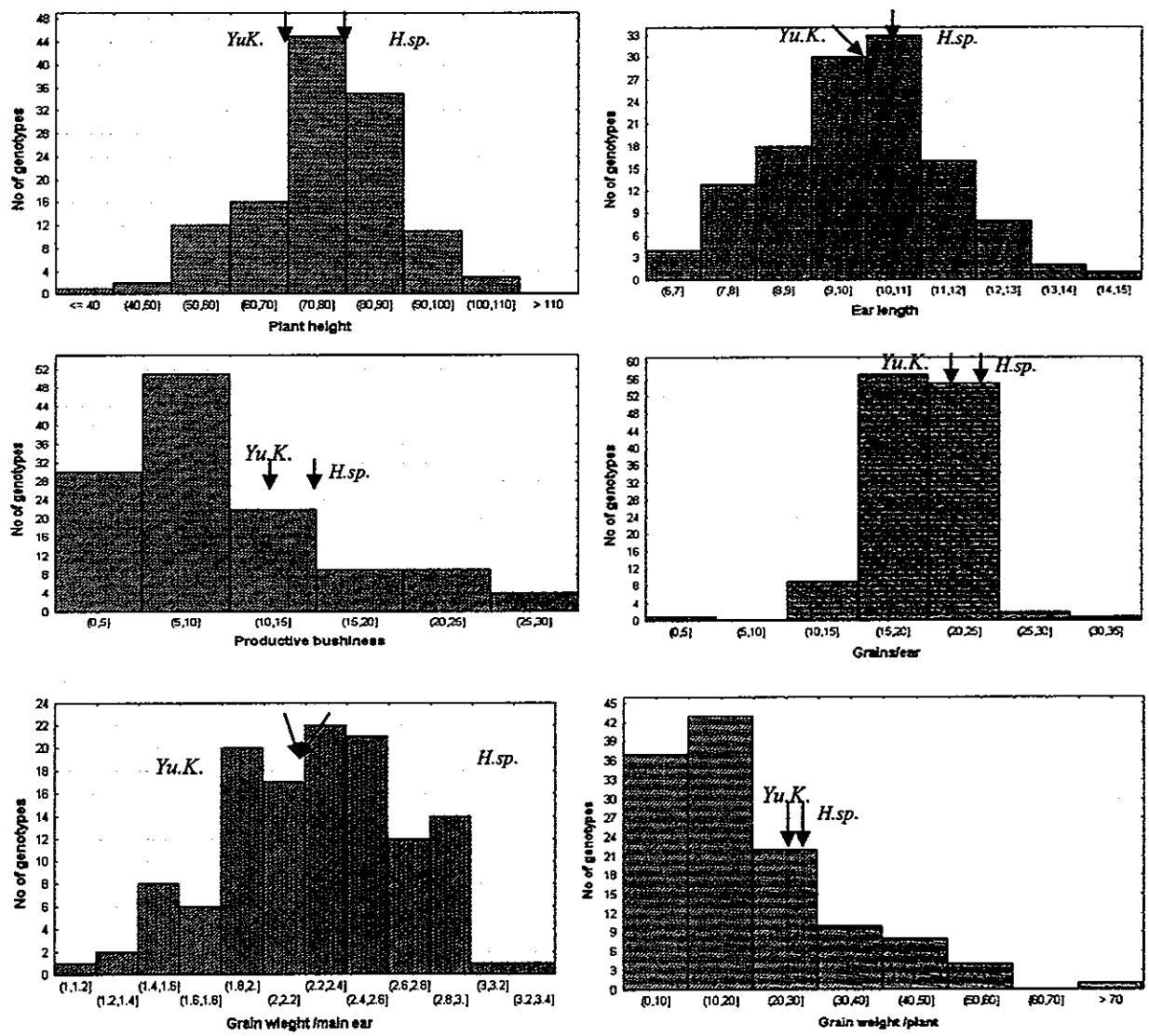


Fig.8. Phenotypic distribution of the Kazakh mapping population (123 genotypes), resulted from a cross between Israeli drought resistant ecotype of *Hordeum spontaneum* and Kazakh elite cultivar YuK43. Note highly transgressive segregation displayed by all of the traits

Appendix

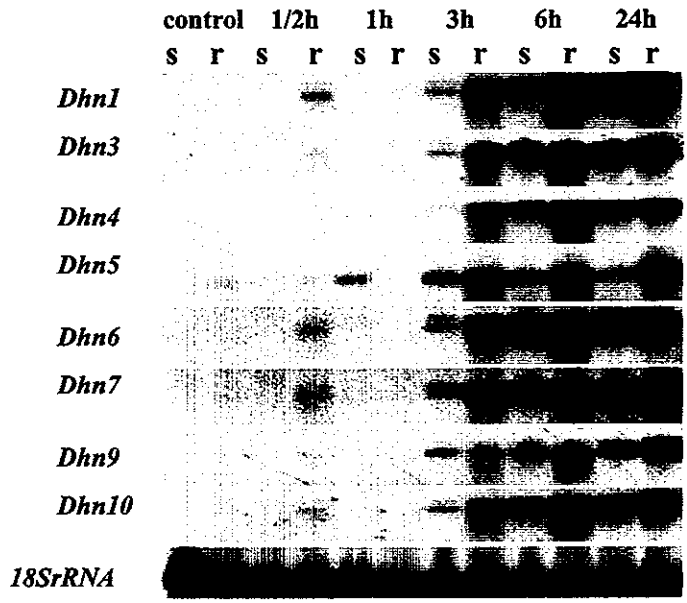


Figure 1. Northern blot hybridization revealing differential expression of *Dhn* genes in *Hordeum spontaneum* originating from xeric (resistant) and mesic (susceptible) populations in Israel.

