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Risk-management strategies and transpiration rates of wild barley in uncertain environments

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Regulation of the rate of transpiration is an important part of plants' adaptation to uncertain environments. Stomatal closure is the most common response to severe drought. By closing their stomata, plants reduce transpiration to better their odds of survival under dry conditions. Under mild to moderate drought conditions, there are several possible transpiration patterns that balance the risk of lost productivity with the risk of water loss. Here, we hypothesize that plant ecotypes that have evolved in environments characterized by unstable patterns of precipitation will display a wider range of patterns of transpiration regulation along with other quantitative physiological traits (QPTs), compared to ecotypes from less variable environments. We examined five accessions of wild barley (*Hordeum vulgare ssp. spontaneum*) from different locations in Israel (the B1K collection) with annual rainfall levels ranging from 100 to 900 mm, along with one domesticated line (cv. Morex). We measured several QPTs and morphological traits of these accessions under well-irrigated conditions, under drought stress and during recovery from drought. Our results revealed a correlation between precipitation-certainty conditions and QPT plasticity. Specifically, accessions from stable environments (very wet or very dry locations) were found to take greater risks in their water-balance regulation than accessions from areas in which rainfall is less predictable. Notably, less risk-taking genotypes recovered more quickly than more risk-taking ones once irrigation was resumed. We discuss the relationships between environment, polymorphism, physiological plasticity and fitness, and suggest a general risk-taking model in which transpiration-rate plasticity is negatively correlated with population polymorphism.

Abbreviations – Chlb, chlorophyll b; CV, coefficients of variation; iCV, index of CV; LA, leaf area; LDW, leaf dry weight; LFW, leaf fresh weight; LTW, leaf turgid weight; QPTS, quantitative physiological traits; $_r$Chlb, relative Chlb; rFW, relative fresh weight; ROS, reactive oxygen species; $_r$RWC, relative RWC; $_r$TR, relative transpiration rate; RWC, relative water content; TR, transpiration rate; TR$_{max}$, maximal transpiration rate; VPD, vapour pressure deficit; WUE, water use efficiency.

Introduction

One of the promising approaches for ensuring the stability of food production involves a thorough understanding of the adaptive behavior of natural populations of the wild relatives of crops such as wheat and barley (Harlan and Zohary 1966, Nevo et al. 2012). Wild-type wheat populations in areas with a moderate amount of rainfall have been found to have relatively high levels of genetic variability, as compared to populations from xeric regions (Peleg et al. 2005). Nevo et al. (2012) showed that in an Israeli wild barley collection, the greatest allelic diversity was found in populations endemic to the Mediterranean coastal climatic zone with a rainfall range of 200–600 mm year^{-1} (Nevo et al. 1979). Both studies indicate a reduction in allelic diversity toward areas in which the level of rainfall is more predictable (either high or low; Peleg et al. 2005, 2008).

Israel, located in the western part of the Fertile Crescent, forms a land bridge between Africa, Asia and Europe and presents a broad environmental gradient, from a Mediterranean climate to a desert climate, over a small geographic area (Hübner et al. 2009). Over a distance of 250 km from north to south, the annual rainfall drops from 1500 mm to 50 mm (respectively) and the duration of the rainy season decreases in the same manner (Goldreich 2003). Dry spells during the rainy season may last for more than a month, causing the soil to dry, whereas long rainy spells may increase flood risk. This fluctuation is currently increasing in southern parts of Israel (Goldreich 2003, Ziv et al. 2013).

The degree of environmental certainty may select for various combinations of phenotypic traits and phenotypic plasticity (Shemesh et al. 2010). Based on the interplay of genetic drift, gene flow and selection, ecotype differentiation is in part a result of local adaptation strong enough to over-ride the effect of gene flow (Volis et al. 2002). Annual plants in arid regions have a preference for escape features, such as early flowering, as opposed to resilience features, such as slow growth and the postponement of the reproductive stage, which are characteristic of well-watered habitats.

Adaptation to variable environments may be accomplished by means of genetic variation, phenotypic plasticity (Schlichting and Levin 1984) or a combination of both. Although wild barley has a self-fertilization rate of about 98%, which results in a very low level of heterozygosity, the effective rate of recombination is not particularly low, and high levels of genetic diversity are maintained (Morrell et al. 2005). In addition, phenotypic plasticity is well known in this species, for example, root allocation is highly affected by water availability and flowering time can change due to a shortage of water or nutrients (Richards 1991, Volis et al. 2004). Higher levels of allelic diversity might be related to more complex water-management behavior, yet the diversity of quantitative physiological traits (QPTs) related to this uncertainty in Israeli wild barley has not been reported.

Plant risk management hypothesis

Decision-making theories in human and animals assume that the likelihood of choosing a risky option is affected by the variability of the option's possible outcomes (Weber et al. 2004). Because water and nutrient availability are both uncertain and crucial to the plant's survival, we consider the availability of those inputs to be a risk factor. In the face of a water or nutrient shortage, a plant must effectively allocate resources to maximize its resource utilization in the short term, while maintaining its long-term survival ability (Shemesh et al. 2010).

Unlike human risk-perception, which may depend on the frequency of the occurrence of events over the short term (Erev et al. 2017), plants are expected to use survival strategies that have evolved based on the real risks to which they have been exposed over long periods. Hence, we assume that a plant's risk management, as observed in common-garden experiments, would be based on the plant's adaptation to its original environment. In particular, precipitation certainty is a major factor in determining an ecotype's water-management behavior. Here, we hypothesize that the Israeli barley populations, that harbor substantial genetic variation (Hübner et al. 2009, 2013), also differ in both their stress-response plasticity and the variation spectrum of their population physiological traits (physiological polymorphism), according to their natural habitat stability. Specifically, we hypothesize that: (1) uncertain water availability (i.e. precipitation uncertainty) will result in increased diversity of water-management behaviour of an endemic ecotype and (2) ecotypes that have evolved in relatively unstable habitats exhibit conservative behavior, whereas those that have evolved in relatively stable environments display relatively risk-taking behavior.

Materials and methods

Plant material

Wild-type barley (*Hordeum vulgare ssp. spontaneum*) seeds were obtained from the original B1K collection using the single-seed descent method (Hübner et al. 2009). They were initially screened from 72 accessions originating from 35 populations of the B1K collection that represented eight different clusters based on both geographic location and genetic distance (Hübner et al. 2009). For further in-depth comparison, we characterized five accessions from the screened populations namely: Mount Meron (B1K−51−07), Mount Arbel (B1K−29−20), Oren Canyon (B1K−30−13), Bet Govrin (B1K−35−08) and Yeruham (B1K−02−10). These wild-type accessions represent five different habitats with distinct environmental conditions spread from north to south in Israel (over a distance of less than 250 km) with a wide range of annual precipitation, and also exhibit a high degree of genetic variation (Hübner et al. 2009). A six-row barley cultivar (*H. vulgare* cv. *morex*) served as the cultivated control for the wild barley.

Experimental conditions

Four experiments were conducted during the winter of 2012−2013 in two adjacent, minimally controlled greenhouses (i.e. no temperature control, natural light, prevention of excessive heat was achieved by activating fans and evaporative wet pads) at the Faculty of Agriculture, Food and Environment in Rehovot, Israel. The greenhouses are equipped with functional phenotyping gravimetric system, PlantArray 3.0 prototype (Plant-Ditech, Rehovot, Israel) with 48 weighing lysimeters [for details see Halperin et al. (2017)]. In each greenhouse, two consecutive experiments were performed during December, 2012 and January, 2013. Environmental conditions were monitored using a temperature and humidity sensor (HC2−S3−L; Rotronic, Switzerland), and with a radiation sensor (LI-COR 190; LI-COR, Lincoln, NE). The temperature range was 16.4−32.7°C, relative humidity was 16.4−83.2% and radiation was 200−1069.2 μmol s^{-1} m^{-2}. The vapour pressure deficit (VPD) was calculated according to Buck (1981) and ranged between 0.3 and 3.9 KPa. These values represent the natural fluctuation in the winter in central Israel.

Preparing plant material for the experiment

The seeds were sown in 10-ml conical trays (one seed in each cone), which were filled with wet peat-based potting medium (Tuff Substrate, kibbutz, marom golan, uziel, Israel). The trays were covered with plastic wrap and aluminum foil and kept at 4°C for 2 weeks. After the dormancy was broken, the trays were placed in a fully controlled glasshouse under short-day conditions (8/16 light/dark, 16/10°C) to encourage germination. After 3 weeks, when the plants had reached the three-leaf stage, the seedlings were transplanted into large conical trays (16 cm length and 208 ml) and those trays were moved into a warmer room (8/16 light/dark, 22/16°C) where the plants were kept for 53−58 more days.

