

Guoping Zhang
Chengdao Li
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Editors

Advance in Barley Sciences

Proceedings of 11th International Barley Genetics Symposium



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PREFACE

The last half century has seen extraordinary progress in barley genetics research. In the first International Barley Genetics Symposium in 1963, scientists just started to discuss a map of barley chromosomes. Today, the International Barley Genome Sequencing Consortium is at the dawn of the completion of the barley genome sequence. The regular International Barley Genetics Symposia provide an important platform for barley breeders and scientists to share their research results and understand the future trends of barley genetics research. The proceedings are not only the permanent record; they also provide key references for interested outsiders at the symposium and future barley research scientists. The organizing committee received around 150 abstracts and put them into a special volume for all symposium participants to have access, which will be helpful for improving the discussions at the poster sessions. Moreover, we have selected 38 full length papers and published them as The Proceedings of 11th IBGS. Hopefully they will provide in depth content of current research and development in barley genetics and breeding.

This is the first time the symposium was held in China. Chinese scientists first participated in the Okayama Symposium in 1986 and increasing numbers of Chinese scientists joined the following symposia. However, the majority of Chinese barley research is still unknown to the international barley community except the fact that China is the world's largest beer producer and malting barley importer. Barley has been a major crop over thousands of years and it is one of the most widely distributed crops in China. The Qing-Tibetan Plateau in southwest China is a unique agricultural region in the world with an average altitude of 4 km above sea level. Barley is still the major food crop for millions of people in this region. The harsh environments have created unique germplasm for low soil fertility, drought, frost and salinity tolerance. Tibetan barley also contains multiple functional components for human nutrition, e.g., high β -glucan and antioxidants. It also has unique enzyme activity and thermo-stability to determine barley malting quality. Tibetan barley germplasm is a highly valuable but underutilized component of the world barley gene pool. This region is arguably another centre of origin of cultivated barley. We hope that the symposium will unveil the secret of Tibetan barley, enhance understanding of China's barley research and promote collaboration between Chinese scientists and the international barley community.

The organizing committee gratefully acknowledges financial support from the Natural Science Foundation of China, the Natural Science Foundation of Zhejiang, Zhejiang University, the KC Wong Education Foundation, Hong Kong. We thank the international organizing committee members for their support and guidance for this symposium. We also appreciate support from the Zhejiang University Press and the Springer Press in publishing the proceedings.

The 11th International Barley Genetics Symposium will be held in the First World Hotel, Hangzhou, China, from 15 to 20 April 2012. Hangzhou is a core city of the Yangtze River Delta and has a registered population of 3.8 million people. The city is located on Hangzhou Bay, 180 km southwest of Shanghai. It has been one of the most renowned and prosperous cities of China for much of the last 1,000 years, due in part to its beautiful natural scenery. The city's West Lake is its best-known attraction and Hangzhou is the oriental leisure capital.

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April 4, 2012

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Evolution of Wild Barley and Barley Improvement

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ABSTRACT: Wild barley, *Hordeum spontaneum*, the progenitor of cultivated barley, *Hordeum vulgare*, originated 5.5 million years ago in southwest Asia, is distributed in the eastern Mediterranean, Balkans, North Africa, central Asia and Tibet. *H. vulgare*, the fourth important world crop, used for animal feed, beer, and human food was domesticated polyphyletically by humans 10,000 years ago in the Neolithic revolution in at least three centers: Fertile Crescent, Central Asia, and Tibet of China. *H. vulgare* with thousands of land races and cultivars, is widespread where other crops can't adapt, yet it deteriorated genetically, especially due to pure breeding, and needs genetic reinforcement. *H. spontaneum*, the best hope for barley improvement, is a hardy ecological generalist, adapted to a wide range of extreme latitudes, altitudes, climates (warm and cold), and soils. Adaptations occur at all levels: *genomically*, *proteomically*, and *phenomically* both *regionally* and *locally*. It displays "archipelago" genetic structure, rich genetically, and harbors immense adaptive *abiotic* and *biotic* resistances precious to barley and cereal improvement. Sequencing the *H. spontaneum* genome will reveal huge, mostly untapped, genetic resources. The current global warming stresses *H. spontaneum*, and so it is imperative to conserve it *in situ* and *ex situ* to safeguard its future immense contribution to barley and cereal improvement, thereby helping to fight hunger.

