Research Paper

Transpiration response of two bread wheat lines differing in drought resilience and their backcross parent under dry-down conditions

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Improving wheat productivity in drylands largely depends on how plants manage limited water resources. Using fraction of transpirable soil water threshold ($FTSW_{Th}$) and drought stress response function, we characterized the water conservation traits of two wheat multiple synthetic derivative lines (MSD53 and MSD345) which both contain introgressed segments from *Aegilops tauschii* but differ in drought resilience. The lines and their backcross parent, 'Norin 61', were subjected to dry-down conditions. MSD53 had a higher $FTSW_{Th}$ for transpiration decrease than 'Norin 61' and MSD345. In terms of drought stress response function, MSD53 had the lowest threshold suction, suggesting a lower drought resilience capacity compared with MSD345. However, MSD53 exhibited an effective-water-use trait whereas MSD345 exhibited a water-saving trait under dry-down conditions. These results are consistent with the reported higher yield of MSD53 in comparison with MSD345 under drought stress in Sudan, and demonstrate that high $FTSW_{Th}$ supports effective water use for improved agricultural productivity in drylands. The differences in water conservation traits between the two MSD lines may be attributed to variation in introgressed segments, which can be further explored for drought resilience breeding.

Key Words: *Aegilops tauschii*, drought, effective water use, genetic variability, transpiration.

Introduction

The reduction in global water availability has caused at least a 20.6% reduction in bread wheat (*Triticum aestivum*) yield within the last 40 years (Daryanto *et al.* 2016). Such drought-induced yield losses have been predicted to worsen in the future because of climate change (Elliott *et al.* 2014). Therefore, there is an urgent need to develop drought-resilient wheat cultivars with improved water conservation traits (World Health Organization 2018).

Drought occurs in different forms across different climatic zones and, as a result, plants need to adapt to region-specific drought conditions (Sherval *et al.* 2014). Such conditions include prolonged drought stress, erratic rainfall, and different groundwater levels. Therefore, to effectively breed drought-resilient wheat varieties, regional climatic and soil peculiarities need consideration. For example, in regions with reachable groundwater table, breeding for long root traits may be beneficial, whereas, in regions with prolonged drought stress and unreachable groundwater table, breeding for water conservation traits may be more meaningful.

Water conservation traits refer to physiological traits that enable plants to optimize water capture and/or use in order to maximize yield under water deficit. These traits are important for increasing water availability to sustain physiological activities, especially during critical stages of development (Gholipoor *et al.* 2012). Many studies have demonstrated the importance of water conservation traits for increasing yield under drought and high vapor pressure deficit in crop plants, including $0.20\,t\,ha^{-1}$ in sorghum (Kholová *et al.* 2014), $2.50\,t\,ha^{-1}$ in lentil (Guiguitant *et al.* 2017), and $1.35\,t\,ha^{-1}$ in maize (Messina *et al.* 2015). Two main water conservation traits have been identified in crop plants: (a) low transpiration rate due to constitutively low plant hydraulic conductance under elevated vapor pressure deficits (Kholová *et al.* 2010), also referred to as water-saving, and (b) early partial stomatal closure when the soil begins to dry (Sinclair 2017), which results in effective water use.

In wheat, Mega *et al.* (2019) reported that water-saving plants overexpressing an ABA receptor (*TaPYL4*) reduced water consumption by up to 20% compared with non-transformed plants, resulting in increased yield per liter of water used. Water-saving wheat cultivars are often grown in drylands, sometimes unintentionally. Schoppach *et al.* (2017) reported that 23 South Australian cultivars conserve water by limiting transpiration, indicating that selection over 100 years resulted in cryptic selection for the limited-transpiration trait. Recent geospatial simulations across

Tunisia found yield increases of up to $1.20\,t\,ha^{-1}$ in wheat genotypes exhibiting water saving traits (Sadok *et al.* 2019). Conversely, the early partial stomatal closure trait ensures that the plant maintains a high transpiration rate when water is available, but quickly reduces transpiration at a relatively high soil water content when the soil begins to dry. That is, the fraction of transpirable soil water threshold ($FTSW_{Th}$)—the soil water content which triggers a drastic reduction in plant transpiration—is higher than in water-saving plants. This trait promotes maximum capture of available soil water which results in effective water use (reviewed in Blum 2009, Sinclair 2018). Variations in transpiration response to evaporative demand and soil water deficit among wheat genotypes have been reported both at the regional (Schoppach and Sadok 2012) and global scale (Tamang *et al.* 2019), with implications for environment-specific breeding.

