ANNUAL REPORT

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

This research was supported under Grant No TA-MOU-97-CA17-001 "Program in U S – Israel Cooperative Development Research Program, Economic Growth, U S Agency for International Development"

PI Prof A Korol

The Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905
Tel (972)-48240-449, FAX (972)-48246-554
e-mail korol@esti haifa ac il

Institutional Administrative Officials

ISRAEL Prof A Ben-Zeev Dean of Research, Research Authority, Haifa University, Tel (972)-4-8240265, Fax (972)-4-8342104,
e-mail zalka@research haifa ac il

TABLE OF CONTENTS

Executive summary 3

SECTION I 4

A) RESEARCH OBJECTIVES 4

B) RESEARCH ACCOMPLISHMENTS 4

Material and Methods 4

(i)Plant material

(ii) Physiological characterization

(iii)Molecular-genetic characterization

(iv) QTL analysis

Results 5

1 Drought related traits

2 Molecular marker analysis

3 Genetic map construction and preliminary QTL analysis

References 7

C) SCIENTIFIC IMPACT OF COLLABORATION 8

D) DESCRIPTION OF PROJECT IMPACT 8

E) STRENGTHENING OF DEVELOPING COUNTRY INSTITUTIONS 8

F) FUTURE WORK 9

SECTION II 9

A) Managerial Issues

B) Budget

C) Special concerns

D) Collaboration, Travel, Training and Publications

E) Request for American Embassy Tel-Aviv or A I D Actions 10

APPENDIXES 11

Appendix no 1 Tables and figures

Appendix no 2 Abstract

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 first year Significant differences were found between the parental genotypes of the mapping population (desert wild barley and cultivated barley) in response to drought stress, manifested in few physiological traits, including osmotic adjustment Currently, a preliminary linkage map of 90 DNA markers have been constructed This allowed us to conduct the first mapping of drought resistance genes evolved in the desert barley (in chromosomes 4, 6 and 7)

Contribution to the project objectives and international development the results of the first 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 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 will generate DNA markers flanking the target genes, enabling marker-assisted introgression of resistance genes from wild progenitor to modern cultivars

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 the project objectives Likewise, members of the Almaty group participated during their training visits in molecular marker scoring These visits proved to be very useful in both learning the techniques and generating data

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 (Nevo 1992) 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) RESEARCH ACCOMPLISHMENTS

Over the past twelve months, the research has been focused on (1) physiological phenotyping and (2) molecular-marker genotyping of the parental lines and one of the two target F4 mapping populations This included

(a) DNA extraction and DNA-template preparation for both sequence-tagged site (STS) and amplified fragment length polymorphism (AFLP) and testing the parental genotypes for polymorphism of molecular markers, STS (sequence tag sites) and AFLP,

(b) scoring the mapping population for molecular markers and construction the molecular map,

(c) physiological scoring of parental genotypes and the mapping population for resistance-related traits at the seedling stage,

(d) preparing a second mapping population based on a cross between Israeli desert genotype of *H spontaneum* and Kazakh cultivar

Important progress has been made, as will be shown in section **Results** (after the section of Material & Methods)

Material and Methods

(i) Plant material Wild barley (*H spontaneum*) genotype 23-39, originated in Wadi Qilt, a desert population from Israel (100-250 mm annual rainfall) was crossed to *H vulgare* cv Mona (Swedish cultivar) A mapping population of 152 F_4 families was established for QTL mapping of physiological characters related to drought stress

(ii) Physiological characterization At the first stage of this project, the differences between the two parents in resistance to a uniform water deficit and the underlying mechanisms were investigated in an environmentally regulated growth chamber The parents and later the F4 mapping population were tested to drought resistance traits and dehydration resistance **(a) *Drought resistance*** the conditions in the growth chamber were 12 h photoperiod (35 W m^2 PAR), 25±1°C, R H 35% day, 60% night Seedlings were grown hydroponically in controlled greenhouse and stressed by polyethylene glycol (PEG 6000) at -0 4 MPa external water potential Water deficit was gradually imposed by PEG to the root nutrient solution PEG was used as an osmolyte because it is an inert, non-toxic and non-penetrating solute in plant research, unlike other osmolytes such as mannitol, sodium chloride and sugar All measurements were made at 37 d after the onset of PEG treatments Assays of physiological responses to a uniform water stress at the whole-plant and cellular levels included Shoot biomass under stress (g), Shoot biomass as % of control, Leaf relative water content (RWC, %), Osmotic potential under stress (measured in MPa), Osmotic adjustment (MPa) Leaf water relations were measured in the middle mature tissues of the last fully expanded leaf The ability to

resist drought was evaluated based on two criteria absolute biomass production under stress (mg per plant) and relative reduction in biomass by stress (%, calculating as (1- B_d/B_c) × 100%, where B_d=biomass under stress and B_c=control biomass) **(b)** ***Dehydration resistance*** Estimated by treatment of severe water deficit, which was imposed at the young seedling stage The following parameters were assayed in the mapping population Viability after rewetting (%), Number of new adventitious roots, Maximal root length (mm), Total root length per seedling (mm)

(iii) ***Molecular-genetic characterization*** **(a)** ***Sequence tagged site (STS)*** 130 pairs of STS primers, kindly provided by Dr T Blake (Montana University), were used to amplify sequences from genomic DNAs of *Hordeum vulgare* cv Mona and *Hordeum spontaneum* Wadi Qilt PCR amplification was done according to Tragoonrung *et al* (1992) and Blake *et al* (1996) The PCR products of samples were separated in 1 2% agarose gels to detect insertion/deletion polymorphisms To detect base substitution polymorphisms, 12 µl of the PCR products after amplification were subsequently digested with 1 5 units of the four-base restriction endonucleases *Mse*I (or *Tru1*I), *Hae*III, *Hha*I, *Hinf*I, *Msp*I, *Rsa*I, *Dde*I, and *Taq*$^{\alpha}$I Digested fragments were separated on 8% polyacrylamide gels (29 1) **(b)** ***Amplified fragment length polymorphism (AFLP)*** AFLP generate a large number of molecular markers without any previous knowledge of the genetic constitution of the genotypes under investigation (Vos *et al* 1995) Compared with other DNA markers, AFLP has proved to be very powerful and efficient for generating high polymorphism in (Qi and Lindbout 1997) The AFLP techniques that we used to screen out the primer combinations which can differentiate parental genotypes in barley are minor modifications of those described by Vos *et al* (1995) and Qi and Lindhout (1997) Genomic DNA from the two parents were double digested with *Eco*RI/*Mse*I at 37°C and then adapters were ligated to the digested DNA For the pre-selective PCR, *Eco*RI+A and MseI+C primers were used Gradient electrophoresis was employed AFLP templates of four reference genotypes in barley (Nudinka, Morex, Stepteo and Proctor) after the pre-selective PCR were kindly provided by Dr Qi (Wageningen), and amplified with the primer combination E35M48 Their AFLP fingerprints were demonstrated in Qi *et al* (1997) at site http //grain jouy inra fr/ggpages/Qi, and here used as internal reference lanes