KEY WORDS: *Hordeum spontaneum*, Polyphyletic domestication, Genetic resources

1. INTRODUCTION

Cultivated barley, *Hordeum vulgare* L., is one of the main cereals of the belt of Mediterranean agriculture, a founder crop of old World Neolithic food production, and one of the earliest domesticated crops (Harlan and Zohary, 1966; Zohary *et al.*, 2012). It is an important crop, ranking fourth (at 136 million tons) in 2007, in world crop production in an area of 566,000 km² (<http://faostat.fao.org/faostat>). Barley is used for animal feed, brewing malts, and human food (in this order). In Mediterranean agriculture, barley is a companion of wheat, but is regarded as an inferior staple and is known as the poor man's bread. Barley has a short season, early maturing grain with a high yield potential, and grows in widely varying environments, including extreme latitudes where other crops can't adapt (Harlan, 1976). It extends far into the arctic, reaching the upper limit of cultivation in high mountains, desert oases, and desert fringes. It is more salt and drought resistant than other

cereals. Barley is a cool season crop. It can tolerate high and low temperatures if the humidity is low, but avoids warm-humid climates. It grows in cold highlands such as Mexico, the Andes, East Africa, and Tibet of China. Major production areas of barley are Europe, the Mediterranean region, Ethiopia, the Near East, Russia, China, India, Canada, United States, and Australia.

2. DOMESTICATION

Barley first appeared in several pre-agriculture or incipient sites in southwest Asia. The remains are of brittle, two rowed forms, morphologically identical with present day *H. spontaneum* wild barley, and apparently collected in nature (Zohary *et al.*, 2012 and their Fig. 16). The earliest records of such wild barley harvest comes from ca. 50,000 years BP Kebara (Lev *et al.*, 2005) and from ca. 23,000 years BP Ohallo II, a submerged Early Epi-Palaeolithic site on the south

shore of the Lake of Galilee, Israel (Kislev *et al.*, 1992; Weiss *et al.*, 2004), as brittle two-rowed forms, morphologically identical with the progenitor of barley, *Hordeum spontaneum*, apparently collected in the wild. Other sites of *H. spontaneum* collection from the wild from 15,500-10,150 BP, including Jericho (Kislev, 1997), appear in Zohary *et al.*, 2012, p. 56). Like the domestication model advocated for wild emmer wheat (Feldman and Kislev, 2007), domestication occurred independently in sites across the Levant. Moreover, recent proposals suggest central Asia (Morell and Clegg, 2007) and Tibet of China (see discussion in Dai *et al.*, 2012) as additional centers of wild barley domestications. According to this view, the linked genes for non-brittleness (Bt_1 and Bt_2) were transferred to numerous wild barley genotypes through multiple spontaneous hybridizations, followed by human selection. The rich genetic variation of the progenitor, *H. spontaneum* (Nevo, 1992), as well as its superb thermogenesis (Nevo *et al.*, 1992), has enabled it to tolerate biotic and abiotic stresses and succeed under cultivation in the warm, dry Near East and in cold, dry Tibet of China. These advantages of wild barley may explain the wider ecogeographic range of wild and cultivated barley as compared to those of wheat. Current archaeological finds show barley as a founder crop of the southwest Asian Neolithic agriculture and as a close companion of emmer and einkorn wheat (Zohary *et al.*, 2012; Morell and Clegg, 2007; Dai *et al.*, 2012). Future studies will highlight the full domestication scenario of *H. spontaneum* in Asia.

3.ORIGIN

The grass family Poaceae originated at the Upper Cretaceous (Prasad *et al.*, 2005). The genera *Hordeum* and *Triticum* diverged about 13 million years ago (Gaut, 2002). The genus *Hordeum* evolved -12 million years in southwest Asia and spread into Europe and central Asia. Multiple intercontinental dispersals shaped the distribution area of *Hordeum* (Blattner, 2006). The divergence of *H. spontaneum* from the Near East and Tibet of China is around 5.5 million years (Dai *et al.*, 2012), whereas barley (*Hordeum vulgare*) was domesticated around 10,000 years ago (see earlier).

The progenitor of barley is wild barley, *Hordeum spontaneum* (Harlan and Zohary, 1966; Zohary, 1969). *H. spontaneum* is an annual brittle two-rowed diploid