Despite recent progress in identifying the variations in transpiration response in wheat under drought stress, not much has been done to investigate the contribution of wild relatives to water conservation in wheat. Wheat wild relatives that are adapted to stress-prone environments are a good alternative to introduce genetic diversity to broaden the gene pool of modern wheat cultivars (Kishii 2019, Ogbonnaya *et al.* 2013). One such wild relative is *Aegilops tauschii*, the D genome progenitor of bread wheat. *Ae. tauschii* is adapted to arid and semi-arid regions and is thus a promising source for wheat improvement, especially for drought stress tolerance (Tsujimoto *et al.* 2015). Sohail *et al.* (2011) reported significant variation in drought tolerance traits among *Ae. tauschii* accessions under drought stress. To transfer this variation to bread wheat, synthetic hexaploid wheat lines developed by crossing *Ae. tauschii* with a durum wheat cultivar were used (Tsujimoto *et al.* 2015). However, due to the wild morphology of the synthetic wheat lines, there was low correlation between their individual performances and those of their corresponding *Ae. tauschii* accessions under drought stress (Sohail *et al.* 2011). Therefore, to effectively utilize the variation in *Ae. tauschii* for wheat breeding, the synthetic wheat lines were crossed with a known bread wheat cultivar and the resulting population was referred to as multiple synthetic derivative (MSD) lines. Elbashir *et al.* (2017) reported high variation in heat tolerance-related traits among the MSD lines under heat stress in Sudan. Similarly, Itam *et al.* (2020) reported higher drought resilience-related traits in some MSD lines compared with their backcross parent ('Norin 61'). They also found that although two of the MSD lines (MSD53 and MSD345) have higher drought tolerance efficiency (the ratio of grain yield under drought to that under well-watered condition) than 'Norin 61', they possess contrasting drought resilience traits: MSD53 has high yield reduction (from 5095 to $3375\,kg\,ha^{-1}$, 33.7%), whereas MSD345 has low yield reduction (from 2031 to $1656\,kg\,ha^{-1}$, 18.4%) under drought stress (Itam *et al.* 2020), suggesting differences in water conservation traits.

However, the transpiration response of these lines in terms of $FTSW_{Th}$ which is regulated by the stomata is not yet known. Also, the overall stress response function of these lines compared with 'Norin 61' has not been systematically investigated. In this study, the term "drought resilience" refers to the capacity of the wheat plant to resist damage and maintain productivity under drought stress.

The objective of this study is to characterize the water conservation traits of MSD53 and MSD345 using the $FTSW_{Th}$ and drought stress response function under dry-down conditions. The dry-down condition is commonly used for studying plant-water relations and involves a systematic reduction of irrigation until wilting point, while measuring the rate of transpiration in the plants (Schoppach and Sadok 2012, Sinclair and Ludlow 1986). The results indicate alternative water conservation traits among the investigated genotypes, with the MSD lines showing two drought resilience mechanisms that can be explored in wheat breeding programs to develop new cultivars with improved drought resilience.

Materials and Methods

Plant materials

The MSD lines were developed by crossing durum wheat (*Triticum turgidum* ssp. *durum*, cv. Langdon, AABB genomes) with *Ae. tauschii* (DD genome), and then crossing and backcrossing once with a bread wheat cultivar, 'Norin 61' (AABBDD genomes) (Gorafi *et al.* 2018, Tsujimoto *et al.* 2015). This resulted in MSD lines containing a recombinant DD genome. The genomic constitution of the MSD lines is 75% 'Norin 61' and 25% synthetic parent origin including *Ae. tauschii* and durum wheat (Gorafi *et al.* 2018). The MSD lines were repeatedly self-pollinated until fixation and are currently at the 8th filial generation (BC_1F_8). The two MSD lines used in this study were developed with *Ae. tauschii* accessions from Iran (MSD53) and Georgia (MSD345), and their pedigrees have been reported in Itam *et al.* (2020). We selected them because of their contrasting drought resilience (Itam *et al.* 2020). For comparison, 'Norin 61' was also investigated in this study.

