



Genomic adaptation to drought in wild barley is driven by edaphic natural selection at the Tabigha Evolution Slope

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Ecological divergence at a microsite suggests adaptive evolution, and this study examined two abutting wild barley populations, each 100 m across, differentially adapted to drought tolerance on two contrasting soil types, Terra Rossa and basalt at the Tabigha Evolution Slope, Israel. We resequenced the genomes of seven and six wild barley genotypes inhabiting the Terra Rossa and basalt soils, respectively, and identified a total of 69,192,653 single-nucleotide variants (SNVs) and insertions/deletions in comparison with a reference barley genome. Comparative genomic analysis between these abutting wild barley populations involved 19,615,087 high-quality SNVs. The results revealed dramatically different selection sweep regions relevant to drought tolerance driven by edaphic natural selection within 2,577 selected genes in these regions, including key drought-responsive genes associated with ABA synthesis and degradation (such as Cytochrome P450 protein) and ABA receptor complex (such as PYL2, SNF1-related kinase). The genetic diversity of the wild barley population inhabiting Terra Rossa soil is much higher than that from the basalt soil. Additionally, we identified different sets of genes for drought adaptation in the wild barley populations from Terra Rossa soil and from wild barley populations from Evolution Canyon I at Mount Carmel. These genes are associated with abscisic acid signaling, signaling and metabolism of reactive oxygen species, detoxification and antioxidative systems, rapid osmotic adjustment, and deep root morphology. The unique mechanisms for drought adaptation of the wild barley from the Tabigha Evolution Slope may be useful for crop improvement, particularly for breeding of barley cultivars with high drought tolerance.

drought adaptation | evolution models | genome resequencing | *Hordeum spontaneum* | microsites

Barley is the fourth largest cereal crop worldwide and is also an excellent model species for genetic and physiological studies (1, 2). Its unique genetic adaptation and tolerance to abiotic stresses are providing insights relevant to the improvement in other cereal crops. Wild barley (*Hordeum spontaneum* L.) is the progenitor of cultivated barley (*Hordeum vulgare* L.) and is a hardy plant species, harboring a myriad of distinctive genes, alleles, and regulators with potential for increasing the resistance of cultivated barley to abiotic stresses such as drought, salinity, and temperature (3–5). Genome sequences, both coding and non-coding, provide a solid basis for understanding biological traits and their regulation, and the recently released, high-quality reference barley genome sequences (6, 7) are providing important resources for comprehensive genetic and genomic studies of cereals. The reference genome provides a basis for positional cloning and functional analysis of key genes, optimizing population genomics and comparative genomic analysis as well as facilitating genome resequencing to investigate relationships between genomic variation and phenotypic differences.

Drought hampers crop production and the global food supply (5, 8). Crops often experience periods of atmospheric or soil

water deficit, which are often accompanied by high temperatures, poor nutrient uptake, and aggravated soil salinity stress (9). Thus, typical features enabling drought tolerance of plants include, but are not limited to, deep and large root systems, thick and complex cuticular waxes, efficient stomatal regulation, and regulation of drought-responsive genes and metabolites (5, 8, 10, 11). As a relatively drought-tolerant crop for dryland agriculture in many countries, several studies aiming to elucidate the mechanisms of drought tolerance have been conducted on barley through genomic approaches (12–15). Using transcriptome analysis, ecotype-specific transcripts have been found in two wild barley ecotypes that differentially adapt them to drought stress (12) and genes related to differential reproduction under drought stress (15). Thus, investigating drought tolerance in wild barley may facilitate a better understanding of the genetic basis of this trait and the identification of effective genetic or genomic approaches toward barley improvement (14).

Significance

Microsite evolution involving ecological divergence due to geological, edaphic, or climatic conditions requires adaptive complexes to environmental stresses. The higher drought tolerance of wild barley populations inhabiting Terra Rossa soil at the Tabigha Evolution Slope has been described, but the underlying genetic mechanisms remain unknown. Using genome resequencing and RNA-sequencing technologies of wild barley genotypes from contrasting Terra Rossa and basalt soil types, we identified genes in selection sweep regions on chromosomes 6H and 7H, showing divergence in the barley populations from Terra Rossa and basalt soils with significant roles in plant drought tolerance. Our results set a solid foundation for future work on gene discovery and on drought adaptation mechanisms in barley related to the rhizosphere environment.

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Data deposition: The sequences of raw data have been deposited in the National Center for Biotechnology Information Sequence Read Archive, <https://www.ncbi.nlm.nih.gov/>, and the data accession numbers are listed in *SI Appendix, Tables S1 and S3*.