($2n=14$), predominantly self-pollinated (but see Brown *et al.*, 1978 reporting on 0-9.6% outcrossing, averaging 1.6%) and a strong colonizer species penetrating central Asia and Tibet of China (Fig. 1). A population-genetic analysis based on 795 loci, in 506 individuals of the progenitor *Hordeum spontaneum*, the cultivar, *Hordeum vulgare*, and their hybrid *Hordeum agriocrithon* concluded that barley cultivars form a distinct species, derived from the progenitor. Numerous cultivars and land races of barley have non-brittle ears. Non-brittle mutations survive primarily under domestication and non-shattering ears, as well as *thresh-1*, the locus of threshability (Schmalenbach *et al.*, 2011) are signatures of cultivation. In spite of these substantial differences between *H. spontaneum* the progenitor, and *H. vulgare* the human derivative, Zohary *et al.*, (2012) concluded that splitting the two entities into separate species is genetically unjustified and that the main cultivated barley types represent races of a single crop species. Notably, Darwin (1859) considered domestication as a gigantic evolutionary experiment in adaptation and speciation, generating incipient species. Domestication was performed by humans primarily during the last 10,000 years mimicking speciation in nature (Wei *et al.*, 2005). It leads to adaptive syndromes fitting human ecology (Harlan, 1992). *H. spontaneum* and *H. vulgare* appear to be both reproductively (hybrids are selected against) and ecologically (occupying separate ecological niches) two independent species. They conform to the biological species concept based on reproductive biology and ecological compatibility. If agriculture was to disappear, then, in all likelihood, cultivated barley would disappear also, along with man-made habitats, with *H. spontaneum* the only surviving species (Wei *et al.*, 2005). Even wild *H. spontaneum* shows signatures of phenotypic and genotypic stresses due to global warming (Nevo *et al.*, 2012).

Hordeum spontaneum is distributed in the east-Mediterranean basin and the west Asiatic countries, penetrating into the Aegean region and North Africa to Morocco. It extends eastwards to central Asian areas (Turkemenia, Afghanistan, Ladakh, and Tibet of China) (Fig. 1). Wild barley occupies both primary and segetal habitats. Its center of origin and diversity was considered, until recently, in the Near East Fertile Crescent, displaying high genetic diversity in Israel, Golan Heights, and Jordan and extending across Asia to Tibet of China (Zohary *et al.*, 2012. See the current

distribution of *H. spontaneum* in Zohary *et al.*, (2012), Morell and Clegg (2007) and Dai *et al* (2012) (Fig. 1). Extensive genetic diversity was found in *H. spontaneum* in Tibet of China using 1,300 markers across the genome (Dai *et al.*, 2012, and their Fig. 1). Zohary (1999) argued that the fixation of independent mutations at nonallelic, non-brittle ear loci in cultivated barley is strongly suggestive of at least two centers of domestications. Chloroplast DNA microsatellites supports a polyphyletic origin for barley (Molina-Cano *et al.*, 2005). Likewise, differences in haplotype frequency among geographic regions at multiple loci infer at least two domestications of barley: one within the Fertile Crescent (Badr *et al.*, 2000; Lev Yadun *et al.*, 2000; Zohary *et al.*, 2012) and the second, 1,500-3,000 km farther east (Morell and Clegg, 2007). Finally, another center of domestication of barley in Tibet of China was suggested, based on 1,300 DArT P/L (Diversity Arrays Technology Pty. Ltd.) (Dai *et al.*, 2012). In the Near East wild barley is adapted primarily to warm and dry climates and only rarely found above 1500 m. However, in Tibet of China it thrives above 4,000 m and is adapted to cold and dry environments, and may have developed after diverging from the Near East adaptive complexes to a cold and dry climatic regime (Dai *et al.*, 2012).

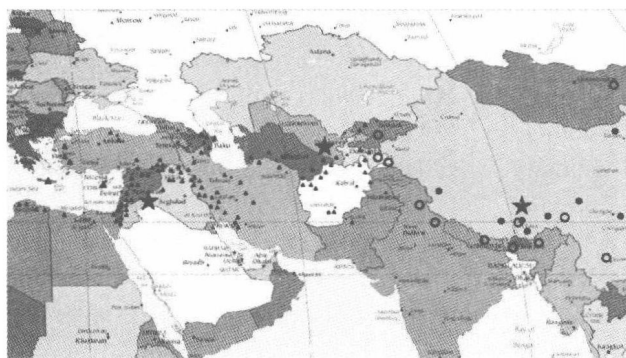


Fig. 1. Distribution of sites of wild barley. Triangle: wild barley in the Near East (left star) and Central Asia (middle star) (Harlan and Zohary, 1966). Dot: wild barley in the Tibetan Plateau (right star) and its vicinity (Ma D.Q., Genetic Resources of Tibetan barley in China, China Agriculture Press, 2000); Circle: wild barley collected by Prof. Kazuhiro Sato (Okayama University, personal communication)

4. GENETIC DIVERSITY IN WILD BARLEY

Genetic diversity is the basis of evolutionary change (Nevo; 1978; 1988; 1998; 2004; 2005; Nevo and Beiles,