Seed sowing and growth conditions

Seeds were cold-treated at 4°C for 5 d in Petri dishes to break dormancy, and then kept at room temperature for 24 h for acclimatization. Three seeds were sown into pots (1.5 L; 1.2 kg of dry soil per pot) containing Kanto loam volcanic soil (6% clay, 44% silt, 50% sand) with a bulk density of $0.84\,g\,cm^{-3}$. The sowing was done on March 29, 2020. The seedlings were thinned at the 3rd leaf stage [Zadoks stage 13 (Zadoks *et al.* 1974)] to two plants per pot. Ten pots were used per genotype. The water retention curve of the soil and experimental setup are shown in **Fig. 1** and **2**, respectively. The plants were grown in a greenhouse with an average day and night temperature of 33°C and 22°C, respectively. This condition attempts to simulate that

of dryland environments, such as Sudan, where high temperatures occur during the wheat growing season (Iizumi *et al.* 2021). Plants were allowed to grow at 100% field capacity for 32 d before the onset of drought treatment,
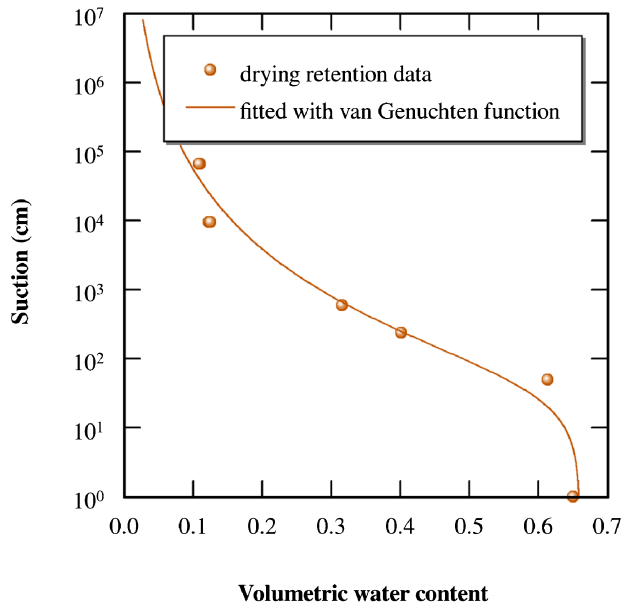

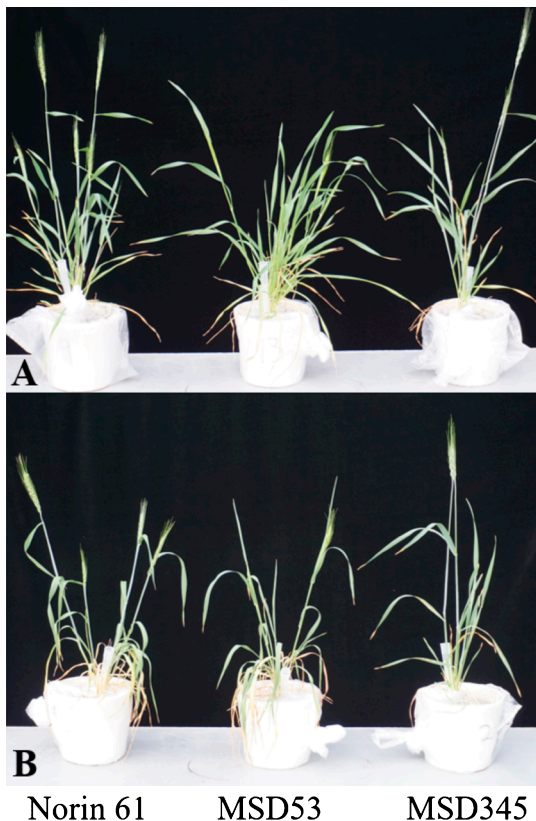**Fig. 1.** Water retention curve for Kanto loam soil.

Fig. 2. Experimental setup showing plants under different water regimes (A) well-watered plants, (B) drought-stressed plants. Photos were taken at 16 days after the beginning of the dry-down treatment.

which followed the dry-down protocol (Sinclair and Ludlow 1986). The 100% field capacity is the amount of water held in the soil after excess water had drained away creating a sufficiently irrigated condition.