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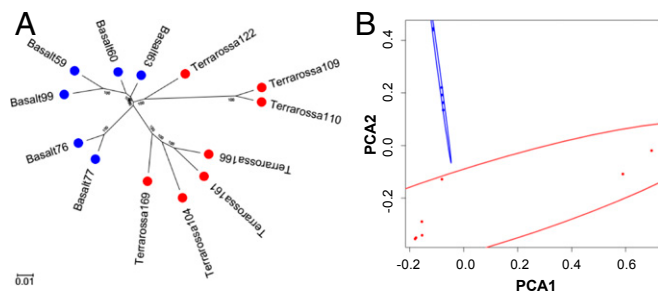


Fig. 1. Phylogenetic tree (A) and PCA (B) of wild barley populations inhabiting Terra Rossa and basalt soils at the Tabigha Evolution Slope. (A) Phylogenetic tree was constructed using the neighbor-joining method, and the percentage of trees from 1,000 bootstrap replications in which the associated taxa clustered together are shown next to the branches. Circles in both A and B indicate barley genotypes from Terra Rossa (red) and basalt (blue) soil types. PCA 1, the first principal component; PCA 2, the second principal component.

The complexity of drought stress requires the investigation of tolerance mechanisms in different targeted environments (5, 16). Wild barley is likely to suffer from different types of drought due to its wide geographic range, i.e., growth under different climate conditions or in different soil types. It was proposed that wild barley has evolved distinctive mechanisms to cope with different types of drought such as those experienced at the Tabigha Evolution Slope located north of the Lake of Galilee in Israel; our study complements those at the Evolution Canyon microsite (see E.N. publications of Evolution Canyons at evolution.haifa.ac.il). At the Tabigha Evolution Slope, Middle Eocene hard limestones weather into Terra Rossa soils and about Pleistocene volcanic basalt flows that weather into basaltic soils; this results in soils that display dramatic chemical and physical differences (17, 18). The basaltic soil possesses a greater water-holding capacity compared with Terra Rossa soil. A pioneering study of edaphic differentiation in wild barley has been made at the Tabigha Evolution Slope (17), taking advantage of the sharp microscale ecological divergence of plants growing on these two soil types. Allozyme polymorphisms were found, which were suggested to be at least partly adaptive and differentiated due to natural selection mediated by edaphic factors rather than by stochastic processes and/or neutrality of allozyme variants (17). A study in *Aegilops peregrina* L. at the same site also demonstrated allozymic divergence in two esterase loci between plants from Terra Rossa and basalt soils (19).

Plants thriving on the calcareous Terra Rossa naturally experience more intense drought than plants inhabiting the moist siliceous clay of the basalt soil, and a study (18) of wild barley populations from these soil types showed divergent phenotypic responses to water stress. A high degree of phenotypic variation was found in the wild barley populations from the Terra Rossa and basalt soils when drought treatments were imposed, resulting in significant genotype \times treatment and soil type \times treatment interactions. Terra Rossa genotypes exhibited significantly better drought adaptation and were more stable under drought stress, important traits for improving adaptation to drought in cultivated barley (18). This contrasting drought tolerance in the wild barley populations at the Tabigha Evolution Slope appears to be related to adaptation to different soil conditions. However, the genomic basis of this difference remains unknown. These studies (17–19) provided the rationale for the present genome-wide comparison of wild barley populations at the Tabigha Evolution Slope, and we extend the limitation of allozymic markers to examine the diversity of the entire wild barley genome in two contrasting populations. We provide strong evidence for edaphic adaptations to drought across the whole genome in wild barley.

Results

Whole-Genome Resequencing and Genomic Diversity of Wild-Barley Genotypes from the Tabigha Evolution Slope. A total of 1,300 Gb of clean data were obtained from 13 wild barley genotypes at the Tabigha Evolution Slope, with an average of 100 Gb for each genotype at 20 \times genome coverage. There were, on average, 88.46% clean reads mapped to the reference genome of barley (cv. Zangqing320, www.ibgs.zju.edu.cn/ZJU_barleygenome.htm), with the mapped ratios ranging from 85.96 to 91.01% (SI Appendix, Table S1).