2011). Wild barley, *H. spontaneum*, is rich in adaptive genetic diversity at the genetic (allozyme and DNA), genomic, proteomic, and phenomic levels (see Nevo, wild cereals at <http://evolution.haifa.ac.il>, and specifically Nevo *et al.*, 1979, 1981, 1983, 1986a-d, 1997, 1998; 2004, 2005; 2009b; Chalmers *et al.*, 1992; Baum *et al.*, 1997; Pakniyat *et al.*, 1997; Forster *et al.*, 1997; Owuor *et al.*, 1997; Li *et al.*, 1998; Vicient *et al.*, 1999; Turpeinen *et al.*, 1999; 2003; Kalendar *et al.*, 2000; Close *et al.*, 2000; Gupta *et al.*, 2002, 2004; Sharma *et al.*, 2004; Ivandich *et al.*, 2002, 2003; Huang *et al.*, 2002; Baek *et al.*, 2003; Owuor *et al.*, 2003).

The regional and local allozyme studies in Israel, Turkey, and Iran highlight the following patterns. *H. spontaneum* in the Near East is very variable genetically. Genetic divergence of populations includes some clinal, but primarily regional and local patterns, often displaying sharply geographic divergence over short distances at both single and multilocus genome organization. The average relative genetic differentiation (GST) was 54% within populations, 39% among populations (range 29% - 48%) within countries, and 8% among the three countries (Table 7 in Nevo *et al.*, 1986c). Allele distribution is characterized by a high proportion of unique alleles (51%) and a high proportion of common alleles that are distributed either locally or sporadically, as well as displaying an “archipelago” genetic structure, where high frequency allele levels can reside *near* low ones or none at all. Discriminant analysis by allele frequencies successfully clustered wild barley of each of the three countries (96% correct classification). A substantial portion of allozyme variation in nature is significantly correlated with the environment and is predictable ecologically, chiefly by a combination of humidity and temperature variables. Natural populations of wild barley are, on average, more variable than two composite crosses and landraces of cultivated barley (Nevo, 2004). Genetic variation of wild barley is not only rich in the Near East, but at least partly adaptive and predictable by ecology and allozyme markers (Nevo, 1987). Consequently, *conservation* and *utilization* programs should optimize sampling strategies by following the ecological-genetic factors and molecular markers as effectively predictive guidelines (Nevo *et al.*, 1986c; 1987; Chalmers *et al.*, 1992; Volis *et al.*, 2001; 2002).

DNA genetic diversity and divergence patterns parallel those of allozymes (Li *et al.*, 2000). This

conclusion suggests that climatic selection through aridity stress may be an important factor acting on both structural protein coding and presumably partly regulatory noncoding DNA regions resulting in adaptive patterns, for example, in intergenic and genic SSRs (Li *et al.*, 2002; 2004). The population structure of *H. spontaneum* is strongly correlated with temperature and precipitation (Hubner *et al.*, 2009). These and other multiple cases indicate that genetic diversity across the genome is driven, to a substantial yet unknown quantity, by natural selection. The latter overrides nonselective forces like gene flow and stochastic factors, revealing how plants respond to stressful environments (Cronin *et al.*, 2007; Fitzgerald *et al.*, 2011; Hubner *et al.*, 2009; Nevo 1992, 2011a-c). Recent increases in the availability of expressed sequence tag (EST) data have facilitated the development of microsatellites (SSR) markers in plants, including cereals, enabling interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye, and rice (Varshney *et al.*, 2005). Development of new microsatellites in barley reinforce genetic mapping (Li *et al.*, 2003). Genomic SSR markers displayed higher polymorphism than EST-SSRs. The latter, however, displayed clearer separation between wild and cultivated barley (Chabane *et al.*, 2005). The EST-SSRs are applicable to barley genetic resources, providing direct estimates of functional biodiversity. EST-SNP are the best markers for typing gene bank accessions, and the AFLP and EST-SSR markers are more suitable for diversity analysis and fingerprinting (Varshney *et al.*, 2007). Analysis of molecular diversity, population structure, and linkage disequilibrium were conducted in a worldwide survey of cultivated barleys (Malysheva-Otto *et al.*, 2006). Low levels of linkage disequilibrium in wild barley were recorded despite the high rate of self-fertilization, -98% (Morell *et al.*, 2005). High resolution genotyping of wild barley and fine-mapping facilitates QTL fine-mapping and cloning (Schmalenbach *et al.*, 2011). This enabled the fine-mapping of the threshability locus *thresh-1* on chromosome 1H. *Thresh-1* controls grain threshability and played an important role in domestication.