(iv) ***QTL analysis*** A novel mapping analysis of multiple trait complexes, which is efficient with correlated quantitative traits, was developed and used to increase the resolution power of QTL mapping This technique is employed on the basis of mixture models with a single QTL and linked QTLs per marked chromosome (Korol 1998, Ronin *et al* 1999) The main algorithms of our multiple trait mapping strategy are implemented in the form of interactive package *MultiQTL* and enable users friendly data treatment assisted by massive Monte-Carlo simulations, bootstrap analysis, etc These tools are developed by collaborative efforts of the Institute of Evolution and a team working in a Technological Incubator (L N Innovative Technologies LTD, Haifa, Israel) under general supervision of Prof Korol The current version of *MultiQTL* can be downloaded from the website *www multiqtl com*

RESULTS

1 Drought related traits The tiller number and the shoot fresh weight of the unstressed control plants differed between the two parents, but other physiological parameters assayed were similar For example, after 5-week growth under control conditions, each shoot in cv Mona weighed 307 9 ± 22 0 mg DW^{1}, not different from 344 2 ± 26 6 mg DW^{1} in Wadi Qilt 23-39 (means ± SE, n=10) However, when subjected to -0 4 MPa root water deficit, shoot growth in cv Mona (on the basis of dry weight) decreased by 85 2% compared with control plants The shoot growth in Wadi Qilt 23-39 was inhibited (74 8%, p=0 01) by the same root water deficit In addition, the stressed plants of Wadi Qilt 23-39 produced much higher biomass at the whole-plant level than Mona (p=0 01) The physiological comparison of cellular and whole plant responses to uniform water deficit between the cross parents *H vulgare* Mona and *H spontaneum* 23-39 is represented in Figure 1 and Table 1 Thus,

the criteria for resistance evaluation indicated that wild desert progenitor is much more drought-tolerant than the cultivar Mona By comparing and contrasting the cellular responses to water deficit in intact plants, the mechanisms underlying drought-tolerance strategy in Wadi Qilt 23-39 appeared to be related to higher ability of osmotic adjustment In the response to -0 4 MPa water deficit, full osmotic adjustment was achieved in the last fully expanded leaf tissues of Wadi Qilt 23-39 In contrast, the expanded leaf tissues of cultivated barley cv Mona compensated for only 62 5% of the 0 4 MPa change in external water potential during the same period As a result, the osmotic potential gradient between the last fully expanded leaf and the root water source in Wadi Qilt 23-39 was 1 33 MPa, that is significantly higher than 1 09 MPa in Mona ($P < 0\ 05$) Leaf relative water contents in the stressed plants of Wadi Qilt 23-39 and Mona were respectively 87 5 ± 1 1% and 82 0 ± 0 9% Both values were also different at the significance level of p=0 01

The foregoing results allow us to **conclude** significant differences in resistance to either moderate drought stress (at -0 4MPa PEG) or severe dehydration stress are manifested between wild barley inhabiting desert environments (Wadi Qilt 23-39) and cultivated barley (cv Mona) Cellular physiological mechanisms underlying their different resistance appeared to be related to their osmotic adjustment ability under drought conditions Distribution patterns of the mapping population relative to the parental genotypes for some of the resistance scores are shown in Fig 2 (see the Appendix)

2 Molecular marker analysis

(1) ***Sequence-tagged-site (STS)*** Of the sets of STS tested primers, 53 primer pairs did not amplify easily discernible fragments from the genomic DNAs of both parents, 11 primer pairs amplified PCR products from only one of the two parents, as shown in Table 2 Totally 66 sets of STS primer pairs gave clear amplification products in both parents, of which five directly differentiated between cv Mona and Wadi Qilt 23-39 based on 1 2% agarose-gel electrophoresis (ABC302 1, BCD402 1, ABG004, ABC156 1, BARG10) For example, amplified DNA fragments from ABG004 primers identified an insertion/deletion polymorphism When this primer pairs was used, a smaller fragment (380bp) was amplified from cv Mona, while a comparatively bigger fragment (450bp) was produced from Wadi Qilt 23-39 In addition, BARG10 primers amplified only one fragment in cv Mona (approximately 350bp) Clearly, the STS primers which generated distinct band-size polymorphism can be directly employed to score the mapping population in barley However, the PCR products amplified with 61 primer sets did not show fragment size polymorphisms between the two parents on 1 2% agarose gels They were consecutively digested with different four-base cutters and electrophoresed on 8% polyacrylamide separating gels Restriction site polymorphisms were identified after the digestion of amplification products with 57 primer sets (Fig 3) On the PCR fragments amplified with the remaining four primer sets (ABC305, ABG378, ABG379 and ABG054), the DNA recognition sequences of eight restriction enzymes here used did not appear to be flanked Thus, after the digestion of amplification products, no restriction fragment site polymorphisms were generated between the two parents

(2) ***Amplified Fragment Length Polymorphism (AFLP)*** With majority of the 42 primer combinations, about 90 DNA fragments were totally generated from the two parents However, the number of amplification products with the same primer combination was significantly different in each parental genotype For example, the six primer combinations labeled with the rare-cutter E45 (*Eco*RI +ATG) amplified visual AFLP fragments of 80 4 ± 4 9 in cv Mona and 69 6 ± 5 3 in Wadi Qilt 23-39 (mean ± SE, n=6) This indicated that the two parents are diverse, in spite of their same genome sizes (1C=5 1×10^9 bp, Bennett and Leitch 1995) On the basis of the number of AFLP fragments (out of 42 tested), the polymorphism rate and the feasibility of marker scoring, eight primer combinations with the best amplification have been screened out to analyze the barley mapping population, which are given below- E33M48, E33M54, E35M48, E35M61, E38M55, E40M54, E42M62, E45M49 (Example see at Fig 4)

3 Genetic map construction and preliminary QTL analysis Currently, the preliminary linkage map of 152 F2 mapping population (genotypes F2 represented by F4 families), constructed by MAPMAKER version 3 0b showed 11 linkage groups assigned to the 7 barley chromosomes At present, this rough map contains 87 AFLP markers and 2 STS locus (*a*ABC160 on chromosome 1H) and covered 1769 2 cM Segregation of alleles at each locus was checked against the expected ratio (3 1 and 1 2 1 for AFLP and STS markers, respectively) using a chi-square test at the level of p=0 05 The main target of this part of our activity for the second year will be to add codominant STS markers (at least 3-4 per each chromosome) Likewise, we plan to take advantage of recently developed for barley microsatellite markers (about 150 such markers are available at the Institute of Evolution due to our international collaborations) Single trait interval mapping analysis was conducted using our new QTL mapping package (currently developed based on funding from Israeli Ministry of Commerce and Industry) The first trials have already revealed four QTLs affecting (a) Dry Weight (DW) on chromosomes 4 (P=0 004), (b) Osmotic Potential (P=0 07) and Relative Water Content (RWC) on chromosome 6 (P=0 011), and (c) Total Root Length after dehydration on chromosome 7 (P=0 008) (see Fig 5) The significance level was evaluated using permutation test with 10,000 permutations for each case (these results were presented as a poster at the 4th Gatersleben Research Conference Plant Genomics, Structural, Functional and applied Aspects June 17-21, 1999 Schlob Meisdorf/Harz and IPK Gatersleben, Germany)

References

Choi D W , Zhu B , and Close TJ (1998) The Barley (*Hordeum vulgare* L) dehydrin multigene family sequences, allelic variation, chromosome assignments and expression characteristics of 11 DHN genes of cv Dictoo (Theor Appl Genet in press)

Korol A , Ronin Y , Nevo E , (1998) Approximated analysis of QTL-environment interaction with no limits on the number of environments *Genetics* 148, 2015-2028