Based on the reads uniquely mapped to the reference genome, we identified a total of 69,192,653 single-nucleotide variants (SNVs) and insertions/deletions, ranging from 16,053,119 to 27,020,003 for each genotype (SI Appendix, Table S1). The large variations have enabled further analysis for population structure and genetic divergence of wild barley. We filtered the raw SNVs and obtained 19,615,087 high-quality SNVs for the construction of a phylogenetic tree (Fig. 1A) and for principal component analysis (PCA) (Fig. 1B), which demonstrated a significant difference between the two wild barley populations inhabiting the two soil types. The PCA clearly divided these barley genotypes into two groups that corresponded with the soil types (Fig. 1B). The phylogenetic tree may involve some mixing due to ongoing gene flow especially near the interface.

Genetic Divergence of Wild Barley Inhabiting the Terra Rossa and Basalt Soils. To identify potentially unique genomic regions modulated by environmental selections, Wright's F_{ST} was calculated based on the 19,615,087 high-quality SNVs. The results show that genetic differences between wild barley populations in Terra Rossa and basalt soils follow a normal distribution, resulting in a cumulative 93.39% with a weighted F_{ST} value of ≤ 0.3 (Fig. 2). This value (0.3) was used as the threshold to conduct a selective sweep analysis.

There were distinct patterns in the distribution of windowed F_{ST} values for each chromosome between the barley populations collected from the two soils (Fig. 3). Higher drought tolerance of the wild barley population inhabiting Terra Rossa soil than that in basalt soil (18) was measured using the fixation index F_{ST} . As a result, 7% of the F_{ST} windows were under strong selective sweeps (Fig. 2). The total length of genomic regions with F_{ST} values above 0.3 was 0.36 Gb and contained 2,577 high-confidence genes (e.g., those related to plant hormones, antioxidants, and osmoprotectants); these

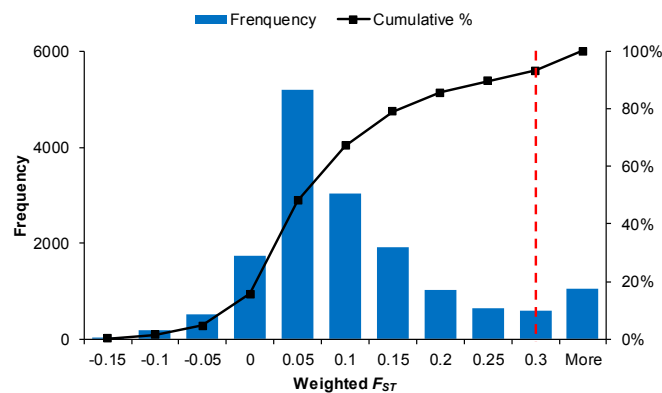


Fig. 2. Distribution of F_{ST} values across the whole genome between wild barley populations inhabiting Terra Rossa and basalt soils at the Tabigha Evolution Slope. The F_{ST} value was calculated in each 1-Mb region with steps of 250 kb. The x axis indicates the value of F_{ST} , and the y axis shows the F_{ST} value frequency (left) and cumulative percentage (right). The red dashed line indicates the threshold value chosen based on the distribution of all windowed F_{ST} .