Two weeks before the experiment start, the seedlings were moved into the greenhouses and transplanted into 3.8-l pots (31.5 × 21.5 × 13 cm). The pots were filled with a commercial growing medium (Matza Gan, Shaham, Givat-Ada, Israel), composed of (w/w) 55% peat, 20% tuff and 25% puffed coconut coir fiber, with a mixture bulk density of 0.3 g cm^{-3}. Each pot was filled with 2.8 kg potting medium. The volumetric water content of the fully drained substrate, namely the 'pot capacity', was 80% (for more details see Halperin et al. 2017). The growing medium is referred to as 'soil.' Each pot was placed on a temperature-compensated load cell, referred to as lysimeters and gravimetric system (Tadea-Huntleigh, 1042 C4, Vishay Intertechnology, Malvern, PA).

The course of the experiment

Each experiment was designed as a single block (four blocks in total) containing 45 to 46 plants in a completely randomized design. In each block, each accession was represented by 4 to 5 biological repetitions for the drought treatment and 3 biological repetitions for the well-irrigated condition (control). Each experiment was divided to 3 phases: pre-treatment, treatment and recovery. Pre-treatment, where all plants received daily night irrigation that consisted of two subsequent irrigations of 15 min each. Each plant was irrigated by two repeated cycles using a on-surface 4-l splitted dripper (Netafim, Tel Aviv, Israel) to ensure uniform water distribution in the pots at the end of the irrigation event and prior to free drainage. During the treatment period, the treated plants were not irrigated while the irrigation of the control plants continued as during the pre-treatment. For the recovery, the daily night irrigation was recommenced for all plants. The first irrigation at the recovery stage included several irrigation cycles to render the soil wet and to ensure a uniform and lateral soil water content (SWC) in the pots. Note that the organic-based growing medium (noted as soil for simplicity) rendered hydrophobic upon drying and extra wetting time is needed to resume its wettability. The irrigation contained fertilizer solution (2% v/v Super Grow 6−6−6 + 3 Hortical, Kadima, Israel).

This functional phenotyping gravimetric system was used for automated continuous measurements of several QPTs [as described in detail in the Technical Advance by Halperin et al. (2017)] during both treated and control conditions. The volumetric SWC of 28 treated pots from each experiment was recorded continuously using soil-embedded probes (EC−5 or 5ET soil sensors; Decagon Devices, Pullman, WA) in each greenhouse. All sensors were calibrated to the specific potting soil before the experiment, according to the manufacturer's instructions. The SWC of fully saturated and well-drained potting soil (peat-based) was 80%, as was reported earlier (Halperin et al. 2017).

For treated (drought stressed) condition, the screening period was divided into three phases during which a physiological profile of QPTs for each plant was measured and assembled: pre-treatment with optimal/well-irrigated condition (5 to 8 days), water deprivation when irrigation was completely stopped for 12 to 20 days until the SWC reached between 20–25% (and maintained for 4 days below its own critical soil-water content), and recovery with optimal/well-irrigation (5 days) as was during pre-treatment period. During the first 24 h of recovery, multiple irrigation cycles were used to ensure that the SWC returned to as was during the pre-treatment period. Then, the irrigation frequency was returned back to the regular schedule as was during the pre-treatment period. The purpose of the pre-treatment period was to measure the normal growth rate and transpiration of any plant, for calculations during the water deprivation period. In addition, for the untreated condition, plants from each accession were continuously grown under an optimal well-irrigated condition in parallel with all the three phases of the treated condition.

Quantitative phenotyping data

Initial plant weight was determined by weighing the plant (with the soil plug) before it was transplanted into the experimental pot (3.8-l), and then subtracting the weight of the soil plug (110 g; measured before sowing). Daily transpiration and plant weight gain were measured between 04:00 and 17:30, based on which all of the following data analysis was done according to Halperin et al. (2017).

On the morning of the last day of each experiment, the canopy of each plant was cut 1 cm above the ground, weighed to evaluate the biomass gain (FW), and finally scanned with a leaf scanner LI-COR 3100 (LI-COR) for the canopy leaf area (LA).

Midday transpiration rate (TR) was calculated as the derivative of a fourth-order polynomial fit for the weight loss measured by the lysimeter (Wallach et al. 2010) between 11:00 and 13:00 as described in Eqn 1, where W_n and W_{n-1} are the load-cell readings at time t_n and t_{n-1}, respectively. The first derivative of the measured load-cell time series was multiplied by −1, assuming that the plant's weight gain during the time used to calculate the TR is negligible and that changes in the plant water use efficiency (WUE) during treated condition are also negligible (Halperin et al. 2017).

$$\mathrm{TR}_n = -dW/dt \approx -\left(W_n - W_{n-1}\right) / \left(t_n - t_{n-1}\right) \quad (1)$$

Whole-plant transpiration (E) of each plant was calculated as the midday TR normalized to the canopy LA in order to eliminate the effect of plant size on TR (Halperin et al. 2017).

Canopy stomatal conductance (G_{SC}) was calculated as the midday TR normalized to the canopy LA and VPD as in Eqn 2, where P_{atm} is the atmospheric pressure (101.3 kPa). VPD was calculated as described in Halperin et al. (2017).

$$G_{SC} = \left(\mathrm{TR} * P_{atm}\right) / (\mathrm{LA} * \mathrm{VPD}) \quad (2)$$

WUE was calculated as the mean slope of a linear regression between cumulative plant weight and cumulative daily transpiration for each plant during the pre-treatment period (5–8 days), for seven to eight biological replicates in each block (Halperin et al. 2017).

Leaf relative water content (RWC) was measured manually as described by Sade et al. (2015). RWC was measured from three leaves (not the flag leaf) that were sampled from each plant every 2–3 days over the course of the drought treatment. Those samples were collected between 11:00–13:00 and were immediately weighed (leaf fresh weight, LFW), dipped into 5 μM $CaCl_2$ in the dark, weighed again after 6 h (leaf turgid weight, LTW) and then weighed for the third time after having been fully dried in a 60°C oven (leaf dry weight, LDW). These manual measurements represent a local evaluation of the water status of the plant carried out in addition to the automatic measurements. The calculation of leaf RWC is described in Eqn 3, where LFW is the leaf fresh weight, LTW is the leaf turgid weight and LDW is the leaf dry weight.

$$\mathrm{RWC} = (\mathrm{LFW} - \mathrm{LDW}) / (\mathrm{LTW} - \mathrm{LDW}) * 100 \quad (3)$$

To estimate the RWC of the treated plants relative to that of the untreated plants, we normalized the measurements of each treated plant to the average of the untreated plants of that same accession at the same time. The relative RWC ($_r$RWC) of Pot (i) of Accession ($\overline{X}$) on Day (d) [$_r\mathrm{RWC}\ (X_i)_d$] was calculated as described in Eqn 4, where R is the RWC; $X_{\text{untreated mean}}$ is the accession under untreated conditions.

$$_r\mathrm{RWC}\ \left(X_i\right)_d = R\left(X_i\right)_d / R\left(\overline{X}_{\text{untreated mean}}\right)_d \quad (4)$$

Chlorophyll concentration was measured using two different methods. For the first one, we used a leaf spectrometer CI–710 (CID Bio-Science, Camas, WA) at the same time when the RWC sampling (11:00–13:00, every 2 to 3 days) was done. These measurements were non-destructive and were taken on the same leaf throughout the whole experiment (leaf number four below the fully expanded flag leaf). The spectrometer was set on transmittance mode, boxcar 10, 1250 ms integration time, and an average of three measurements for each record value was used. The instrument was calibrated for chlorophyll b ($\mathrm{Chl_b}$) using untreated control plants. Based on the transmitted values received at 649 nm, the chlorophyll concentration was calculated from a chlorophyll extraction protocol with DMSO (>99.7%, Thermo Fisher Scientific, Waltham, MA). The extraction protocol included sampling three 0.6-cm discs from the leaf after measuring with the leaf spectrometer, and then storing the collected tissue at −20°C for at least 24 h (Moran 1982). Each frozen sample was then combined with 2 ml DMSO and incubated in 50°C water for 2 h (Novák et al. 2013). After incubation, the samples were moved to a dark box with ice to prevent chlorophyll degradation. The OD of 1 ml of each sample was measured at two wavelengths: 665 and 649 nm (V−1100D spectrophotometer; MRC, Tel-Aviv, Israel). For the second method, the absorbance of the extracts was measured at $\mathrm{OD_{665\,nm}}$ and $\mathrm{OD_{649\,nm}}$ using a spectrophotometer (V−1100D spectrophotometer; MRC, Tel-Aviv, Israel) and the chlorophyll concentration was calculated based on the equation described by Wellburn (1994). The correlation between the leaf spectrometer measurement data and the extracted $\mathrm{Chl_b}$ is described in Eqn 5.

$$\mathrm{Chl}_{\mathrm{b}\ (\mu g\ \mathrm{ml}^{-1})} = 3.7813e^{-0.074*(\text{leaf spectrometer at 649 nm})}, \quad R^2 = 0.6559 \tag{5}$$

To estimate the relative $\mathrm{Chl_b}$ ($\mathrm{_rChl_b}$) of the treated plants relative to that of the untreated plants, we normalized the measurements of each treated plant to the average of the untreated plants of that same accession at the same time. The $\mathrm{_rChl_b}$ of Pot (i) of Accession ($\overline{X}$) on Day (d) [$\mathrm{_rChl_b}\ (X_i)_d$] was calculated as described in Eqn 6, where C is the $\mathrm{Chl_b}$; $X_{\text{untreated mean}}$ is the accession under untreated conditions.

$$\mathrm{_rChl_b}\ \left(X_i\right)_d = C\left(X_i\right)_d / C\left(\overline{X}_{\text{untreated mean}}\right)_d \tag{6}$$

Critical soil-water content (Θ_{crt}) is defined as the SWC at which the physiology of a plant starts getting affected during stress. To define the exact stages of the initiation of the drought stress, we used a piecewise linear function (as described in Halperin et al. 2017) to find the best fit between midday TR and SWC. The function yields the maximal transpiration rate ($\mathrm{TR_{max}}$) and the critical SWC (Θ_{crt}) at which the water shortage affects the TR and the TR decreases with a slope (b). Effective drought stress for each accession was defined by the mean Θ_{crt} of all the plants of the accession in each block, using only plants that had a fitness level of $R^2 > 0.5$. Initial drought stress for each accession was defined as the first 4 days after the SWC dropped below the mean Θ_{crt}. Severe drought stress was defined as at least 5 days of drought stress at SWC below 15%.