Nevo E , (1992) Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum*, in the Fertile Crescent *In Barley Genetics, Molecular Biology and Biotechnology, Shewry, P (ed) C A B International* Pp 19-43

Qi X , Lindhout P , (1997) Development of AFLP markers in barley *Molecular and General Genetics* 254, 330-336

Ronin Y , Korol A , Nevo E , (1999) Single- and multiple-trait analysis of linked QTLs some asymptotic analytical approximation *Genetics* 151, 387-396

Vos P , Hogers R , Bleeker M , Reijans M , Lee T V D , Hornes M , Frijters A , Pot J , Peleman J , Kuiper M , Zabeau M , (1995) AFLP a new technique for DNA fingerprinting *Nucleic Acids Research* 23, 4407-4414

c) SCIENTIFIC IMPACT OF COLLABORATION

The first year has demonstrated a fruitful collaboration between the partner teams proved by the richness of the generated data 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 maker 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 In practice, two members of Almaty group participated during their training visits in molecular marker scoring (Mr S Sersenbayev) and scoring physiological traits (Dr N Ryabushkina) The accumulated experience will be used by these scientists for advancing the second mapping population Likewise, the scientists at Almaty conducted preliminary field scoring of the candidate mapping populations that will assist in making a justified choice of the second mapping population as well as in further scoring of both mapping populations under drought stress conditions characteristic to Kazakhstan

D) DESCRIPTION OF PROJECT IMPACT

Although it is premature to expect a real usage of the obtained results after the first year of the project start, it is clear that 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 These will be of importance not only for barley improvement, but for all cereals Note, that the foregoing barley genotypes are able to cope with as low as 100 mm rainfall per year 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 Due to technical obstacles they have got the money for equipment relatively recently, so that only for the second year of the project the ordered equipment will generate data This will allow to conduct large-scale PCR analysis as well as other marker genotyping at Almaty

(2) **Training the personel** 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 During this time they learned techniques and participated in scoring the molecular markers and physiological traits, it is noteworthy that one of them is a very young student (conducting his M Sc project), and this policy to involve young students will be continued In addition to experimental techniques, they had the opportunity to learn how to use new information technologies including QTL mapping software

(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 will be 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

(1) **Linkage map** we will improve the density and information content of the linkage map by complementing the dominant AFLP markers with STS markers which proved to be polymorphic between the parents, as well as available microsatellites
(2) **Candidate genes** a series of dehydrin genes (Choi et al , 1998) are now being mapped on our mapping population in order to relate the positions of detected QTLs with those of candidate genes related to drought resistance, in particular osmotic adjustment Likewise, we start to test for differential expression of the candidate genes in the response of the parental genotypes to drought stress, based on Northern analysis
(3) **Drought resistance scoring** further characterization of the mapping population for the resistance at the whole plant level will be conducted (in the lab, at Haifa, and in the field, at Almaty)
(4) **QTL analysis** a detailed multiple trait analysis will be conducted using a revised map with added codominant markers and additional scores of resistance traits
(5) **Characterizing the second mapping population** a few candidate mapping populations F2 have been established based on crosses between Israeli desert genotypes of *H spontaneum* and Kazakh cultivars One of these will be chosen as the second target mapping population for the project and will be characterized for molecular markers (mainly, STS and microsatellites), as well as for resistance traits

SECTION II

A) **Managerial Issues** (a) The Kazakh group required to assist in managing the training budget from Haifa This problem was addressed to the Project Administrator Mr Boaz Ayalon who approved this application In light of the difficulties in managing such problems in Almaty, this decision appeared to be very important to provide efficient management of the whole training part of the project (b) The Kazakh group was interested in managing all the purchases (equipment and chemicals) via Haifa University The reasons were (i) higher efficiency in ordering the items from the providing companies, and (ii) lower dependence on voluntarism of the local administration The PI of the project was ready to take the responsibility for that, but it appeared that such a management does not fit the interior rules of Haifa University

B) **Budget** The financial part of the report will be provided the next week by the management of the Research Authority of the University of Haifa (by 3-5 of July)

C) **Special concerns** No such issues have arisen

D) Collaboration, Travel, Training and Publications
(1) **Collaboration** Fruitful collaborative relationships have been established between the participating groups 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 maker 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 This complementary expertise was already used to achieve the project objectives of the first year Namely, the first mapping population that was established by Haifa group has been characterized at Haifa for molecular markers and cell and seedling level resistance traits, whereas whole plant traits were partly characterized at Almaty Likewise, a thorough test of several candidate mapping populations established by crossing of Israeli resistance genotypes of barley to Kazakh cultivars, is conducted at Almaty

(2) Travel and Training Two members of Almaty group participated during their training visits in molecular marker scoring (Mr S Sersenbayev) and scoring physiological traits (Dr N Ryabushkina) Altogether, their training visits at Haifa continued for five months, August-October 1998 for Sersenbayev and March-April 1999 for Ryabushkina These visits proved to be very useful in both learning the techniques and generating data

(3) Publications The obtained results demonstrate a remarkable success of the project already after the first year we have produced a preliminary QTL map for drought resistance evolved in a desert ecotype *of H spontaneum* These results were presented at a European conference on Plant Genomics (Gatersleben) (see Appendix 2 for the Abstract of the presentation) After the completion of genotyping of additional 8-10 STS markers, a revised map will be constructed, and the first two-three papers will be submitted for publication We expect that the first manuscript will be submitted no later than by September-October 1999 Here is the list of publications (in preparation, besides the first one)

(a) Lu Z , Krugman T , Sersenbayev S , Turuspecov Y , Nevo E , and A , Korol 1999 Physiological characterization and genetic mapping of drought resistance in wild barley, *Hordeum spontaneum* Poster, presented at the 4th Gatersleben Research Conference Plant Genomics, Structural, Functional and applied Aspects June 17-21, 1999 Schlob Meisdorf/Harz and IPK Gatersleben, Germany

(b) Lu Z , Krugman T , Turuspekov Y , Ronin Y , Nevo E and A Korol 1999 QTL mapping of drought resistance evolved in a Negev desert ecotype of wild barley, *Hordeum spontaneum* (in preparation)

(c) Zamorzaeva I Korol A , Krugman T , and E Nevo 1999 **D**ifferential response of dehydrine genes to drought stress in drought-resistance and drought-susceptible genotypes of barley (in preparation)

(d) Lu, Z Y A Korol, T Krugman, Ronin, Nevo Comparison of barley genetic maps *H vulghae* x *H vulgare* versus *H vulgare* x *H spontaneum* (in preparation)

E) Request for American Embassy Tel-Aviv or A I D Actions We have no special requests The helpful assistance of the Project Administration is highly appreciated

Appendixes

Appendix No 1 Tables and Figures

Table 1 Physiological comparisons of cellular and whole plant responses to uniform water deficit between the cross parents

Table 2 Category of PCR products amplified with STS primer pairs in barley parent

Figure 1 Comparison of Osmotic Adjustment and Relative Water Content in *Hordeum vulgare* cv Mona and *Hordeum spontaneum* Wadi Qilt 23-39 induced by –0 4 MPa PEG

Figure 2 Distribution of drought and dehydration related traits in 152 F4 families (a) Relative water content % (b) Osmotic potential induced by –0 4 MPa PEG (c) Total length of new adventitious roots after treatment of severe water deficit at young seedling stage