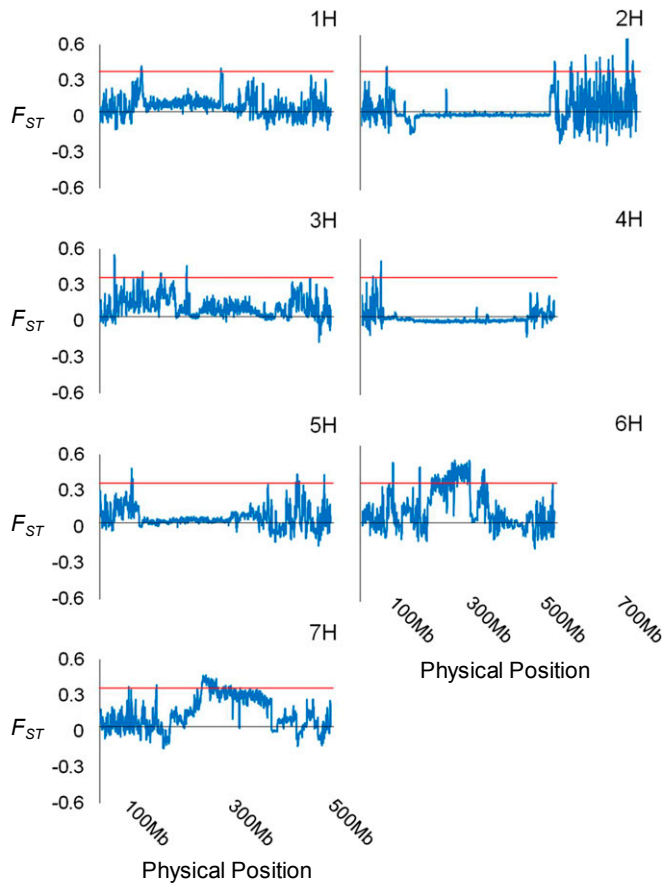


Fig. 3. Distribution of windowed F_{ST} values along each chromosome between wild barley populations from the Terra Rossa and basalt soils at the Tabigha Evolution Slope. The x axis indicates the physical position, and the y axis shows the value of F_{ST} for each 1-Mb genomic region with steps of 250 kb. The red solid lines indicate the threshold F_{ST} value (0.3) delimiting regions considered to be under strong selective sweeps.

selected regions were assumed to be subject to diversifying selection (*SI Appendix*, Table S2 and Dataset S1). We found evidence of strong environmental selection in two genomic regions, each about 250 Mb in length located around the centromeres of chromosome 6H (between 194,500,001 and 302,750,000 bp) and chromosome 7H (between 219,250,001 and 382,750,000 bp) (Fig. 3).

To clarify the genetic differences between wild barley populations inhabiting Terra Rossa and basalt soils, we conducted a genetic diversity analysis using the SNVs of the 2,577 selected genes (Dataset S1). There were 881 genes carrying 6,926 SNVs with no missing sites for polymorphism analysis in each of the 13 wild barley genotypes, including 3,396 SNVs showing genetic diversity. Among these, we further selected the genes with all SNVs having no missing sites and obtained 77 genes showing genetic diversity between the two barley populations (Dataset S2). These genes included those encoding protein phosphatase 2C (PP2C, MLOC_15036), MIZUKUSSEI 1 (MIZ1, MLOC_54802), alkaline neutral invertase CINV2 (MLOC_7525), and ECERIFERUM 1 (CER1, MLOC_11693). PP2C is an important negative regulator in ABA signaling and drought tolerance (20), MIZ1 plays a role in lateral root development by maintaining auxin levels (21), CINV2 regulates sugar-mediated root development by controlling sucrose catabolism in root cells (22), and CER1 participates in epicuticular wax biosynthesis (23). However, sequences of these genes in genotypes from basalt soil showed no genetic diversity (*SI Appendix*, Table S2 and Dataset S2).

The average π value, an indicator of genetic diversity, for the wild barley population inhabiting the Terra Rossa soil ($\pi = 0.208$) was much higher than that of the population from the basalt soil ($\pi = 0.147$). In addition, there were 1,050 and 214 unique SNVs in the wild barley populations inhabiting Terra Rossa and basalt soils, respectively (Fig. 4A). Moreover, the Terra Rossa population showed a large number of unique SNVs in chromosomes 6H and 7H (Fig. 4B). We randomly chose 50-Mb regions on chromosome 5H (without selection signature) and chromosomes 6H and 7H (with genetic signature of selective sweeps) to conduct genetic diversity analysis. The average π value of SNVs in 50-Mb regions on chromosome 5H was 0.214 for the population inhabiting the Terra Rossa soil and 0.130 for the population on basalt. The results indicated that the genetic diversity of the populations inhabiting Terra Rossa soil is larger than that from the basalt soil, a phenomenon reported in other higher-stress environments (24).

Transcriptome Analysis of Wild-Barley Populations from Evolution Canyon I at Mount Carmel Under Drought Treatment. Drought or soil water availability is one of the key climatic stresses that may distinguish the environment of the tropical hot and dry African Slope (AS) from the abutting temperate cool and humid forested European Slope (ES) of the Evolution Canyon I (ECI) at Mount Carmel, Israel, and 70 km west from the Tabigha Evolution Slope (25–28). Therefore, we conducted RNA sequencing to explore differential gene expression profiles of five wild barley genotypes each from the AS and the ES from ECI under drought treatment (*SI Appendix*, Fig. S1). In total, 3.47 billion raw reads (519.96 Gb) for 60 samples of the 10 wild barley genotypes (with two treatments and three biological replicates) were obtained by transcriptome sequencing. On average, 97.82% were clean reads, with the mean clean reads of 57,424,477 for each genotype, and 93.20% of the clean reads on average were mapped to the barley reference genome (1) (*SI Appendix*, Table S3).