Relative transpiration rate ($\mathrm{_rTR}$): to estimate the transpiration of the treated plants relative to that of the untreated plants, we normalized the measurements of each treated plant to the average of the untreated plants of the same accession at the same time. The $\mathrm{_rTR}$ of Pot (i) of Accession ($\overline{X}$) on Day (d) [$\mathrm{_rTR}\ (X_i)_d$] was calculated as described in Eqn 7, where T is the TR; $X_{\text{untreated mean}}$ is the accession under untreated conditions.

$$\mathrm{_rTR}\ \left(X_i\right)_d = T\left(X_i\right)_d / T\left(\overline{X}_{\text{untreated mean}}\right)_d \tag{7}$$

Recovery rate is defined as the rate at which the plant returns back to their normal daily transpiration, growth rate and other physiological activities under optimal growing conditions during revival from stress. This was calculated as a linear regression (slope ± SE) between the $\mathrm{_rTR}$ and the time (days) from the last day of drought stress through 5 days of recovery (fully irrigated). This calculation was performed only for plants that experienced severe drought stress and presented at least a 75% decrease in TR on the last day of the drought stress ($\mathrm{_rTR} < 0.25$).

Estimation of the fitness loss point: We estimated the fitness as the canopy fresh weight (FW) at the end of each experiment, and the loss of fitness (due to drought stress) as the relative fresh weight ($\mathrm{_rFW}$) of drought treated plants to the control plants of the same accession. The calculation of the fitness loss point of any accession ($\Delta_r\mathrm{FW}_X$, Eqn 8), the relative fitness, is similar to the drought-susceptibility index described by Fischer and Maurer (1978), with the exception of the subtraction of grand mean (as opposed to division in order to keep the values around 0, see Fig. 6J). In this manner, we ranked the accession populations.

The fitness loss point of any accession ($\Delta_r\mathrm{FW}_X$) was evaluated relative to the general loss-of-fitness point. The mean $\mathrm{_rFW}$ of all the barley accessions represents the mean loss of fitness under the specific experimental conditions, and was calculated by subtracting the mean $\mathrm{_rFW}$ (of all the accessions) from the $\mathrm{_rFW}$ of that accession, as described in Eqn 8.

$$\Delta_r\mathrm{FW}_X = {}_r\mathrm{FW}_X - \left(\text{mean }{}_r\mathrm{FW}_{(\text{all accessions})}\right) \tag{8}$$

Climate analysis was based on precipitation measurements from meteorological stations located near the locations at which the five accessions were collected. The data were taken from the Israeli meteorological service (data available from http://data.gov.il/ims), with documentation from 1940 through 2013. Not all the five sites had documentation that was continuous or started in the same year, so we analyzed data from several nearby sites. Partially documented data (data for only part of the season) were removed from our database. The total number of seasons included in the statistical analysis was 59 for Yeruham, 61 for Mt. Meron, 66 for Oren Canyon, 68 for Bet Govrin and 82 for Mt. Arbel. A season is defined as the time period between the first effective rain event (for barley germination, an accumulation of at least 5 mm) at August, after the summer through May when the barley is dry and rain is no longer useful to the crop (Goldreich 2003). A rain event was taken into consideration only if at least 0.1 mm of rain were recorded. Drought duration is the time (days) between two rain events. Seasonal accumulated rainfall and the duration of the rainy season were calculated on a multi-year basis; multi-year average drought duration was calculated based on each year's mean and SD.

Statistical analyses

A factorial (B1K accession × irrigation regime) block design with four replicates was applied. Data from the gravimetric system, greenhouse probes and soil probes were organized and subjected to a preliminary analysis performed using MatLab 12 (MathWorks, Natick, MA). Each block consisted of two main plots (for two irrigation treatments), with each main plot split into three well-irrigated plants and four to five treated plants for each accession. The grand mean was calculated as the mean of all accession replicates in each block over four blocks; days of the experiment were considered as technical repetitions (during drought).

Means and coefficients of variation (CV) were calculated for single-day measurements for all biological repetitions of each accession during the drought treatment (different days for each plant), as described in Schlichting and Levin (1984). The mean values of traits were calculated for repeated technical repetitions of the plant over 4 days for each accession in each block. A grand mean was calculated for each accession for all four blocks. The index of CV (iCV) was calculated as the ratio between the treatment CV and the non/pre-treatment CV of a parameter (Fridman 2015). iCV was calculated for each accession in a block and a grand mean was then calculated for each accession across all blocks. The calculation for an accession (x) over four blocks is described in Eqn 9.

$$\text{iCV}\left(_{\text{X}}\right) = \text{Mean treatment CV}_{\left(\text{X}\left(\text{blocks } 1-4\right)\right)} / \text{Mean pre-treatment CV}_{\left(\text{X}\left(\text{blocks } 1-4\right)\right)} \quad (9)$$

Exceptional measurement data that had been affected by extreme weather, including cloudy days with radiation lower than 200 μE m^{-2} s^{-1} at midday, or by electronic noise were removed from the database.

ANOVA was used to examine the differences between the accessions (one-way) and the effect of drought stress compared to well-irrigation (two-way) in plants. Differences between the least squares means were examined using Tukey's (HSD) test, and the values were considered significantly different at $P < 0.05$. These statistical analyses were performed using JMP 10 Pro (SAS Institute Inc., Cary, NC). Multiple linear regressions with a dummy variable were used to examine differences between regression coefficients (slope) and Student's *t*-tests were performed for every two lines. Differences were considered significant at $P < 0.05$ and the data were analyzed with STATA 11 (StataCrop, College Station, TX).

Results

Habitat risk characteristics

Rainfall was found to be randomly distributed in space and discharged as rain on a localized area in the southernmost, arid part of Israel (Sharon 1972). Many studies have shown a negative correlation between the rainfall-variation and mean annual rainfall in deserts and semi-arid regions. However, it cannot be generalized that desert precipitation is more variable than precipitation in the non-deserts, and therefore emphasize the importance of measuring variability for each study site of interest (Davidowitz 2002). Rain amounts decrease significantly as one move from north to south and from west to east in Israel (Fig. 1A). The multi-year average precipitation in the Mt. Meron area (north) is 9.6 times higher than that seen in the Yeruham area (south); those values are 876 and 91 mm, respectively. The minimum amount of accumulated rainfall at Mt. Meron is 1.8 times the maximum amount in Yeruham. Multi-year average rainfall accumulation was 633 mm in Oren Canyon, 465 mm at Mt. Arbel and 391 mm at Bet Govrin.

The length of the rainy season also decreases from north to south. Yet, significant differences were found only between Yeruham (5.2 months) and the rest of the habitats (6.6–6.9 months; Fig. 1B). Drought duration (rain dispersal) is described by the mean duration of dry periods between two consecutive rain events during the rainy season. Drought duration figures follow the same pattern and increase from north to south (Fig. 1C). Yeruham had significantly longer average drought duration (10.9 days) than all other habitats. In contrast, the shortest drought duration (7.2 days) was observed at Mt. Meron and that figure is significantly different from that observed for Bet Govrin (8.5 days) and Yeruham (10.9 days). One way to estimate the stability of rainfall patterns is by using the CV. Ben-Gai et al. (1994) showed that, in Israel, the CV of seasonal rain accumulation