Figure 3 Examples of segregation of STS-PCR markers in the barley mapping population

Figure 4 Examples of AFLP fingerprinting of the barley mapping population

Figure 5 LOD scores for single trait interval analysis of 4 QTLs

Appendix no 2

Abstract of poster, presented in the 4th Gatersleben Research Conference Plant Genomics, Structural, Functional and applied Aspects June 17-21, 1999 Schlob Meisdorf/Harz and IPK Gatersleben, Germany

Table 1 Physiological comparisons of cellular and whole-plant responses between *Hordeum vulgare* cv Mona and *Hordeum spontaneum* Wadi Qilt 23-39 to a uniform water deficit. Water deficit at -0 4 MPa PEG was imposed at the seedling stage The aerated root medium nutrient solutions containing PEG were replenished weekly and all the measurements were made at 37 d after the onset of PEG treatments Water potentials of fresh leaf tissues and osmotic potentials of frozen thawed tissues were determined by thermocouple psychrometry using 5 leaf sections of 5-mm length per leaf cutter probe and a 3-h equilibration period at 27°C in a water bath as described in Snir & Neumann (1997) Turgor values in the newly expanded leaves were obtained by subtracting osmotic potentials from water potentials Relative water contents were determined as in Matin *et al* (1989) and water loss rates of excised-leaves as in Clarke & McCaig (1982) No significant differences between the parental control plants were found in the assayed physiological parameters except tiller number and shoot fresh weight Each value represents the mean ± SE for 10 plants

Physiological assay	cv Mona (Sweden barley)	23-39 (wild barley)
Leaf water status		
Water potential (MPa)	-0 51 ± 0 02	-0 70 ± 0 06 **[†]
Osmotic potential (MPa)	-1 12 ± 0 05	-1 36 ± 0 04 *
Turgor pressure (MPa)	0 61 ± 0 03	0 66 ± 0 02 [NS]
Ψ_{ow} - Ψ_{iw} (MPa)	0 08 ± 0 02	0 27 ± 0 06 **
Osmotic adjustment		
(MPa)	0 25 ± 0 05	0 41 ± 0 04 *
(%)	62 5	102 5 *
Relative water content (%)	82 0 ± 0 9	87 5 ± 1 1 **
Water loss after excision (mg g^{-1} H_2O)	694 7 ± 21 3	683 4 ± 4 2 [NS]
Shoot growth		
Leaf development	2 7 ± 0 1	4 3 ± 0 1 ***
Number of tiller	0	1 5 ± 0 1 ***
Plant height (mm)	222 0 ± 12 5	193 7 ± 8 2 [NS]
Fresh weight (mg/plant)	197 5 ± 24 3	432 3 ± 31 9 ***
Dry weight (mg/plant)	45 7 ± 4 3	79 7±6 0***
Root growth		
Root length (mm)	308 0 ± 6 3	575 0 ± 20 8 ***
Fresh weight (mg/plant)	349 6 ± 31 4	456 4±37 0*
Dry weight (mg/plant)	67 3 ± 4 1	77 8 ± 5 0 [NS]
Root/shoot dry matter ratio	1 47 ± 0 02	0 98 ± 0 06 ***
Biomass production		
Fresh weight (mg/plant)	547 1 ± 10 7	888 6 ± 57 7 ***
Dry weight (mg/plant)	113 0 ± 4 9	157 5 ± 10 5 **

[-] Ψ_{ow} and Ψ_{iw} represent the external water potentials of the root medium and the internal water potentials of expanded leaf tissues, respectively

[+] [NS] * ** and *** indicate differences between means of two parents which are non-significant or significant at P < 0 05 0 01 and 0 001 respectively

Table 2 Category of PCR products amplified with STS primer sets in barley parents The sequences of sets of STS primers as in Table 1 PCR reactions were performed according to Tragoonrung *et al* (1992) and Blake *et al* (1996) and amplified products were detected in 1 2% agarose gels with 1× TBE In brackets M and W represent cv Mona and wild barley 23-39, respectively The numbers in brackets are the molecular weight size (base pair) of amplified DNA fragments or the number of bands

Type of amplifications	STS-primer set
No amplified products	ABA001, ABA004, ABC151, ABC156 5, ABC156 7, ABC168, ABC255, ABC261, ABC302 1, ABC310, ABC323, ABC451, ABC455, ABC468, ABG005, ABG010, ABG020 1, ABG020 2, ABG055, ABG057 1, ABG058, ABG059, ABG064 ABG065 ABG070, ABG075 ABG317, ABG320 ABG356, ABG366 ABG377 ABG45, ABG459 ABG460 ABG466 ABG471 ABG484, ABG498 ABG57 3 ABG570 3 ABG616 ABG654 ABG694 ABG712 BCD269, BCD828, BCG57 1, BG123 BTA002 CDO213 CDO474, CDO475 D14 MST126, WG232 1 WG541 WG686, WG940 WG996
Amplified products in only one parent	ABA001 (M) ABC253 (M), ABC717 (M) BCD402 7 (W) PST337 (W) ABG452 (M) ABG493 (M), WG464 (W) ABG57 4 (M) ABG053 (M) ABG153 (M)
Amplified products in both parents	ABA003 ABA005 ABC152 ABC153 ABC153, ABC156 1, ABC156 1* ABC156 2 ABC156 5 ABC160 ABC164 ABC252 ABC302 1 ABC302 3 ABC303, ABC305, ABC306 ABC451, ABC454, ABC465, ABC483, ABC718 ABG065, ABG155 ABG156 1, ABG170, ABG379, ABG380 ABG391, ABG396 1 ABG397 1, ABG4, ABG458 1, ABG472 ABG473 1 ABG473 11 ABG484 ABG498, ABG500, ABG54, ABG57 2, ABG602, ABG603, ABG608 ABG613, ABG618, ABG701 ABG711, ABG712, ABG715 ABG716, BARG10 BCD175 1 BCD269, BCD402 1, BG123, CD0673, MST101, MST102, MST107, MST108, MST109, MST126 MST510, RRN5S1, RRN5S2, WG669
Polymorphic products in 1 2% agarose gels	ABC302 1 (M 350bp, W 450bp), BCD402 1 (M 350bp W 450bp), ABG004 (M 380bp W 450bp) ABC156 1 (M, 1 band, W 4 bands) BARG10 (M. 1 band, W 2 bands)