Phylogenetic analysis (*SI Appendix*, Fig. S1), Venn diagram (*SI Appendix*, Fig. S2), and volcano plots of differentially expressed genes (DEGs) (*SI Appendix*, Fig. S3) at ECI showed clear divisions between the two populations. We identified 276 and 285 DEGs in five genotypes from both the AS and the ES; 59 DEGs were common to all 10 genotypes (*SI Appendix*, Fig. S24). About 75.58% of the DEGs specific to the AS genotypes were up-regulated, while nearly 74.34% of the DEGs specific to the ES genotypes were down-regulated (*SI Appendix*, Fig. S24), suggesting contrasting modes of response to drought stress between the barley genotypes from these tropical hot and dry AS and the temperate cool and humid ES. Furthermore, phenotypic analysis

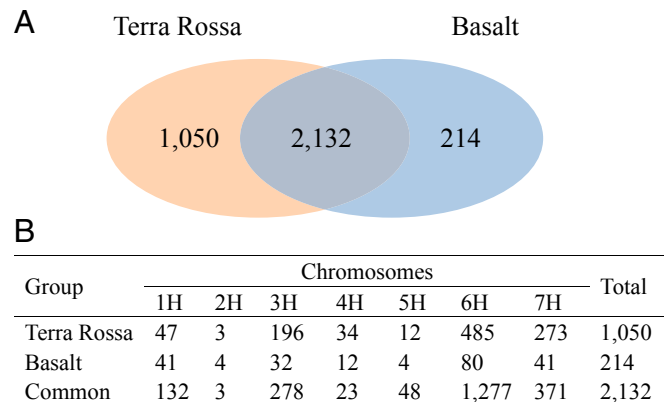


Fig. 4. Genetic diversity of two wild barley populations inhabiting Terra Rossa and basalt soils at the Tabigha Evolution Slope. (A) Venn diagram shows unique and common SNVs. (B) Distribution of SNVs with genetic diversity along chromosomes for each population.

of these genotypes from the contrasting AS and ES showed that AS genotypes have significantly lower stomatal conductance and higher intrinsic leaf water-use efficiency at a population level (*SI Appendix, Fig. S4*).

Mechanisms Underlying the Genetic Difference of Drought Tolerance in Wild Barley at the Tabigha Evolution Slope. To help understand the mechanisms of drought tolerance in wild barley from the Tabigha Evolution Slope, we analyzed the 2,577 genes in the selective genomic regions of genotypes from Terra Rossa and basalt soil types (*Dataset S1*), and the 502 DEGs of the genotypes from the AS and ES at ECI (*Dataset S3*), resulting in 22 common genes (*SI Appendix, Fig. S2B*), including one related to proline metabolism (MLOC_52074) (*Datasets S1 and S3*). Interestingly, we identified specific genes for drought adaptation of wild barley from the Terra Rossa soil.

Overall, many drought-responsive and ABA-signaling genes were identified in the selected genomic regions of wild barley from the Terra Rossa soil. For example, there were genes associated with stomatal development [mitogen-activated kinase kinase kinase, YODA (MLOC_51546), transmembrane leucine-repeat receptor (LRR)-like protein, TMM (MLOC_17359)], ABA synthesis and degradation [Cytochrome P450 protein, CYP (MLOC_7007), ABA receptor, PYL2 (MLOC_49654)], protein kinases [SNF1-related kinase, SnRK (ZLOC_8934), CBL-interacting protein kinase, CIPK9 (ZLOC_12175), serine threonine kinases (e.g., BLUS1: MLOC_57740), G-type lectin S-receptor-like serine threonine-kinases (MLOC_67098), lectin-domain-containing receptor kinases (MLOC_21948)], and protein phosphatases [serine threonine phosphatase, PP1 (MLOC_64374), phosphoinositide phosphatase, SAC, (MLOC_44139)] (*SI Appendix, Table S2 and Dataset S1*). These genes also encoded transcription factors [MYBs (MLOC_6171), NACs (MLOC_39910)], reactive oxygen species (ROS) and nitric oxide (NO) signaling [superoxide dismutase, SOD (MLOC_17760), peroxiredoxins, PRX (ZLOC_22743), nitrate reductase, NIA (MLOC_3293)], and Ca²⁺ binding and signaling [calmodulin-like (ZLOC_1443)] (*SI Appendix, Table S2 and Dataset S1*). Moreover, major ion channels [slow anion channels SLACs (MLOC_67347), SLAH1s (ZLOC_30254), SLAH2s (ZLOC_21671)], K⁺ transporters (MLOC_11780), cotransporters [ATP-binding cassette, ABC (MLOC_56261), nitrate transporter, NRT (MLOC_60308), high-affinity K⁺ transporter, HKT (MLOC_55066), cation/H⁺ exchanger, CHX (ZLOC_25185), cation-chloride cotransporter (MLOC_64607)], pumps [vacuolar H⁺-ATPase, VHAs (MLOC_72577), Ca²⁺-ATPase, ACAs (MLOC_34557)] were also found in the selective genomic regions of wild barley populations from the Tabigha Evolution Slope (*SI Appendix, Table S2 and Dataset S1*).