5. ADAPTIVE COMPLEXES IN THE NEAR EAST

5.1 Phenotypic Adaptations

Israeli populations of *H. spontaneum* display dramatic

variation in phenotypic traits across Israel in accordance with climatic and edaphic variations from robust mesic phenotypes to slender xeric genotypes. The genetic basis of this phenotypic variation in ten variables, including germination, earliness, biomass, and yield, was identified in common garden experiments in the mesic (Mount Carmel, Haifa) and xeric Avedat and Sede Boqer in the northern Negev desert (Nevo *et al.*, 1984, including several figures demonstrating the variation). Adaptive variation patterns of germination and desiccation of mesic and xeric phenotypes include longer seed dormancy, roots, and desiccation tolerance in xeric plants (Chen *et al.*, 2002; 2004a, 2004b). Likewise, small and dark kernels characterize xeric and high-solar exposed populations (Chen *et al.*, 2004c). The genetic basis of wild barley caryopsis dormancy and seedling desiccation tolerance at germination was described by Zhang *et al.* (2002; 2005). Xeric phenotypes had deeper dormancy but less seedling salt tolerance (Yan *et al.*, 2008). Fifteen agronomic, morphological, developmental, and fertility traits differentiated at the 100 m Tabigha microsite, subdivided into 50 meters wetter basalt and 50 meters drier terra rossa soil (Ivancic *et al.*, 2000). Terra rossa genotypes had better resistance to drought than basalt genotypes.

Edaphic natural selection strongly diverge phenotypes and genotypes at microscales as was also shown in the microclimatic divergent microsites of Newe Ya'ar (Nevo *et al.*, 1986a) and "Evolution Canyon" (Nevo *et al.*, 1997). Growth characteristics diverge distinctly in wild barley from different habitats (van Rijn *et al.*, 2000) associated with AFLP markers (van Rijn *et al.*, 2001; Vanhala *et al.*, 2004), growth rates (Verhoven *et al.*, 2004a; 2004b), and seedling desiccation tolerance (Zhang *et al.*, 2002; 2005; Yan *et al.*, 2008; 2011).

5.2 Genotypic Adaptations

5.2.1 Abiotic Genetic Resources of Drought and Salinity Resistances in *Hordeum spontaneum*

Drought and salinity are the major abiotic stresses that threaten food supplies around the world. Wild relatives of wheat and particularly barley harbor immense potential for drought and salt tolerance. *Triticum dicoccoides* (Gustafson *et al.*, 2009; Nevo, 2011a), but particularly *Hordeum spontaneum* (Chen, 2005), the progenitors of cultivated wheat and barley, respectively, developed rich genetic diversities for

drought and salt tolerance (Nevo and Chen, 2010) with great potential in plant breeding for stress environments (Blum, 1988). Drought resistance in wild barley from Israel, including physiology, gene identification, and QTL mapping, was extensively studied by Chen (2005) and Chen *et al.*, (2002; 2004a; 2004b; 2004c; 2009; 2010; 2011a; 2011b). Multilevel regulation and signaling processes associated with adaptation to terminal drought in wild emmer wheat was analyzed by Krugman *et al.*, (2010 and references therein), followed by transcriptomic and metabolomic profiles in drought adaptation mechanisms in wild emmer (Krugman *et al.*, 2011). A total of 5,892 transcripts were identified in this study between drought-resistant and drought-susceptible genotypes. Two hundred and twenty-one well studied genes involved 26% regulatory genes including transcriptional regulation, RNA binding, kinase activity, calcium and abscisic acids signal affecting stomatal closure. Additional adaptive genes were involved in wall adjustment, cuticular wax deposition, lignification, osmoregulation, redox homeostasis, dehydration protection, and drought-induced senescence. Tolerant genes, gene networks, and QTLs within a multidisciplinary context, will play an increasing role in crop breeding programs to develop drought- and saline-tolerant crops, especially with the ongoing global warming associated with drought (IPCC, 2010). A huge amount of literature, impractical to cite here, describes patterns and mechanisms of genetic resources from wild relatives as candidate genes for crop improvement. I will briefly overview three in-depth studies involving dehydrins and two novel genes, *Hsdr4* and *Eibi 1*, studied at the Institute of Evolution.