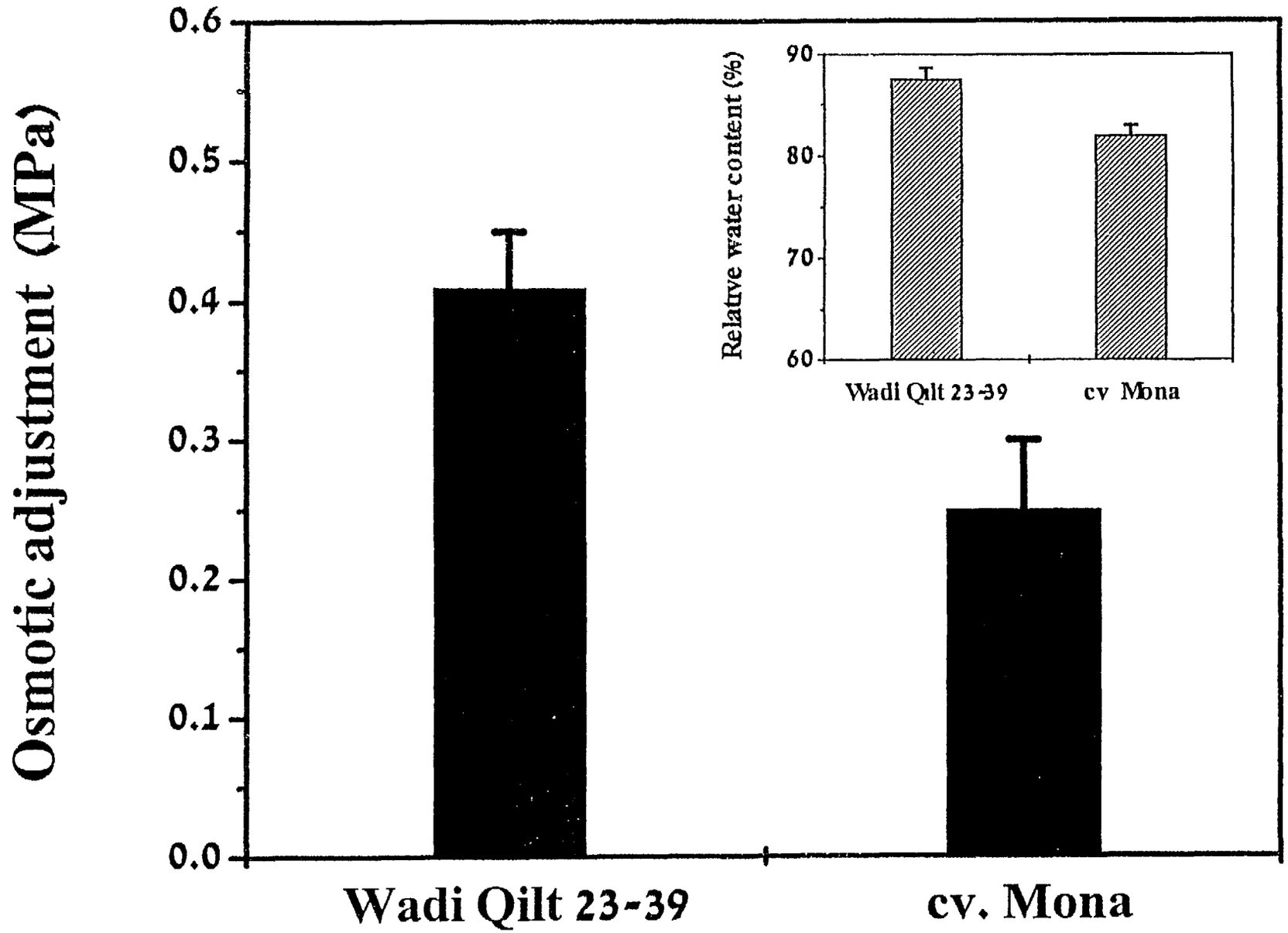

Fig. 1. Physiological responses comparison of the cross parents Mona and Wadi Qilt 23-39 to uniform water deficit: Osmotic adjustment (Mpa) and Relative Water Content (%)

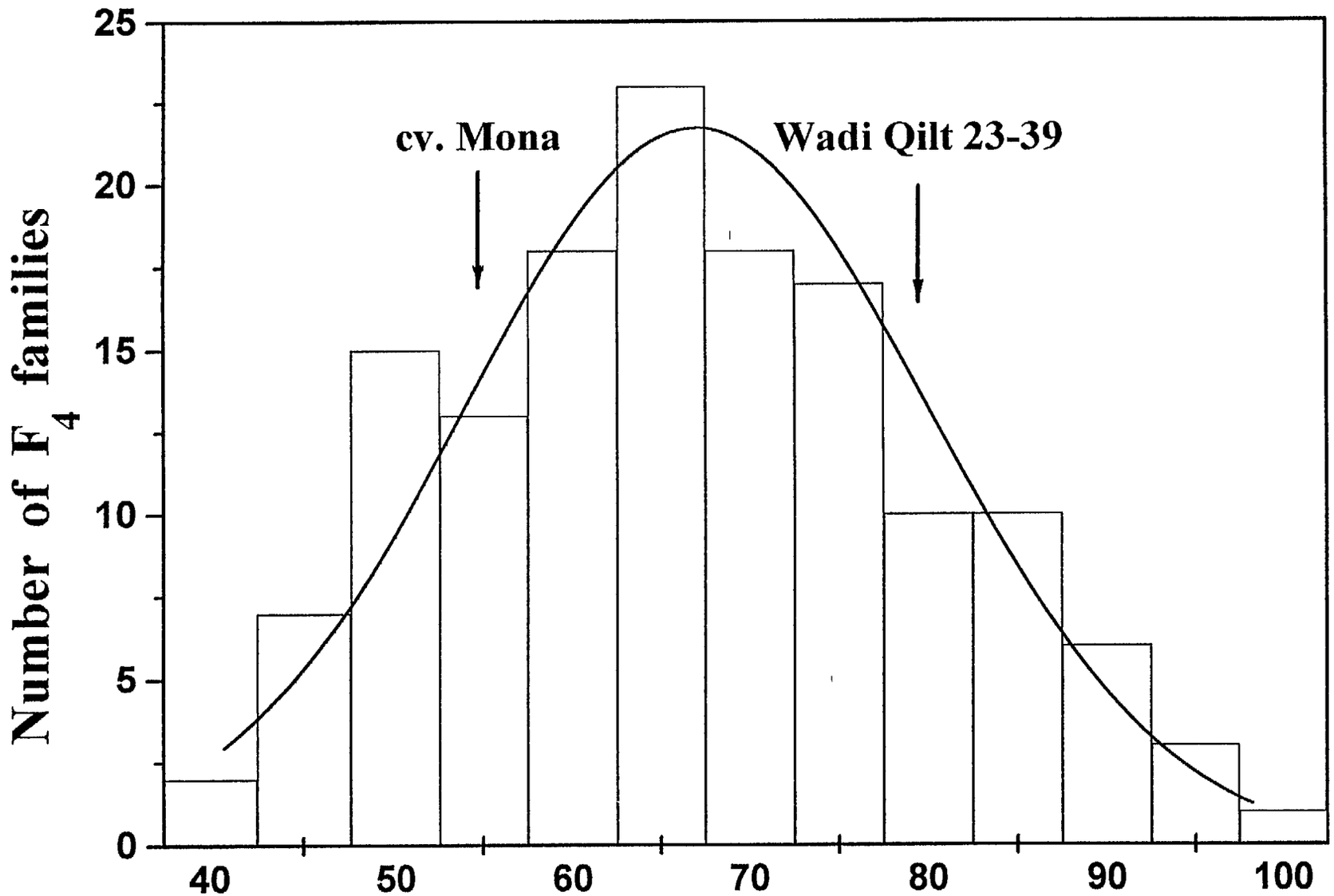

Fig. 2a. Relative water content [%]