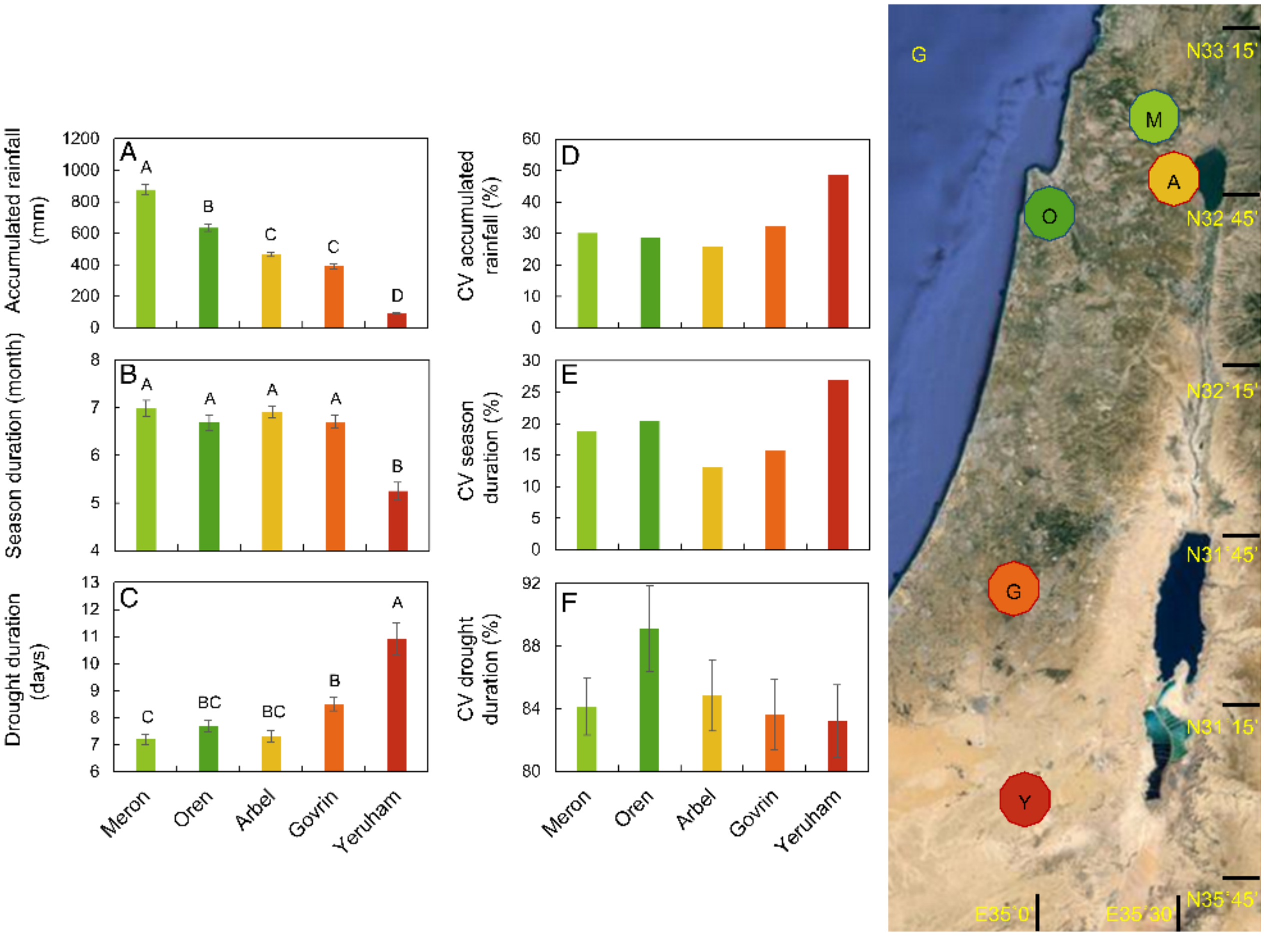

Fig. 1. Rainfall patterns in the native habitats of the five wild barley accessions. In Israel, the natural barley growing season is the winter. The season starts at the first rainfall event of more than 5 mm (accumulated) after the summer (August) and ends in May. Using meteorological data collected in the different native habitats, we calculated the (A) seasonal mean amount of accumulated rainfall, (B) the mean duration of the rainy season and (C) the mean duration of drought stress between consecutive rain events involving accumulation of more than 0.1 mm. To estimate the multi-year stability of these parameters, we calculated the (D) CV of the accumulated rainfall, (E) the CV of the length of the rainy season (both were calculated on a multi-year basis) and (F) the mean CV of duration of drought stress between consecutive rain events (multi-year average was calculated based on each year's mean). (G) A satellite image of Israel showing the geographic location of the native habitats of the five ecotypes. Presented data are means ± SE; letters indicate significant differences as determined using Tukey's HSD test ($P \leq 0.05$). Means were calculated for 62 seasons at Mt. Meron (M), 73 seasons at Oren Canyon (O), 60 seasons at Mt. Arbel (A) and 63 seasons in Bet Govrin (G) and Yeruham (Y).

decreases sharply as one moves toward the desert. Calculating the stability of seasonal precipitation revealed that the Yeruham area has the least stable seasonal precipitation with a CV of 48.6% for average seasonal rain accumulation, as compared to the more stable precipitation figures of the more northern habitats with Mediterranean climates (i.e. a CV of 25.7% for Mt. Arbel and a CV of 32.3% for Bet Govrin; Fig. 1D). The CV of the average length of the rainy season (Fig. 1E) had a similar pattern, in which the length of the rainy season in Yeruham was the most variable (with a CV of 26.9%) and the length of the rainy season at Mt. Arbel was the least variable (with a CV of 13.1%). Nevertheless, to estimate the variability within a season (i.e. the certainty of the next rain event), we looked at the CV of drought duration (Fig. 1F). Here, we found overall greater variability (high CV values): Yeruham with the least variability (83.1%) and Oren with a CV of 89.1%. The differences between the CV values for the different areas were not statistically significant.

Physiological characteristics of barley

We defined the risk-management strategy of each accession under uncertain water-availability conditions based on the physiological characteristics (phenotypes) measured under well-irrigated and drought stressed conditions. Our initial screening included the examination of few QPTs under well-irrigated conditions in

72 accessions of 35 *Hordeum spontaneum* populations collected from eight different locations. The screening revealed a wide range of frequency of these QPTs between all accessions and suggested a high degree of polymorphism (Fig. S1). To simplify our experimental approach and to increase the number of biological repetitions (in order to increase the statistical power of our analysis) we proceeded with only five wild-type barley accessions which represented five distinct sets of environmental conditions, along with one cultivated barley variety as control (Hübner et al. 2013).

We compared several QPTs among plants of these six accessions grown together under optimal conditions. There were no significant differences among the average plant weights for the six accessions (Fig. 2A). However, concentrations of Chl_b were significantly lower in the leaves of the southern accessions (Fig. 2B). Comparing the WUE of the different accessions revealed that the cultivated line, Morex, had the highest WUE of 0.058 g ml^{-1} (i.e. 5.8% biomass gain per ml transpired) and the Oren accession had the lowest WUE of 3.7%. Despite the geographic distance between the native habitats of the different accessions and the distinct annual rainfall patterns of those habitats (Hübner et al. 2009), the Mt. Meron and Yeruham accessions showed similar WUE values of 4 and 4.3%, respectively (Fig. 2C).

Comparing changes in the transpiration of different plants over a period of drought stress is challenging since plants that transpire more will use up the available water in the soil more quickly and will face earlier water shortages than plants that transpire less (Fig. S2). Thus, it seemed more appropriate to examine midday TR values relative to SWC instead of time, as those values have particular physiological relevance when the transpiration regulation of different plants is compared.

Examination of the whole-plant transpiration response to changes in SWC revealed a pattern of high TR under the given ambient conditions and a sharp decrease in TR at a critical SWC point (Fig. 3A). This critical SWC value was reported by Halperin et al. (2017) as the Θ_{crt}. Note that, the Mt. Meron, Yeruham and Bet Govrin accessions even increased their TR before reaching their soil-water limitations. Beyond Θ_{crt}, SWC acts as a limiting factor for plant transpiration, causing it to decrease in a linear fashion (we refer to this slope as 'b'). The grand mean values of all four biological repetitions revealed differences in TR between the wild-type and cultivated barley varieties (Table S1). Moreover, comparisons of TR values reflect the definite (absolute) water loss and the differences between plants' water-management strategies under given conditions. This phenotype might also be influenced by plant size. Nevertheless, measuring the whole-plant transpiration (E; TR normalized to the canopy LA) and canopy stomatal conductance (G_{SC}; TR normalized to the canopy LA and VPD) revealed a similar pattern. The only difference was the smaller G_{SC} of the Oren accession, which is a result of its larger canopy LA (Fig. 4).