5.2.2 Differential Expression of Dehydrins in Wild Barley at Regional and Local Scales

Dehydrins (DHNs; Lea D-11) are water-soluble lipid vesicle-associated proteins involved in adaptive responses of plants to drought, low temperature, and salinity (Close *et al.*, 2000). The assembly of several domains into consistent permutations resulted in DHN polypeptide lengths from 82 to 575 amino acid residues. Allelic variation in *Dhn* genes provides a rich repertoire for drought-stress tolerance in barley and other Triticeae species. Regionally, tolerant and sensitive genotypes were identified from Israeli and Jordanian wild barley *H. spontaneum* populations in dehydrin genes (*Dhn* 1, 3, 5, 6, and 9) (Suprunova *et al.*, 2004) (Fig. 2). The five *Dhn* genes were up-

regulated by dehydration in resistant and sensitive wild barley genotypes and remarkably so in *Dhn1* and *Dhn6* genes, depending on the duration of dehydration stress. *Dhn1* reacted earlier, after 3 hours, and displayed higher resistance (at 12 h and 24 h) in resistant compared to sensitive genotypes. The expression level of *Dhn6* was significantly higher in the resistant genotypes at earlier stages of stress, but after 12 h and 24 h *Dhn6* expression was relatively higher in sensitive genotypes. These results indicate adaptive responses of these genes in dehydration tolerance *regionally* in wild barley (Fig. 2). We continued to test *Dhn* genes *locally* at “Evolution Canyon”. “Evolution Canyon” I, at lower Nahal Oren, Mount Carmel, Israel, is a natural micro scale model for studying *evolution in action* highlighting biodiversity evolution, adaptive radiation, and incipient sympatric speciation across life (Nevo list of “Evolution Canyon” at <http://evolution.haifa.ac.il> and reviewed in Nevo (1995; 2006a; 2009a; 2011b; 2011c; 2012). Wild barley, *Hordeum spontaneum*, is a major model organism at ECI, displaying interslope adaptive molecular-genetic divergence (Nevo *et al.*, 1997; Owour *et al.*, 1997), and incipient sympatric speciation (Parnas, 2006; Nevo, 2006a). The adaptive divergence occurs between the “African” xeric, tropical south-facing slope (AS=SFS) and the “European”, mesic, temperate north-facing slope (ES=NFS), separated, on average, by 200 m. *Dhn1* of wild barley was examined in 47 genotypes at “Evolution Canyon” I, 4-10 individuals in each of 7 stations (populations) in an area of 7000 m². The analysis was conducted on sequence diversity at the 5' upstream flanking region of the *Dhn1* gene. Rich diversity was found in 29 haplotypes, derived from 45 SNPs in a total of 708 bp sites. Most haplotypes, 25 of 29 (86.2%), were represented by one genotype, i.e., unique to one population. Only a single haplotype was common to both slopes. Nucleotide diversity was higher on the AS (Fig. 3A) (as in 64% of other model organisms tested at ECI (Nevo, 2009a). Haplotype diversity was higher on the ES. Interslope divergence was significantly higher than *intraslope* divergence, and SNP neutrality was rejected by the Tajima test. *Dhn1* expression under dehydration displayed interslope divergent expression between AS and ES genotypes (Fig. 3B), unfolding the adaptive nature of *Dhn1* drought resistance. Microclimatic natural selection appears to be the most likely evolutionary driving force causing adaptive interslope *Dhn1* divergent evolution at ECI.