Some of these genes in the populations from the Tabigha Evolution Slope were also differentially in the populations from the AS and ES sites (*Dataset S3*). For example, there were nine genes and five DEGs encoding CYPs in wild barley of the Tabigha Evolution Slope and ECI; two (MLOC_23018, MLOC_64838) were significantly up-regulated in the wild barley from the AS and three (MLOC_51434, MLOC_7476, MLOC_65039) were down-regulated in genotypes from the ES (*Datasets S1 and S3*). BTB/POZ/MATH proteins function as a negative regulator, affecting stomatal behavior and responses to ABA in *Arabidopsis* (29). There were seven selected genes and three DEGs encoding BTB-POZ-MATH proteins in wild barley of the Tabigha Evolution Slope and ECI; MLOC_38371 was significantly up-regulated in wild barley from the AS, and two (MLOC_63926, MLOC_51464) were down-regulated in accessions from the ES (*Datasets S1 and S3*). H₂O₂ and NO are two key secondary messengers mediating signal transduction in response to many biotic and abiotic stresses (30–32). We identified 7 genes encoding peroxidases and 2 encoding glutaredoxins, 10 encoding glutathione synthesis and 3 genes encoding SOD, respectively, under selection in wild barley from

the Tabigha Evolution Slope (*SI Appendix, Table S2 and Dataset S1*), but none was found in wild barley materials from ECI. LRR receptor-like serine threonine-kinases are key regulators of ABA signaling (RPKs), plant innate immunity (FLSs), and coping with adverse soil conditions (GSOs) (33–35). There were 12 LRR receptor-like serine threonine-kinases including the major RPKs, FLSs, and GSOs in the unique genomic regions of the wild barley from the Tabigha Evolution Slope, and two LRRs (including GSO1) were found in the wild barley from ECI (*SI Appendix, Table S2 and Dataset S1*).

In addition, there were differences in genes from the selective genomic regions encoding osmoprotectants. Seven of the 10 genes associated with proline accumulation were located on 6H and 7H of the genotypes from the Tabigha Evolution Slope (*SI Appendix, Table S2 and Dataset S1*), providing evidence of strong environmental selection in the two genomic regions. In contrast, none of the three DEGs associated with proline in the genotypes from ECI were located on these two chromosomes (*Dataset S3*). We also identified three genes related to root development [e.g., *Root Hairless 1 (RTH1)*] and four genes associated with auxin signaling from the selective genomic regions of the genotypes from the Tabigha Evolution Slope (*SI Appendix, Table S2 and Dataset S1*). In contrast, very few DEGs in ECI were related to these genes, which might contribute to the drought tolerance mechanisms in the wild barley inhabiting Terra Rossa soil (*Dataset S3*).

Discussion

Adaptive Edaphic Selection of Wild Barley at the Tabigha Evolution Slope. Wild barley has adapted to various ecological and environmental conditions due to long-term adaptation driven by natural selection, including differences in water availability, soil type, temperature, and altitude (3, 17, 36–38). The average dry weight of wild barley from Terra Rossa was reduced by only 22.7% after a water stress treatment was imposed whereas a 78.0% decrease was detected for accessions from basalt soil, showing a significant genotypic difference between these populations (18). In this study, we performed genome resequencing of two wild barley populations adapted to these two contrasting soil types at the Tabigha Evolution Slope. Significantly, we identified two large genomic regions, 18.54% of chromosome 6H and 24.85% of chromosome 7H, that have been strongly subjected to environmental selection (Fig. 3). The results indicated a large genomic diversity between the two wild barley populations. This interslope genetic diversity at Tabigha between genotypes from calcareous Terra Rossa and siliceous basalt, a large part of which may involve adaptive pathways rich in drought resistance, is driven by edaphic differences, particularly in the drier Terra Rossa, highlighting the importance of edaphic factors in adaptive evolution.