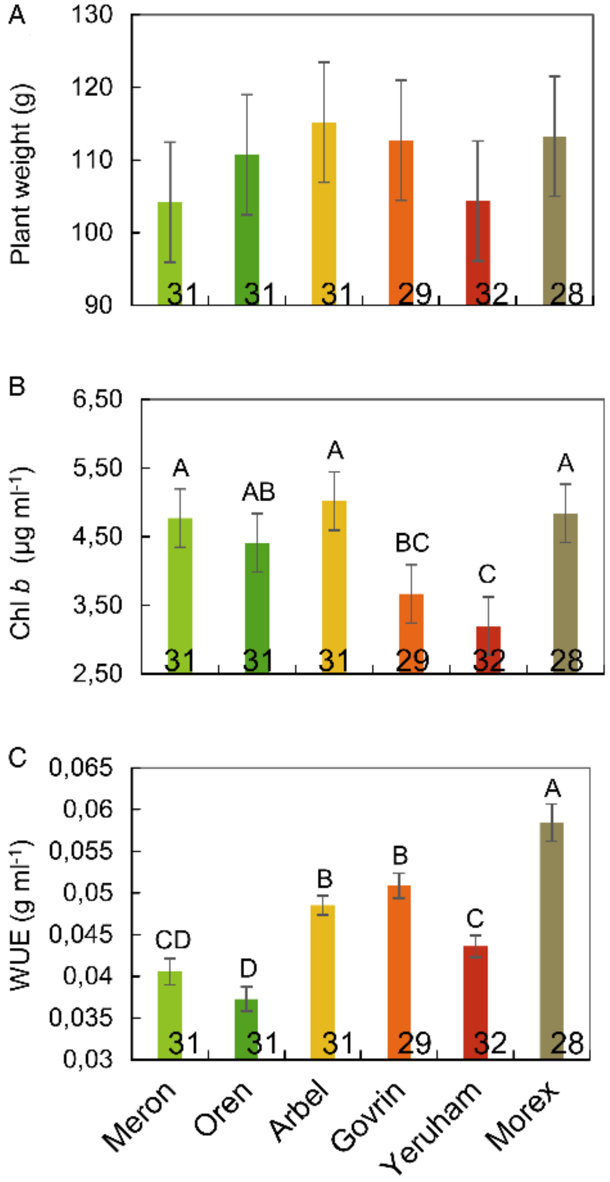

Fig. 2. Physiological characteristics of wild barley grown for 74–79 days under optimal/ well-irrigated (Pre-treated) conditions. (A) Mean plant weight, (B) mean Chl_b concentration (leaf 4) and (C) mean water-use efficiency (see section Materials and methods). All of the presented data are means of four blocks. For (A) and (B), presented data are least square means ± SE; letters indicate significant differences as determined using Tukey's HSD test ($P \leq 0.05$). For (C), all linear regressions (see section Materials and methods) are significant ($P < 0.001$); letters indicate significant differences as determined using Student's *t*-test ($P \leq 0.05$). The number of biological repetitions is shown at the bottom of each column.

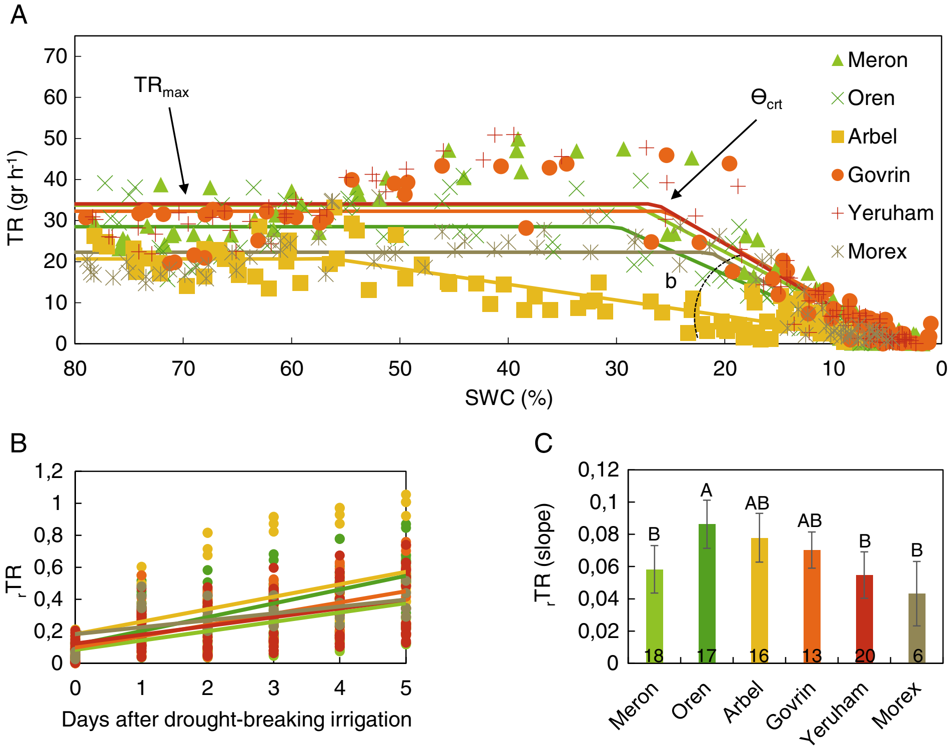

Fig. 3. Midday whole-plant TR as a function of volumetric SWC over the pre-treatment (well-irrigated) and drought stressed periods of the experiment (same plants and experimental condition as in Fig. 4) and the rate of recovery following drought-breaking irrigation (recovery start). (A) A piecewise linear model describes the transpiration rate before and after the Θ_{cr} of each accession in Block 3. The linear model fit the data for all biological repetitions with an R^2 value greater than 0.5. There were four repetitions for Bet Govrin and Morex and five repetitions for Meron, Oren, Arbel and Yeruham. The grand means of the blocks and repetitions are presented in Table S1 in Appendix S1. (B) $_r$TR during the last day of the drought stress (day 0) and the 5 days of subsequent irrigation. (C) The recovery rate linear regression (slope ± SE) calculated with a dummy variable. The same color-coding is used to represent the different barley lines in each graph. All linear regressions are significant ($P < 0.001$). Letters indicate significant differences as determined using Student's *t*-test ($P \leq 0.05$). The number of biological repetitions is shown at the bottom of each column.

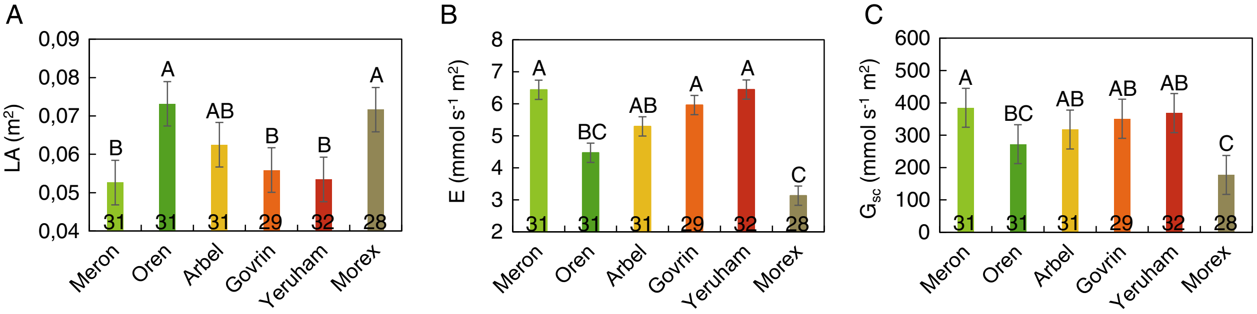

Fig. 4. Physiological characteristics of wild barley accessions grown for 74–79 days under well-irrigated (untreated) conditions. (A) Canopy LA, (B) whole-plant transpiration (E) and (C) whole-canopy stomatal conductance (Gs_C). Presented data are least squares means ± SE. Different letters indicate significant differences as determined using Tukey's HSD test ($P \leq 0.05$) for the four blocks. The total number of biological repetitions is shown at the bottom of each column.

Recovery from drought stress

Recovery characteristics were calculated using only plants that experienced severe drought stress (i.e. more than 5 days under Θ_{crt}, SWC of less than 15% and a reduction in transpiration of at least 75%). The faster the TR of the treated plant returned to the non-treatment level (higher $_r$TR), the faster the rate of recovery was. Fig. 3B shows that Arbel plants displayed a maximum $_r$TR of 1.05 within only 5 days of recovery. The maximum $_r$TR for other varieties in decreasing order is as follows: Oren accession with 0.87, Bet Govrin accession with 0.75, Yeruham accession with 0.74 and Mt. Meron accession with the maximum $_r$TR of 0.67. The cv. Morex had the lowest maximum $_r$TR of 0.51. We calculated the recovery rate (Fig. 3C) on the basis of the relation between $_r$TR and time, from the last day of treatment (day 0) through 5 days after recovery. The recovery rates, calculated as the linear regression line slope of $_r$TR over 5 days after recovery revealed that, the Oren Canyon accession had the fastest recovery rate of 0.08 $_r$TR/d, which was significantly different from that observed for Mt. Meron, Yeruham and cv. Morex accessions. The Mt. Arbel and Bet Govrin accessions showed an intermediate recovery rate of 0.07 $_r$TR/d.

Plasticity of QPTs

Since higher CV values indicate greater plasticity (Fridman 2015) and vice versa, CV can serve as a tool for the standardized comparison of traits' 'norm of reaction' values, i.e. to measure QPT-plasticity (Dalal et al. 2017). The CV of TR (CV_{TR}) was calculated for each accession under optimal conditions (i.e. 4 days before Θ_{crt}) and under initial drought stress (4 days after Θ_{crt}). CV_{TR} almost doubled under drought stress for the Mt. Arbel and Morex plants (reflected in their high CV_{TR} values of 1.9 and 1.7, respectively); whereas the Mt. Meron and Yeruham accessions maintained low CV or low iCV_{TR} values of 1.02 and 1.3, respectively.

iCV_{TR}, which serves as an estimation of the transpiration stability of an accession, revealed a different trend. We noted a trend between iCV_{TR} and seasonal rainfall according to which the accessions from less stable environments (higher CV of drought duration) had higher iCV_{TR} values than the accessions from the more stable regions (areas that were more consistently wet and areas that were more consistently dry; Fig. 5).