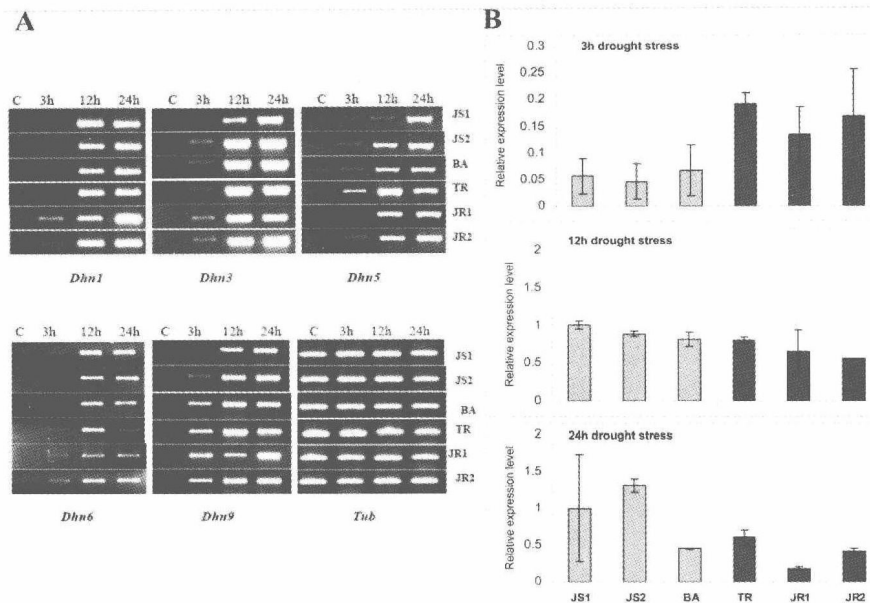


Fig. 2. A: Differential expression patterns of *Dhn1,3,5,6* and *9* detected by RT-PCR. The RT-PCR was carried out with gene specific primers, using cDNA obtained from six wild barley genotypes (JS1, JS2, BA, JR1, JR2, TR) after 0 (control, C), 3, 12, and 24 h of dehydration. As a control for relative amount of DNA, RT-PCR with gene specific primers for α -tubulin (*Tub*) was performed. **B:** Expression of *Dhn6* detected by quantitative real-time PCR. Real-time PCR was carried out with cDNA obtained from six barley genotypes (JS1, JS2, BA, TR, JR1, and JR2) after 3, 12, and 24 h of dehydration. Quantification is based on C_I values that were normalized using the C_I value corresponding to a barley (housekeeping) α -tubulin gene. Two independent plant samples for each genotype were examined in triplicate. Each value is the mean \pm SE (n=2). (From Suprunova *et al.*, 2004)

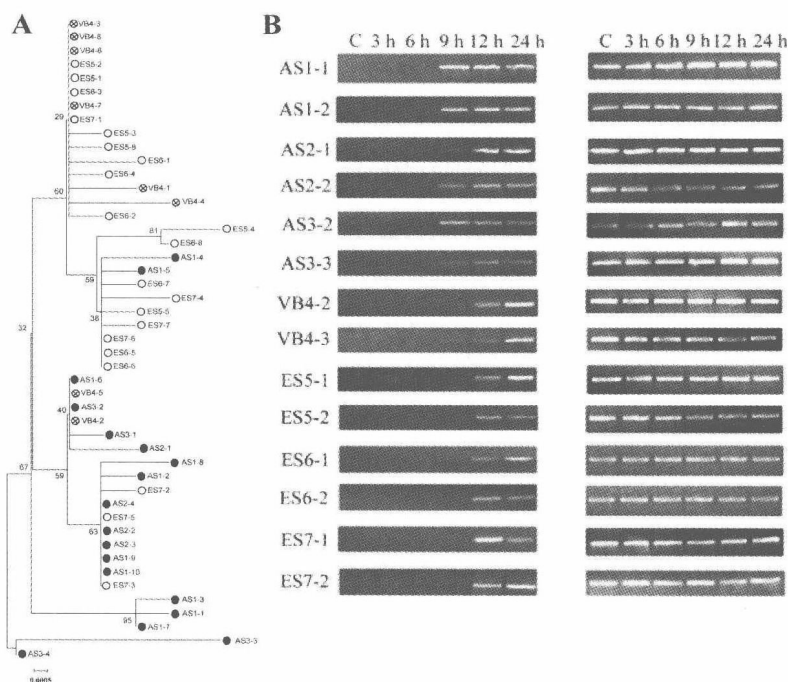


Fig. 3. A: Dendrogram of the genetic relationships of 47 genotypes of wild barley, *H. spontaneum*, from seven populations representing the AS (black circles), ES (blank circles) and VB (x-circles) in "Evolution Canyon"1, Israel. These genotypes were obtained from the dehydrin 1 alignment sequence, based on Nei's calculated nucleotide diversity (p-distance) values (see scale), using the neighbour-joining method. Numbers on branches are percentage values from bootstrap analysis (1000 replicates). **B:** Differential expression patterns of *Dhn1* detected by RT-PCR. The RT-PCR of *Dhn1* was using the cDNA from the 14 genotypes (2 from each station) after 0 (control, C), 3, 6, 8, 12, and 24 h after dehydration with α -tubulin (*Tub*) as a control. (Figs. 4 and 5 in Yang *et al.*, 2008)

We also examined the genetic pattern of *Dhn6* in 48 genotypes of wild barley at ECI (Yang *et al.*, 2012) because it is also strictly related to drought resistance in barley. A recent insertion of 342 bp in 5'UT primarily at the upper more xeric stations of the opposite slopes, AS and ES, was associated with earlier up-regulation of *Dhn6* after dehydration. Both coding SNP nucleotide and haplotype diversity (see Fig. 2 in Yang *et al.*, 2012) were higher on the AS than on the ES, and the applied Tajima D and Fu Li tests rejected neutrality of SNP diversity. Differential expression patterns of *Dhn6* were detected after different hours of dehydration. The interslope genetic divergence of amino acid sequences indicated significant positive selection of *Dhn6*. Clearly, *Dhn6* diversity was subjected to microclimatic divergent natural selection and was adaptively associated with drought resistance of wild barley at "Evolution Canyon" I, paralleling *Dhn1* (Yang *et al.*, 2012).