Total RNA from xeric (r) and mesic (s) of barley after 0 (control), ½, 1, 3, 6, 24 hours. Northern blots were performed with eight *Dhn* probes amplified from genomic DNA of barley (*Hordeum vulgare* L. cv Noga) by gene-specific primers (Choi et al., 1999). As a control for relative amount of RNA, Northern blot was performed with *18S rDNA* probe.

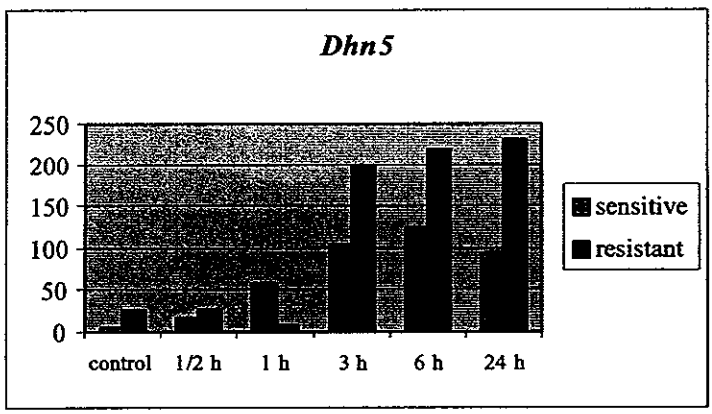


Figure 2. Differential expression of *Dhn5* in *Hordeum spontaneum* originating from xeric (resistant) and mesic (sensitive) populations.

Quantification of hybridization signals of *Dhn5* intensity were measured and compared with *18S rDNA* by densitometric analysis with imager version ImageJ. Histogram shows the normalized values of *Dhn5* (after standardization to *18S* ribosomal signal intensities) presented as percentage of the highest value.

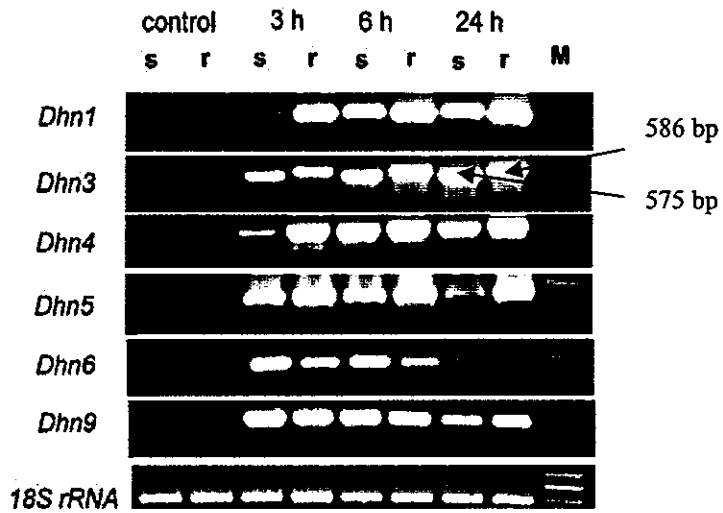


Figure 3. Analysis of differential expression patterns of *Dhn 1, 3, 4, 5, 6* and *9* by RT-PCR.
Total (Dnase I-treated) RNA extracted from resistant (**r**) and susceptible (**s**) plants of barley. First-strand cDNAs were synthesized by SuperScript II reverse transcriptase (GIBCO-BRL) with universal oligo(dT)$_{15}$ primer. PCR were carried out with gene-specific primers. As a control for relative amount of RNA we performed RT PCR with gene-specific primers for *18S rRNA*.

H. spontaneum Dhn3
Mesic (S)
RVDEYGNPVAGHGVGTGMG[redacted]TGAAAGGHFQPTREEHKAGGILQRSGSSSSS
Xeric (R)
RVDEYGNPVAGHGVGTGMG[redacted]TGAAAGGHFQPTREEHKAGGILQRSGSSSSS

H. vulgare Dhn3
cv. Dictoo
RVDEYGNPVAGHGVGTGMG[redacted]TGAAAGGHFQPTREEHKAGGILQRSGSSSSS
cv. Himalaya
RVDEYGNPVAGHGVGTGMG[redacted]TGAAAGGHFQPTREEHKAGGILQRSGSSSSS

Figure 4. Comparison of the amino-acid sequences of two alleles of *Dhn3* from xeric and mesic *Hordeum spontaneum* and *Dhn3* allele of the cultivars Dictoo (NCBI ID # 4105107) and Himalaya (NCBI ID # 118487).