In contrast, the long, nondiverse regions of chromosomes 4H and 5H show a close genetic relationship between these two wild barley populations (Fig. 3), which is consistent with the minor branches of the neighbor-joining tree (Fig. 14). This is to be expected as gene flow is likely to occur in both directions primarily in the vicinity of the interface between the two soils at the Tabigha Evolution Slope (17). Thus, our results revealed that the wild barley genotypes from the two soils have a close genetic relationship at the whole-genome level but, interestingly, have distinct selection sweep regions due to the adaptation to different soil types, which, as discussed below, may be primarily mediated by drought, which is higher on the Terra Rossa than the basalt soil (17–19).

Drought Tolerance Mechanisms of Wild Barley at the Tabigha Evolution Slope. Plants have evolved many strategies to maintain growth and development when water availability is restricted or unpredictable (5, 8). We have detected a set of genes relevant to drought adaptation in the wild barley population inhabiting the Terra Rossa soil in the Tabigha Evolution Slope in the selection sweep genomic regions (*SI Appendix, Table S2 and Dataset S1*). Coincidentally,

several quantitative trait loci (QTL) associated with drought tolerance (39–42) have also been identified in the same chromosomes in field-grown barley, such as four QTLs on chromosome 6H and two QTLs on chromosome 7H controlling relative water content (42).

To obtain more supporting evidence for drought mediating the selection sweep, we also analyzed the drought-responsive DEGs of wild barley from ECI, another classic evolution microsite in Israel (28), for comparative analysis of the specific drought-tolerance mechanisms in materials from the Tabigha Evolution Slope. ECI provides a microcosmic ecological model of life with contrasting biodiversity, where the AS is characterized by higher solar radiation, higher temperature, less water, and wider spatiotemporal heterogeneity and fluctuation than the ES (28). Therefore, drought-adaptive mechanisms in wild barley genotypes at ECI may result mainly from climate conditions (28) and may be different from those found at the Tabigha Evolution Slope. More than 75% of the DEGs in the genotypes from the AS were up-regulated, while around 75% of those in genotypes from the ES were down-regulated (*SI Appendix, Fig. S2A*). Therefore, the up-regulation of these key drought-adaptive genes in wild barley genotypes from the AS may be responsible for overcoming drought.

Comparison of these 502 DEGs from wild barley from ECI with the 2,577 genes from wild barley from the Tabigha Evolution Slope demonstrated that most are found in the selective genomic regions of the wild barley from the Tabigha Evolution Slope, but also show distinct expression in the wild barley genotypes from ECI (*Datasets S1* and *S3*). We summarize here a few highlights of the novel mechanisms of drought tolerance in the wild barley from the Tabigha Evolution Slope. It was reported that drought tolerance in barley may be attributed to ABA accumulation, osmotic adjustment, dehydrin expression, stomatal regulation, and root elongation of postgermination seedlings (9, 10, 13, 43). We suggest that the wild barley population inhabiting Terra Rossa soil from the Tabigha Evolution Slope has adapted to drought conditions by fine-tuning the ABA-modulated stomatal aperture opening to balance water use efficiency and CO₂ assimilation. The ABA-signaling pathway is one of the major pathways regulating drought tolerance and seed germination in plants (9, 11, 44). ABA can bind to the PYR/PYL/RCAR ABA receptors to inhibit clade A type 2C protein phosphatases (PP2Cs), thus releasing SnRK2 protein kinases from inhibition by PP2Cs and activating downstream signaling components for stomatal closure (20, 45). Activated SnRK2s can phosphorylate downstream effectors, such as SLAC1, and then trigger stomatal closure (46). Genes within the selection sweep regions may participate in this pathway.

Moreover, ABA-mediated stress responses also involve Ca²⁺-dependent and -independent signaling and ROS and NO signaling, regulating anion channels and other transporters for stomatal closure under drought stress (9, 30, 31). H₂O₂ is the primary ROS (32, 47) and can cause oxidative cell damage such as lipid peroxidation and membrane damage (48). To protect cellular systems from cytotoxic ROS, plants express peroxidase and superoxide dismutase and antioxidants, such as glutathione (47, 49). There were seven genes related to ROS and NO signaling under selection in the wild barley from the Tabigha Evolution Slope (*SI Appendix, Table S2* and *Dataset S1*). Nitrate-reductase-generated NO production, mediated by genes such as *NIA1* and *NIA2*, regulates stress-responsive genes, such as TFs and enzymes, either through modification of cysteine residues of proteins (S-nitrosylation) or by direct or indirect interaction with biomolecules like fatty acids or hormones (30, 31). Significantly, drought-induced down-regulation of *NIA*s in the wild barley of the ES suggests that these plants may not have efficient regulation of NO production during drought stress (*Dataset S3*). In summary, many genes at key nodes of the ABA-signaling pathway were found in the selected regions of wild barley from Terra Rossa soil, suggesting that they have important roles in drought-tolerance mechanisms.