5.2.3 *Hsdr4* Novel Gene Involved in Water-Stress Tolerance in Wild Barley

In search of drought-resistant genes in wild barley we identified a novel gene, *Hsdr4* (Suprunova *et al.*, 2007). Gene expression profiles of water-stress tolerant vs. water-stress sensitive wild barley genotypes were compared under severe dehydration stress applied at the seedling stage using cDNA AFLP analysis. Seventy out of 1,100 transcript-derived fragments (TDFs) displayed differential expression between control and stress conditions. Eleven of them showed clear up- or down-regulation differences between tolerant and susceptible genotypes. These TDFs were isolated, sequenced, and tested by RT-PCR. TDF-4 was selected as a promising candidate gene for water-stress tolerance. The corresponding gene, designated *Hsdr4* (*Hordeum spontaneum* dehydration – responsive), was sequenced. The deduced amino acids were similar to the rice Rho-GTOase-activating protein-like with a Sec 14 p-like lipid binding domain. Analysis of the *Hsdr4* promoter region revealed a new putative miniature inverted repeat transposable element (MITE) and several potentially stress-related binding sites for transcription factors (MYC, MYB, LTRE, and GT-1), suggesting a role of the *Hsdr4* gene in plant tolerance of dehydration stress. The *Hsdr4* was mapped to the long arm of chromosome 3H within a region that was previously shown to affect osmotic adaptation in barley. Transgenic work will validate the role of *Hsdr4* in resisting dehydration stress. This study indicates the great potential for identifying novel candidate genes related to water-stress tolerance in wild barley.

5.2.4 Wild Barley *eibil* Mutation Identified a Gene Essential for Leaf Water Conservation

The colonization of land by water plants necessitated the evolution of a cuticle, a cutin matrix embedded with and covered by wax, sealing the aerial organ's surface, thus protecting the plant from uncontrolled water loss. The incidental discovery by Guoxiong Chen of a spontaneous wilted mutant (*eibil*), hypersensitive to drought in a desert wild barley in Israel, led to the identification of a major gene contributing to the generation of cutin and enabling land life (Chen *et al.*, 2004a-c; 2005; 2009-2011a,b). *eibil* showed the highest relative water-loss rate among the known wilted mutants, indicating that it is one of the most drought-sensitive mutants. *eibil* had the same abscisic acid (ABA) level, the same ability to accumulate stress-induced ABA, and the same stomatal movement in response to light, dark, drought, and exogenous ABA as the wild type. Thus, *eibil* was neither an ABA-deficient nor an ABA-insensitive mutant. The transpiration rate of *eibil* was closer to the chlorophyll efflux rate than to stomatal density, demonstrating that the cuticle of *eibil* was cutin-defective (Fig. 4A, B). A fine-scale genetic mapping of the *eibil* locus on chromosome 3H is perfectly collinear with the equivalent region on rice chromosome 1. Gene prediction revealed that this rice segment harbors 16 genes. Some of them were proposed as candidate genes of *eibil* (Chen *et al.*, 2009). Map-based cloning revealed that *eibil* encodes a HvABCG31 full transporter. The gene is highly expressed in the elongation zone of a growing leaf (the site of cutin synthesis), and its gene product also was localized in developing, but not in mature, tissue (Chen *et al.*, 2011a). A *de novo* wild barley mutant, named "*eibil.c*", along with two transposon insertion lines of rice, mutated in the ortholog of HvABCG31, were also unable to restrict water loss from detached leaves. HvABCG51 was hypothesized to function as a transporter involved in cutin formation, and the *eibil* mutant resulted in the loss of function of HvABCG31, which is an ABCG full transporter. The sequence of the wild-type and mutant alleles revealed a single nucleotide difference in exon 14, predicted to alter a tryptophan codon into a TAG stop codon (Fig 4B), and thereby induced a probable loss-of-function mutation. The full-length wild-type *eibil* sequence consisted of 11,695 bp arranged in 24 exons, producing a transcript length of 4,799 (Fig. 4Bb with an ORF of 4,293 bp (1,430 residues) (Fig. S1 in Chen *et al.*, 2011). *eibil* epidermal cells contain lipid-like droplets consisting presumably of cutin monomers that have not been transported out of the cells (Chen *et*