These comparative measurements between the accessions revealed how canalized the transpiration trait is (based on iCV) in each accession. Yet, they did not reveal the plasticity of the trait, which may have an important role in the plant risk-response behavior. In order to better understand the trait plasticity, we looked at the $_r$TR values of all the varieties at the same time point. Hence, during initial drought stress, Morex and the Mt. Arbel accession had relatively high $_r$TR of 0.62 and 0.52, respectively (lower trait plasticity), as compared to the accessions from Yeruham and Meron which had $_r$TR values of 0.35 and 0.41, respectively (higher trait plasticity; Fig. S3A). However, there was no significant difference among the ecotypes. Thus, the lower the plant's $_r$TR, the higher the plasticity of that trait and the higher is its risk-behavior estimation.

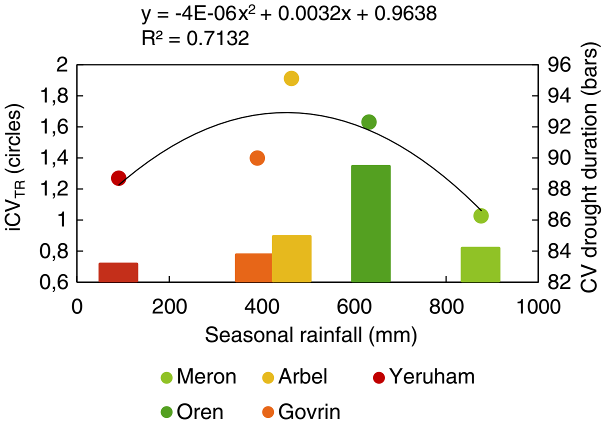

Fig. 5. Barley transpiration polymorphism (iCV_{TR}, circles) in relation to mean seasonal rainfall (mm) and the predictability of the duration of dry spells duration in the endemic habitats of the wild barley (bars, based on Fig. 1 and Table S1). For the five accessions, the relationship between iCV_{TR} and mean rainfall is described by a second-order polynomic function ($P < 0.3$).

Leaf RWC, which represents the critical water level of the plant and exhibits a very narrow range of variation, as compared to other QPTs, (Bartlett et al. 2012, Sade et al. 2012, Dalal et al. 2017), was used as another indicator of plant risk-behavior. An examination of relative RWC ($_r$RWC, calculated in the same way as $_r$TR) showed that the cv. Morex plants had a high $_r$RWC of 0.95 (lower risk), which was significantly different than the $_r$RWC of 0.82 observed for the Oren and Yeruham ecotypes. With a common $_r$RWC of 0.86, the Mt. Meron, Mt. Arbel and Bet Govrin accessions were not significantly different from the other ones (Fig. S3B).

The degradation of chlorophyll represents a biochemical response of the plant to the stress that is hardly reversible (compared with TR or RWC), and thus can be used as an additional way to assess plant risk-response. Analysis of the $_r$Chl$_b$ concentration ($_r$Chl$_b$, calculated in the same way as $_r$TR) revealed higher levels of chlorophyll degradation in the southern accessions (Fig. S3C). The Yeruham and Bet Govrin accessions were the only two accessions with $_r$Chl$_b$ values lower than 0.75, with $_r$Chl$_b$ values of 0.6 and 0.63, respectively. However, there was no significant difference among the accessions.

Under severe drought stress (i.e. more than 5 days below Θ_{crt} and SWC less than 15% till the last day of treatment), the TR dropped approximately 90% compared to the non-treatment (Fig. S3D). The cv. Morex and the Mt. Arbel accession maintained their conservative behavior, showing the highest $_r$TR levels (low plasticity). However, there was no significant difference among the accessions. Interestingly and unlike what was

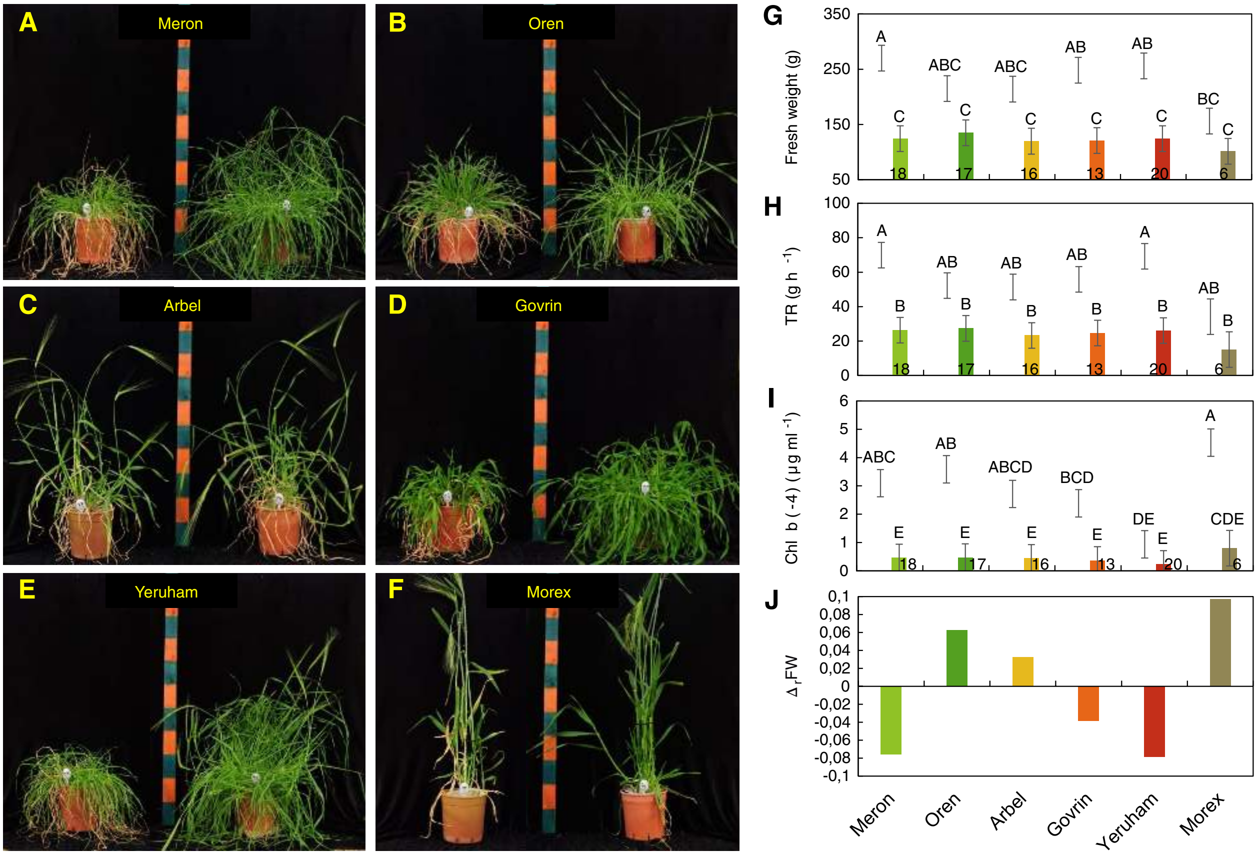

Fig. 6. The effect of drought stress on wild barley phenotypes and fitness. (A-F) Representative images of 106-days old drought-stressed plants (10 days after recovery; on the left) and well-irrigated plants (on the right). (A) Meron, (B) Oren, (C) Arbel, (D) Bet Govrin, (E) Yeruham and (F) cv. Morex. The measuring stick in each photo is divided into 11 sections that are each 10 cm long. Physiological characteristics of treated and untreated plants on the last day of the experiments: (G) canopy FW, (H) mid-day TR, (I) Chl_b concentration and (J) relative fitness of wild barley in response to drought stress. The solid bars represent non-treatments and striped bars represent treatments. Presented data are least square means ± SE. Letters indicate significant differences ($P \leq 0.05$) in a two-way ANOVA model, including the factors, lines, treatments and their interaction; 'experiment' was considered a random effect. The model was applied for all four blocks of the wild-type varieties (there were two blocks for cv. Morex, due to its low numbers of severely stressed plants). The total number of biological repetitions is shown at the bottom of each column; all untreated plants had three repetitions in each block.

observed for initial drought stress, the $_{r}$RWC value of the Morex accession during severe drought stress was significantly different than those of the Mt. Meron, Mt. Arbel and Bet Govrin accessions (Fig. S3E), but not significantly different than the values observed for the Oren Canyon and Yeruham accessions. 'Morex' maintained low chlorophyll plasticity as well (compared with initial stress) with high $_{r}Chl_b$ (Fig. S3F). However, the wild barley lines showed substantial plasticity as compared to their response to the initial stress, with $_{r}Chl_b$ values between 0.29 and 0.13, though there was no significant difference among the accessions.