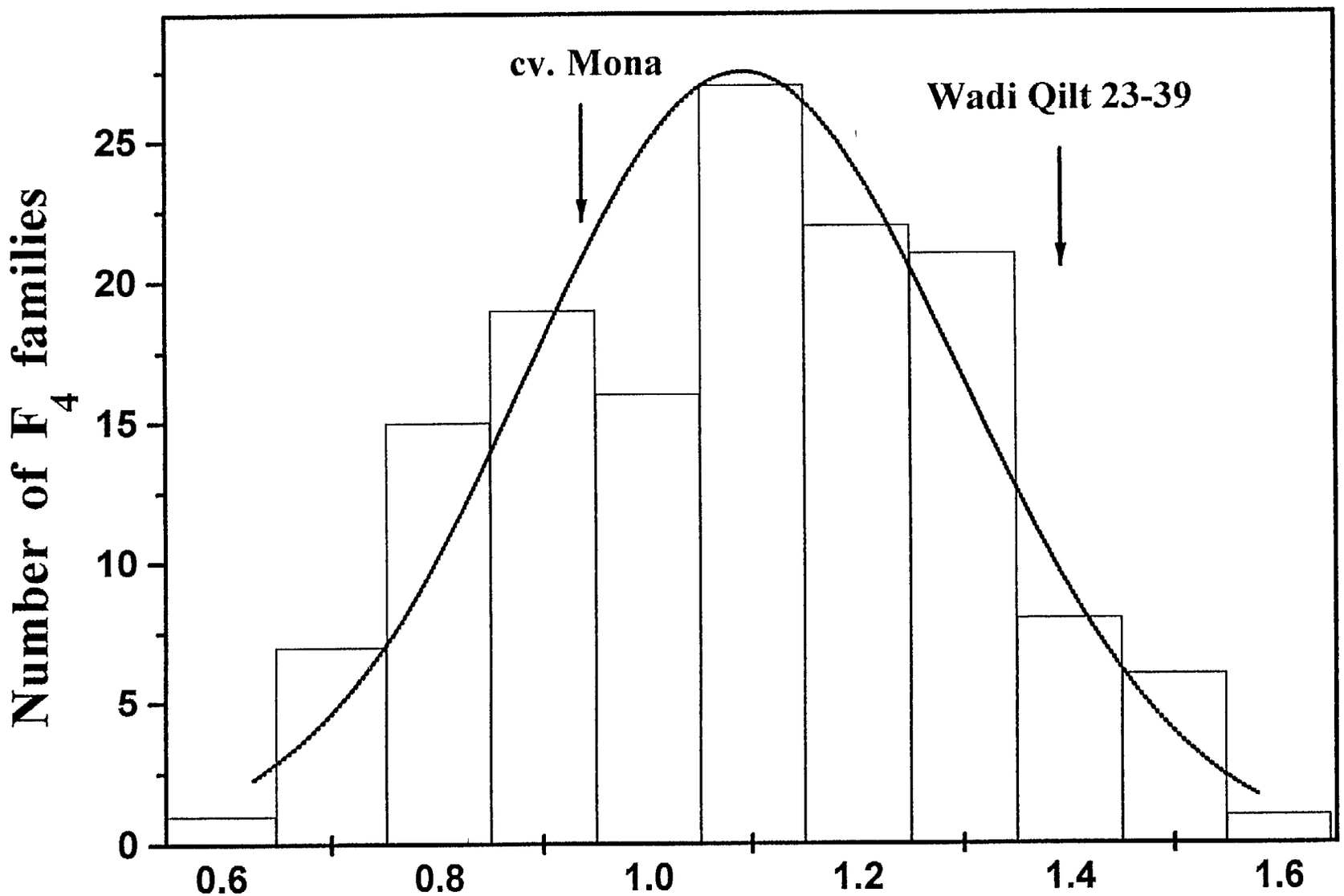

Fig. 2b. Osmotic potential under stress [-MPa]

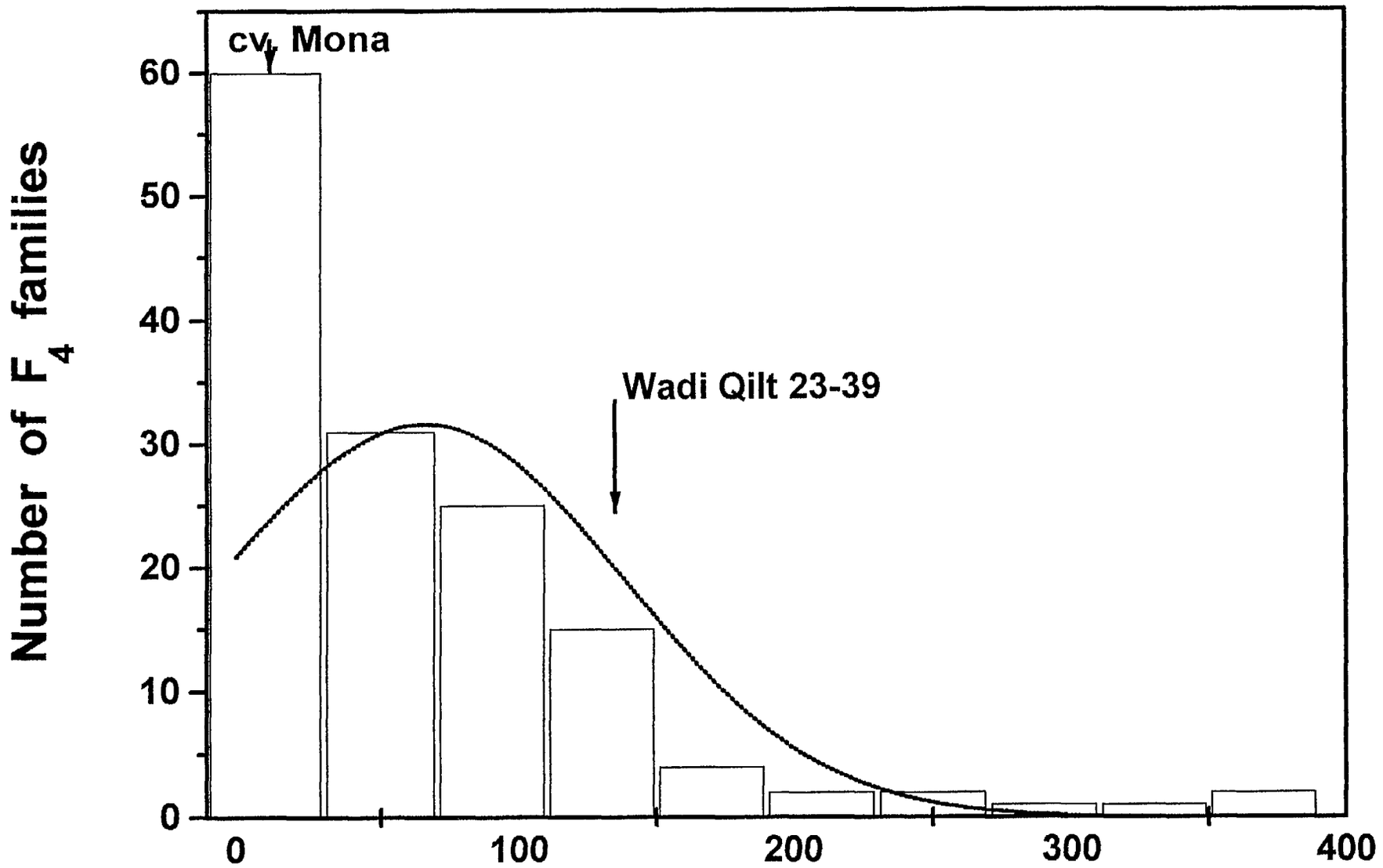

Fig.2c. Length of new adventitious roots [mm]