Soil conditions have a strong influence on plant root morphology. To adapt to water limitation, wild barley can sacrifice root number for deeper-growing roots to obtain water from lower soil levels (6). Drought tolerance in wild barley seedlings has been attributed to the root length of postgermination seedlings and the formation of root hairs (13, 50). In the current study, we identified a gene encoding *RTH1* and two genes encoding *Root Primordium Defective 1 (RPDI)* under selection at the Tabigha Evolution Slope. Auxin also plays an important role in root formation (51). We detected four genes related to auxin under selection at the Tabigha Evolution Slope, and all of them were located on chromosome 7H. To adapt to dry and poor soils, plants form a Casparian strip as a hydrophobic barrier on endodermal cells that restricts lateral diffusion of ions and water between the root vascular bundles and the soil. Sulfated peptides, Casparian strip integrity factors (CIFs), are required for contiguous Casparian strip formation in *Arabidopsis* roots (33). These CIFs specifically bind to the endodermis-expressed leucine-rich repeat receptor kinases GSO1 and GSO2 for Casparian strip regulation and serve as active facilitators to cope with adverse soil conditions (33). Both *GSO1* and *GSO2* were found in the selective sweep genomic regions of the wild barley from the Tabigha Evolution Slope (*SI Appendix, Table S2* and *Dataset S1*), and *GSO1* was up-regulated by drought in the wild barley of the AS (*Dataset S3*), implying an active strategy for roots to control water and nutrient uptake under drought. Our results suggest that root morphology adjustment is another crucial adaptive mechanism for the wild barley genotypes inhabiting Terra Rossa at the Tabigha Evolution Slope that allows them to cope with drought stress.

Conclusions and Prospects. In conclusion, wild barley populations inhabiting Terra Rossa soil at the Tabigha Evolution Slope have evolved a suite of drought-tolerance mechanisms under the long-term natural selection mediated by soil conditions. These mechanisms include the expression and regulation of genes related to ABA signaling, antioxidative defense systems, and root morphology for a genomic adaptation of wild barley to drought resistance at the Tabigha Evolution Slope. The comparison with the transcriptome of wild barley in ECI suggests unique genomic mechanisms for drought tolerance in the wild barley at the Tabigha Evolution Slope. Future studies could examine in depth the unique functional drought resistance found at the Tabigha Evolution Slope at genetic, genomic, and epigenomic levels.

Materials and Methods

Plant Materials. We used 13 wild barley (*H. spontaneum*) genotypes from the Tabigha Evolution Slope (*SI Appendix, Table S1*) to conduct whole-genome resequencing, including 7 genotypes from Terra Rossa and 6 genotypes from basalt soils. All of the materials were collected and previously characterized by Nevo and coworkers (17, 18, 52).

Selective Sweep Analysis and Genetic Diversity Analysis. Selective sweep analysis is one of the major methods used to detect selection signatures at the genomic level in many organisms where the value of F_{ST} is used to detect the signals of strong recent selection with reduced pooled heterozygosity (53). Selective sweep analysis was conducted by measuring the patterns of allele frequencies in each 1-Mb fragment with a step of 250 kb along all chromosomes, using the 19,615,087 high-quality SNVs randomly distributed on chromosomes. Genomic regions under selective sweeps were measured by the F_{ST} using VCFtools v0.1.13 (54) with parameters of “-fst-window-size 1000000 -fst-window-step 250000.” Genomic regions with F_{ST} values >0.3 were considered under strong selective sweeps. Genetic diversity (π) was calculated using VCFtools v0.1.13 (54).

Population Structure Analysis. A phylogenetic tree was constructed using FastTree (55) with 1,000 replicates for bootstrap confidence analysis. MEGA v5.05 (56) was applied to draw the constructed tree. PCA was performed by SNPrelate v1.10.2 (57) and Car v2.1.5 (58) packages of R (version 3.4.0).

Additional experimental details of drought treatment and transcriptome sequencing analysis of wild barley genotypes from ECI, DNA library preparation,

deep sequencing, reads mapping, and SNVs and insertions/deletions calling can be found in *SI Appendix, SI Materials and Methods*.

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