Assuming that all of the physiological phenotypes described so far are related to the plant's fitness in its natural environment, we looked for a way to assess the impact of drought stress on fitness. Visually, the impact of the stress was clear when we compared untreated plants with treated plants (plants under severe drought stress recovered over 10 days; Fig. 6A–F). While the most severe, visible differences were seen among the Mt. Meron, Bet Govrin and Yeruham accessions, greater similarity between the treated and untreated plants were observed among the other accessions. These differences were shown to be statistically significant when QPTs such as FW, TR and Chl_b concentration were measured (Fig. 6G–I, respectively). For all of those characteristics, no significant differences were observed among the treated plants. When we compared treated with untreated plants for each accession, we found that the Mt. Meron, Bet Govrin and Yeruham accessions had significantly lower FW (Fig. 6G), Meron and Yeruham were the only accessions with significantly lower TR (Fig. 6H) and only Yeruham did not have any significant differences in Chl_b concentration (Fig. 6I).

To summarize and rank the specific observations as a 'modified fitness index' (see section Materials and methods), we used the mean ratio between treated FW and untreated FW (mean of all lines ratio). This enabled easier comparison of the effect of drought stress as part of the accession potential, which we consider as the relative (to optimal conditions) fitness (Δ_rFW) in our experiments (Fig. 6J). In this index, the mean value ('0' value) is the 'loss point,' with accessions with values above that point (cv. Morex, Oren Canyon and Mt. Arbel) showing less biomass loss than the average of all accessions, in response to the stress described in these experiments. The Bet Govrin, Mt. Meron and Yeruham accessions each had a ratio smaller than the mean ratio (i.e. losses greater than the average), indicating that they are relatively less adjusted to the stress examined in this study. It is possible that other stress scenarios might induce different behaviors.

Discussion

In general, our results support the basic postulation of this study that the stability of environmental conditions affects the variation of plant physiological traits, and that uncertain water availability (i.e. precipitation uncertainty) is positively correlated with increased diversity of water-management behavior of an endemic accession.

Variation of TR, plasticity and stability

Comparison of the QPTs of all the barley ecotypes from eight different geographic locations (Hübner et al. 2009) under well-irrigated (untreated) conditions revealed wide ranges of basic physiological characteristics (Fig. S1). This phenotypic variation, which corresponds to the natural variation in climate across different regions (Fig. 1), supports our general hypothesis. The Israeli B1K habitat is distributed over a relatively small geographic area (less than 22 000 km^2). To gain a better understanding of the interplay between physiological phenotypic plasticity and environmental stability, we decided to focus on five accessions from distinct environments, which are characterized by differences in rainfall patterns (Fig. 1).

To estimate the stability of each environmental parameter, we chose to work with CV, which is a unit-less value that can be used as a proxy for standardized comparison and prediction of environmental risk factors, as opposed to variance or standard deviation. We chose to focus on midday TR as a phenotypic trait that represents a plant's behavioral response to the environment. This decision was made due to the rapid response (sensitivity) of TR to environmental conditions. Moreover, TR is a non-normalized characteristic (unlike E or G_{SC}, which normalize TR to morphological and environmental conditions) and so represents a genotype-integrated behavioral response (which is the combined outcome of developmental and physiological characteristics such as leaf and root size, chlorophyll content, stomatal density, cuticle thickness, stomatal aperture, hydraulic conductance, etc.). TR also takes into account the residual transpiration where both leaf osmolality and the amount of total cuticular wax are involved in controlling cuticular water loss from barley leaves under well irrigated conditions (Hasanuzzaman et al. 2017). Any normalization of the absolute water loss will not be objective, as we do not know the relative contribution of the different parameters to the whole-plant behavioral response. In addition, it is relatively easy to accurately measure TR for a large number of plants simultaneously.

Our challenge was to quantitatively define drought stress that would resemble the plants' objective responses to similar stress scenarios in the wild. From a physiological point of view, drought stress starts at the point at which soil-water availability begins to limit a plant's ability to reach its full growth and productivity potential. This point is very hard to define as the plant response to stress is comprised of multiple biological processes, each with its own sensitivity threshold and unique characteristics. Therefore, we set the drought stress start point (Θ_{crt}; Fig. 3A) based on the plant's physiological response to the drought stress rather than a time scale (based on Halperin et al. 2017). This clear separation between the non-treatment and treatment allowed us to evaluate the diversity, stability and plasticity of the plants' responses.

Interestingly, under unstressed conditions, both TR and its CV (CV_{TR}) were similar across all of the B1K accessions. Yet, while the CV_{TR} of some accessions increased under drought stress, other accessions exhibited similar CV_{TR} figures across both conditions (i.e. optimal/ well-irrigation and drought stress; Table S1). If we consider CV as an adaptive trait in the face of environmental change, then non-substantial changes in CV values in response to stress (i.e. iCV approximately 1) suggest high stability of a trait, which is considered as a characteristic of environmental canalization (Fridman 2015). However, canalized traits might represent risk-averse behavior and vice versa.

Our examination of the relation between the iCV_{TR} values of the different accessions and the stability of their native environments revealed an interesting trend. Accessions from intermediate environments (i.e.

intermediate annual rainfall at high certainty) such as Mt. Arbel, exhibited greater phenotypic variation than accessions from locations characterized by extreme conditions, such as Mt. Meron and Yeruham (i.e. large amount of rain at high certainty or low amount of rain with high certainty, respectively; Fig. 5).

Our polynomial model describes a phenomenon in which high phenotypic variation is associated with intermediate amounts of rainfall. This relation explains 71.3% of the phenotypic variation (Fig. 5). If we assume that higher phenotypic variation corresponds to greater genetic polymorphism, this could be similar to the relation between genetic polymorphism of wild barley populations and annual rainfall as described by Nevo et al. (1979), or to the relation between allelic diversity of wild emmer wheat and average annual rainfall as described by Peleg et al. (2008). These studies suggest that populations endemic to intermediate and more variable environments maintain higher levels of polymorphism than populations that are native to habitats that are extreme, yet more predictable.

Here we present a novel functional approach in assuming that high level of genetic polymorphism is indicative of a conservative risk-taking strategy for coping with environmental uncertainty. It describes the translation of genetic polymorphism into behavioral variation. In addition, our measurements conducted on single-seed-descended plants from each accession, suggest that at least some of the observed behavioral variation could be maintained and transferred via self-fertilization. Moreover, we found that the high polymorphism (iCV) of TR in an accession is inversely correlated to this QPT-plasticity (${}_{r}$TR; Fig. 7A). This relation outlines a trade-off between the plasticity of TR and the whole-accession polymorphism, explaining 68.9% of the polymorphism variance at a significance level of 90% ($P < 0.1$). It is important to note that, in this comparative presentation, the location of the accession on the regression line defines the level of risk-taking and relative to the other plants under the same set of experimental conditions. These results raise questions whether ${}_{r}$TR and iCV_{TR} are indicative of the optimal risk-taking behavior of certain genotypes under certain environmental conditions, as well as questions about the optimal risk-management behavior for survival in particular environments.

What is the optimal model for risk-management behavior?

The prospect theory of human economic behavior and the energy-budget rule (a special case of risk-sensitivity theory in animals) predict risk-aversion when animals are not in danger of starvation (domain of gains), but risk-tolerance when there is a risk of starvation (domain of losses; Weber et al. 2004). However, it is likely that, in plants, this trait is less dynamic than in animals. This might be due to the effect of local microenvironment on plant behavior, and therefore risk assessment in plants should be considered from an evolutionary perspective.

The fitness of annual plants is determined by their total reproductive output and the quality of their offspring (Aarssen and Taylor 1992, Volis et al. 2004). Assuming that optimal risk-taking behavior of a plant will lead to optimal fitness under predictable conditions, we wanted to get a good estimation of the relative benefit or loss associated with a certain strategy in a particular environment. Taking plant biomass as an indicator of fitness (Sultan 2000), we calculated the fitness index (Δ_rFW; Fig. 6J) of each accession. Similar indices could be calculated for other physiological parameters (e.g. TR). Thus, the relative benefit of different strategies is defined as the point at which the regression line intersects zero on the fitness-index (Δ_rFW) axis (Fig. 7A).

In this study, the Mt. Arbel accession and cv. Morex exhibited a conservative approach of minimal TR plasticity and maximal phenotypic polymorphism, which endowed them with beneficial plasticity. This behavior might be worthwhile under conditions of relatively lower and less certain water availability, but will result in relatively lower yields under predictable and abundant water available conditions, when compared to the more risk-taking behaviour. Risk-taking behavior was observed for the Mt. Meron and Yeruham accessions, which had high levels of phenotypic plasticity and minimal phenotypic variation (i.e. canalization of the risk-taking behaviour within the accession). This behavior of these plants could also be seen in their increasing TR despite a gradual decrease in SWC (Fig. 3A).