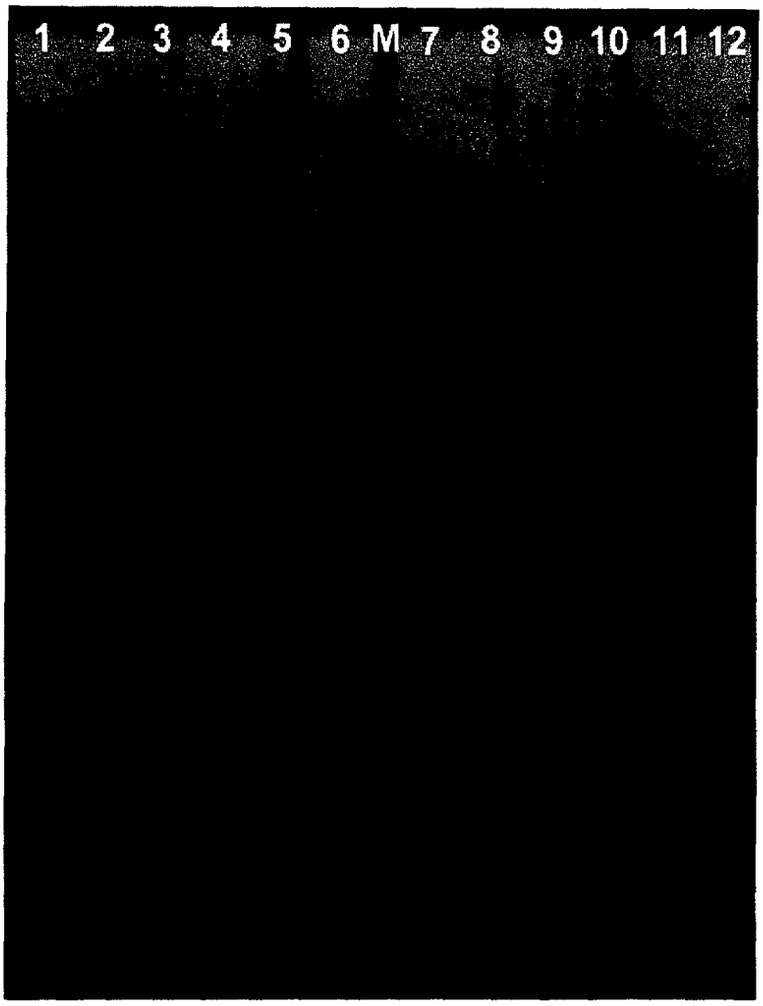

Fig 3 Examples of endonuclease digestions of STS-PCR products amplified from the two parents with different primer sets From left to right lanes primer set no 1-12 represents ABA397 1 ABC718 ABG473 1 ABG378 ABG715, ABG473 11, ABG396 1, ABG609 ABC160 ABG484 ABC305 and WG669 respectively Each set of STS primers amplified the DNA templates from *Horduem vulgare* cv Mona (the first lane) and *Hordeum spontaneum* Wadi Wilt 23-39 (the second lane) respectively Amplification products were cleaved with *Hae*III at 37 °C for 2 h and then separated on a 8% polyacrylamide gel at 210 V for 3 5 h DNA size markers (100 bp Bio-Rad) was shown as M in the middle lane

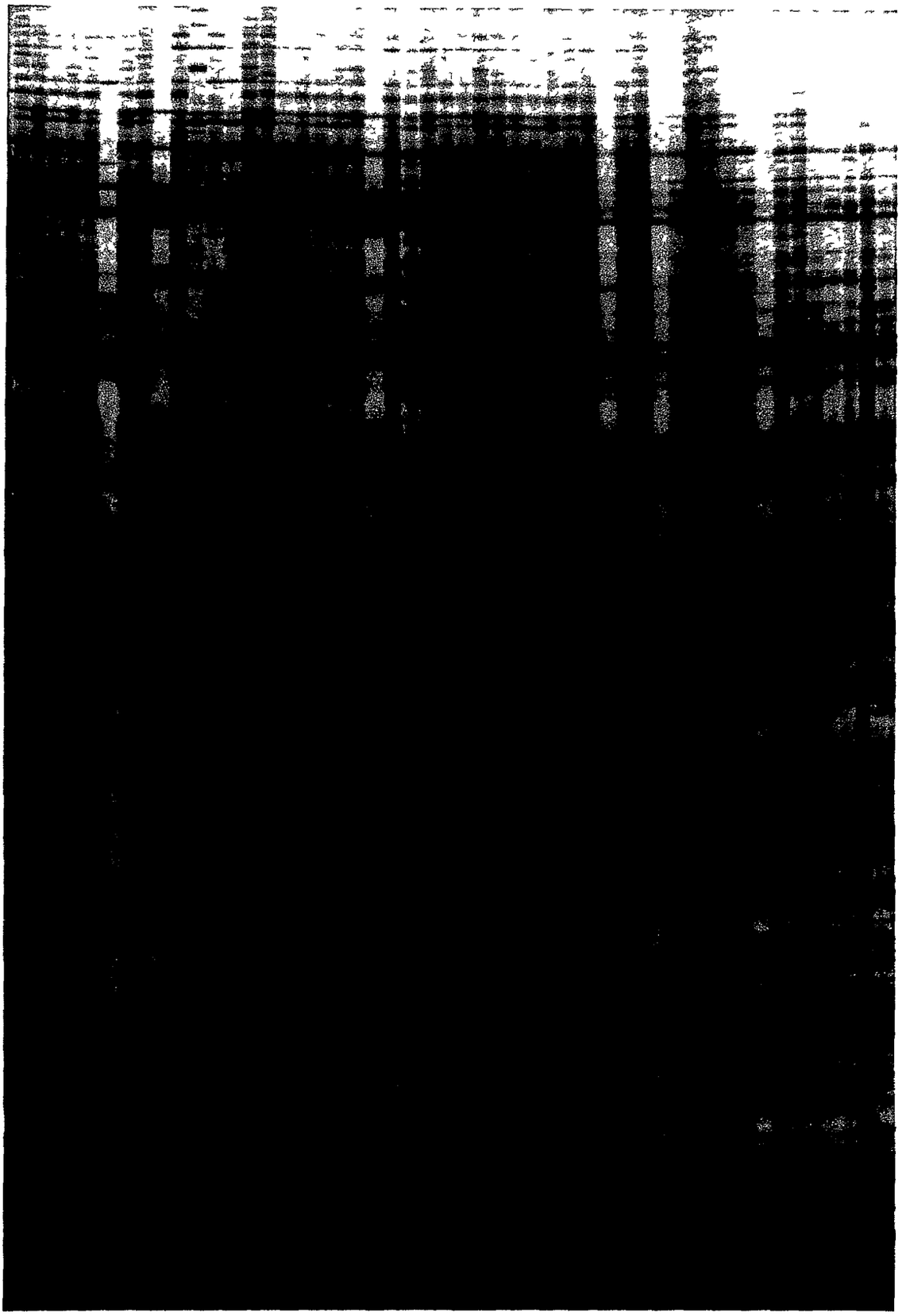

Fig 4 Examples of AFLP fingerprinting of the barley mapping population The genomic DNAs of F4 families derived from the cross of cv Mona with Wadi Qilt 23-39, were amplified with the primer combination E33M54 namely *Eco*RI + AAG /*Mse*I + CCT Each lane represents the AFLP fingerprint of one family The molecular weight size range of the fingerprints is from 45 to 500 nucleotides