The advantage of this behavior for the Mt. Meron accession is clear, as it is native to a high-rain, high-certainty environment. Nevertheless, it will suffer major losses under conditions of prolonged drought stress, with a slow reconciling of levels of Chl_b and a slow rate of recovery (Figs S3 and 3B,C, respectively). The Yeruham accession, on the other hand, comes from a dryer and shorter season. Thus, we suggest that its canalized high risk-taking behavior is a type of ‘drought escape’ and is correlated with early flowering regardless of stress conditions (reviewed by Negin et al. 2016). Yeruham is an early flowering ecotype (Volis 2011, Hübner et al. 2013) and exhibits a slow recovery rate (Fig. 3B, C). Moreover, Yeruham’s rapid chlorophyll degradation while maintaining relatively high RWC under prolonged stress (Fig. S3) are types of

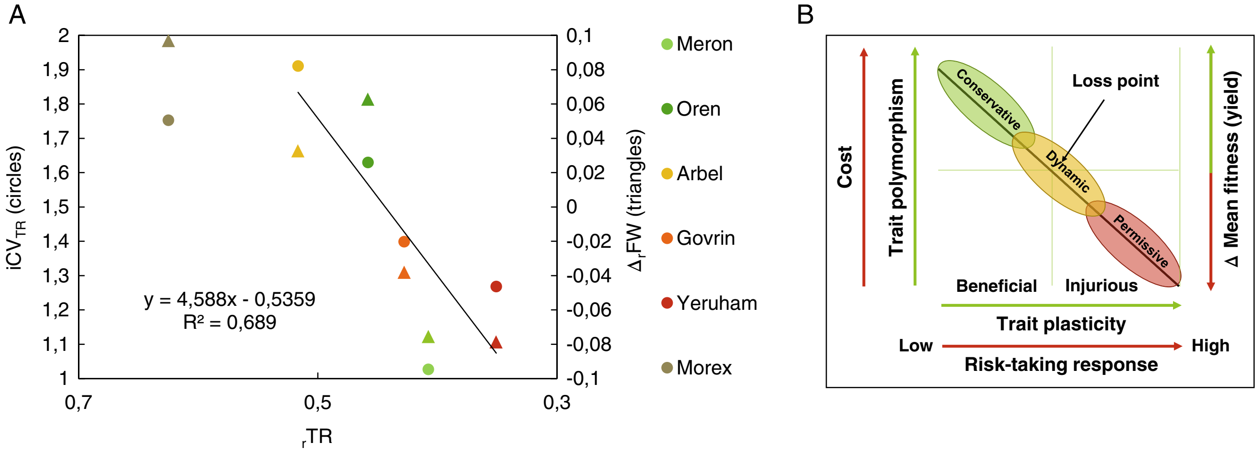

Fig. 7. (A) Transpiration polymorphism (iCV$_{TR,}$ circles) and relative fitness of barley accessions (Δ$_r$FW, triangles) in $_r$TR in response to drought stress. Data are based on Table S1 in Appendix S1, Figs 6J, Fig. S3A in Appendix S1 (respectively). The regression line describes the relation between iCV$_{TR}$ and $_r$TR for the five wild barley accessions, with a significance level of 90% ($P < 0.1$). (B) Our portrayal of a general risk-taking model for water management in plants. A physiological parameter (transpiration rate) of a phenotypic polymorphism (canalization) as a function of its plasticity (reaction norm) in reaction to stress (drought), and the relative effect on its fitness (yield or biomass). As single-plant trait plasticity increases, the accession polymorphism decreases, in accordance with increases in relative fitness and risk levels up to the loss point. Past the loss point, the risk continues to increase and relative fitness decreases. The loss point for each barley line was validated only for a specific set of experimental conditions and only relative to the other examined plants. The overall range of plasticity is divided by the loss point into beneficial and injurious sub-ranges of greater and lower fitness than the average, respectively. Taking a risk might cause a loss of fitness under specific experimental conditions. The combination of the three axes defines the risk-taking level of the plants as conservative, dynamic or permissive and also defines its compatibility with the experimental conditions. High productivity carries high risks.

escape behaviour, assumed to facilitate the translocation of nutrients from the senescent leaves to the grains. An additional explanation might be that the Yeruham ecotype does not reflect adaptation to a general desert climate, but rather to the microclimate found along small desert streams (Wadis), which is a moderate climate with greater water availability. Even if this is the case, we still see other parameters for Yeruham adaptation such as low chlorophyll concentration (Fig. 2B) and rapid chlorophyll degradation due to drought stress (Fig. S3C), the maintenance of high RWC through severe drought stress (Fig. S3E) and a relatively short flowering time (Hübner et al. 2009, 2013). The Oren Canyon and Bet Govrin accessions had intermediate $_r$TR and iCV$_{TR}$ values. Oren, with its more conservative behavior, manages to maintain relative higher fitness and a faster recovery rate; whereas Bet Govrin, with its more permissive behavior, exhibits a level of fitness that is below average.

Cost-effectiveness of polymorphism

The plasticity of TR has a major role in controlling the plant's 'potential losses.' A relatively high TR at Θ_{crt} is considered a risky behavior that may lead to high productivity (in case of immediate water refilling), that is beneficial plasticity. However, it may also lead to plant desiccation in case of terminal drought stress (i.e. harmful plasticity). In this manner, relative TR values were used to estimate TR plasticity (similar to a reaction-norm slope) or the 'risk-taking' behavior of the plants ($_r$TR). As long as the plant shows good recovery rates, the lower the $_r$TR, the higher is its plasticity and 'risk-taking' behaviour. Interestingly, the $_r$TR values showed a similar pattern as the iCV$_{TR}$ values. The Mt. Arbel accession had the lowest levels of TR plasticity (very similar to that of the control cultivated variety); whereas the Mt. Meron and Yeruham accessions had the highest levels of TR plasticity (unlike the control cultivated variety; Fig. S3A). Maintaining high phenotypic polymorphism may increase an ecotype's chances of survival in an uncertain environment at the cost of relatively low production, as described in Figs 6G and 7A.

Plasticity requires the maintenance of sensory and regulatory mechanisms, which may entail an energetic or genetic burden that can prevent the plant from reaching its full production potential (DeWitt et al. 1998). On the other hand, canalized ecotypes with less phenotypic polymorphism and greater phenotypic plasticity allocate all of their resources to biomass production at the risk of withering before the end of the season. In this scenario, their risk-taking behaviour is considered relatively harmful plasticity (Alpert and Simms 2002, Sade et al. 2012). This behavior is characteristic of cultivars that have undergone phenotypic selection in an optimal growing environment (irrigation, fertilization and pest control) for traits such as yield, as opposed to stress resistance. This domestication is expected to diminish genetic diversity and reduce fitness in natural environments (Warschefsky et al. 2014). Interestingly, the cultivated barley, cv. Morex, which was developed for specific traits such as malt extract, alpha amylase and grain protein for the malt industry in Minnesota, USA (Rasmusson and Phillips 1997), exhibits exceptional non-risk taking behaviour and relatively low biomass loss due to stress. It is challenging to indicate the reason for this exceptional behaviour and it is likely that few mechanisms are involved. Morex was also reported as relatively tolerant to salinity stress and it was suggested that proteins responsible to detoxification of reactive oxygen species (ROS) were more abundant in it compared with another more sensitive Barley cultivar (Witzel et al. 2009). This anti-ROS regulation mechanism, together with lower transpiration but wider LA and higher WUE may explain, at least partially, a tolerance mechanism.

Conclusions

Climate change and global warming are resulting in increasing water shortages for agriculture, which likely reduces crop growth and production (Yoo et al. 2009, Nevo et al. 2012). While crop breeding has led to the development of high-yielding cultivars, progress toward the development of abiotic stress tolerant crops has been very slow. Thus, the need for decreasing the ‘yield gap’ and increasing yield certainty under different stress conditions is of strategic importance for assuring food security (Araus et al. 2002, Cattivelli et al. 2008, Sadras and Richards 2014). Our results describe the relation between the phenotypic plasticity (i.e. reaction norm) of the yield-related quantitative physiological trait, phenotypic polymorphism (canalization) and relative fitness (e.g. biomass) in response to stress (Fig. 7A, B). We suggest that this model can be developed to a new breeding tool, enabling a quantitative approach for measuring the G × E plasticity of yield-related physiological traits. This tool can be integrated into breeding programs, to assess the risk behavior associated with a particular trait in the presence of a specific selection factor (e.g. disease, limited availability of water and/or nutrients, etc.).

Author contributions

E.G. and M.M. conceived the idea, designed methodologies and developed the models; E.G. performed most of the experiments, while A.D. with A.E. performed part of the physiological phenotyping experiments; E.G. and M.M. performed data analysis with the help of I.K. and R.W.; E.F. contributed to the Barley seed collection; E.G., A.D. and M.M. led the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The grand mean values of all four experimental repetitions.

Fig. S1. Phenotypic distribution of 35 wild-type barley populations.

Fig. S2. Representative variation in different whole-plant parameters along a soil–atmosphere water gradient.

Fig. S3. Phenotypic change in plants exposed to initial and severe drought conditions, relative to control plants.

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