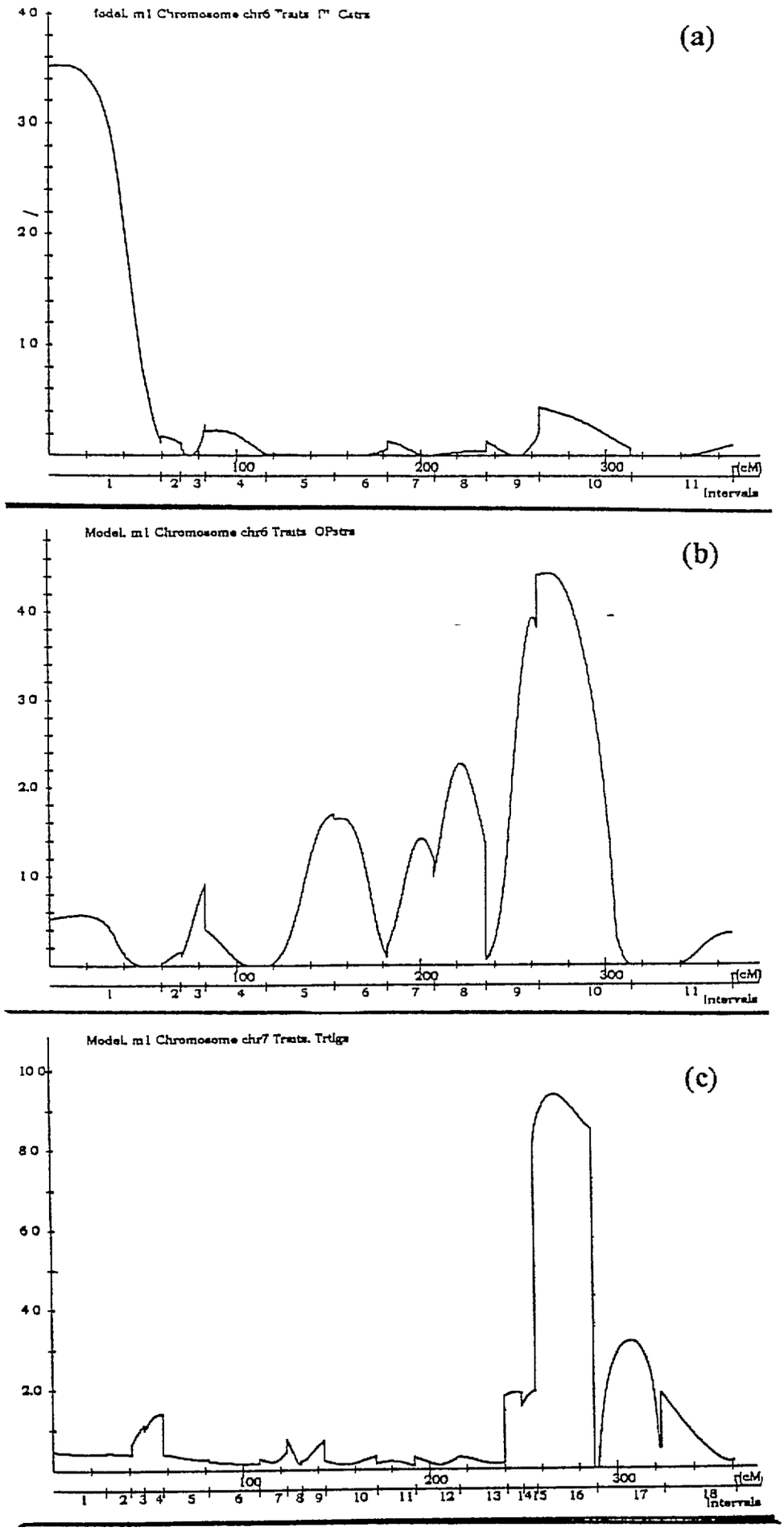

Fig 5 LOD scores for single trait interval analysis Single trait interval mapping analysis revealed four QTLs affecting (a) Relative Water Content on chromosome 6 (P=0 013, d/σ=10.3/11 9=0.86); (b) Osmotic Potential on chromosome 6 (P=0 01, d/σ=0.261/0 161=1 6) and (c) Root Length after dehydration on chromosome 7 (P=0 008, d/σ=250.2/49 0=5 1)

Appendix no 2

Physiological characterization and genetic mapping of drought resistance in wild barley, *Hordeum spontaneum*

Lu Z [1], T Krugman[1], S Sersenbayev[2], Y Turuspecov[2], E Nevo[1] and A Korol[1]

[1] *Institute of Evolution, Haifa University, Mt Carmel, Haifa, 31905, Israel*
[2] *Institute of Plant Physiology, Genetics and Bioengineering, National Biotechnology Center, Almaty, Kazakhstan*

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 wide range 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 of wild barley, *Hordeum spontaneum,* from Israeli desert populations, as a potential source for a radical improvement of drought resistance This includes identification of specific 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

Wild barley (*H spontaneum*) genotype 23-39, originated in Wadi Qilt (100-250 mm annual rainfall) was crossed to *H vulgare* cv Mona (Swedish barley cultivar) The differences between the two parents in resistance to a uniform water deficit were investigated in a growth chamber Seedlings were grown on hydroponic substrate and stressed by polyethylene glycol (PEG 6000) at -0 4 MPa external water potential Leaf water relations (water potential, osmotic potential and turgor) were assayed in the middle mature tissues of the last fully expanded leaf When subjected to water stress, the shoot growth (DW) in cv Mona decreased by 85 2%, as compared with the control plants, while Wadi Qilt 23-39 was significantly less inhibited (74 8%, $P<0\ 01$) By comparing and contrasting the cellular responses to water deficit in intact plants, in response to -0 4 MPa water deficit, full osmotic adjustment was achieved in the last fully expanded leaf tissues of parent 23-39 In contrast, Mona compensated for only 62 5% of the 0 4 Mpa As a result, the osmotic potential gradient between the last fully expanded leaf and the root water source in Wadi Qilt 23-39 was 1 33 MPa, significantly higher than 1 09 MPa in cv Mona ($P<0\ 05$) Leaf relative water content in 23-39 and cv Mona, were 87 5±1 1% and 82 0±0 9% ($P<0\ 01$) respectively A mapping population of 154 F_4 families was established for QTL mapping analysis of the revealed differences as well as change in whole plant performance in response to drought stress The AFLP technique was used to produce molecular markers across the genome On the basis of the number of AFLP fragments, polymorphism rate and the reliability of marker scoring, eight primer combinations (out of 42 tested) were chosen for analyzing the barley mapping population In addition, 133 STS primer pairs were tested for polymorphism between the parental lines Of these, 66 STS markers produced clear amplification products in both parents, five STS markers directly differentiated between the parents and the remaining 61 manifested fragment size polymorphisms after restriction analysis using 4-base cutters The results of QTL mapping analysis of the foregoing drought resistance scores obtained in controlled conditions will be presented in this report Another mapping population derived from a intraspecific cross of mesic and desert ecotypes of *Hordeum spontateum* is also being currently analyzed for mapping of drought resistance genes

Acknowledgment
This research was supported under Grant No TA-MOU-97-CA17-001 "Program in U S –Israel Cooperative Development Research Program, Economic Growth, U S Agency for International Development"