Before the dry-down experiment, all pots were over-watered and allowed to drain overnight (about 18 h). On the following morning, the pots were wrapped in polyethylene bags to prevent evaporation from the soil surface. To facilitate watering, a 10-mL pipette tip was inserted between the plant tillers and the end of the polyethylene wrap, and was secured with a twist tie (**Fig. 2**). After wrapping, the initial pot weights were measured.

Ten pots were used per genotype (except 'Norin 61' which had nine pots), three pots were designated as well-watered and maintained at a target weight for sufficiently irrigated condition, while six pots for 'Norin 61' and seven pots for each MSD line were subjected to drought treatment. The target weight for sufficiently irrigated condition refers to the pot weight at high moisture content near 100% field capacity, as defined in the next section. To designate pots for the well-watered condition, we ranked the mean transpiration rates of the nine or ten pots for each genotype, and then chose three pots each from the high, middle, and low ranks. Water supply was withheld from the six pots for 'Norin 61' and the seven pots for each MSD line to induce drought stress. The drought treatment began on May 2, 2020.

Determination of target weight and dry-down condition

Pot weight during the first 48 h was used for optimization for transpiration rate. For example, to set a uniform target weight for sufficiently irrigated condition for each genotype and avoid water logging, the average water loss during the first 24 h (May 3, 2020) was subtracted from the initial pot weights measured on May 2, 2020. The first 24 h was chosen because it represents the highest moisture content after the dry-down process had begun. The subtracted values were 37 g, 44 g, and 19 g for 'Norin 61', MSD53, and MSD345, respectively. At the same time, only 40% of the water lost was replaced in the drought-treated pots, creating a 60% drought stress (i.e., 22 g, 26 g, and 11 g were subtracted from the water to be added to the drought-treated pots of 'Norin 61', MSD53, and MSD345, respectively). On May 4, 2020, the drought intensity was doubled by subtracting 44 g, 52 g, and 23 g from the water to be added to the drought-treated pots of 'Norin 61', MSD53, and MSD345, respectively. Pots were weighed daily (between 11:00 and 12:00) and the amount of water lost was replaced in the well-watered pots to maintain the target weight. To prevent rapid dehydration and ensure gradual drought stress for the drought-treated pots, the drought intensity was adjusted when necessary so that beyond May 4, 2020, daily net water loss did not exceed 50 g (Gholipoor *et al.* 2012). The daily pot weights are shown in **Supplemental Table 1**. Measurements before and after the experiment are shown in **Supplemental Table 2**. The pot weight difference between successive days was considered as the daily

Table 1. Segmented regression fit for normalized transpiration ratio (NTR) in response to decreasing fraction of transpirable soil water (FTSW) for two wheat multiple synthetic derivative lines and their backcross parent, 'Norin 61'

Genotype	Extracted water (g)	Transpiration rate (g plant^{-1} day^{-1})	Duration (days)	FTSW threshold	Confidence interval (95%)	S.E.	Threshold suction (cm)
Norin 61	524.85 ± 22.34[a]	37.1 ± 11.9.[a]	18.40 ± 0.89[c]	0.38	0.35–0.40	0.06	617
MSD53	513.64 ± 11.42[a]	43.9 ± 6.0[a]	16.28 ± 1.25[b]	0.52	0.48–0.57	0.08	333
MSD345	518.81 ± 15.22[a]	19.4 ± 7.6[b]	37.33 ± 0.51[a]	0.32	0.30–0.33	0.07	511

Different letters (column wise) indicate significant ($P < 0.05$) differences between genotypes according to Tukey's HSD test.

transpiration rate (**Table 1**). Finally, the number of days taken to extract all the available water in the soil was recorded as duration (**Table 1**).

Determination of normalized transpiration ratio (NTR) and FTSW$_{Th}$

The NTR is transpiration ratio (TR) centered on a value of 1.0 which allows for comparison among different genotypes. The FTSW$_{Th}$ is the soil water content which triggers a drastic reduction in plant transpiration. To calculate the daily TR of each pot and minimize day-to-day variations in transpiration rate, the transpiration rate for each pot was divided by the average transpiration rate for the three well-watered pots of the same genotype (Gholipoor *et al.* 2012).

To account for plant-to-plant variation, the TRs were normalized using the equation:

$$NTR = \frac{TR}{iTRave} \quad (1)$$

where *NTR* is the normalized transpiration ratio of a particular genotype on a particular day, *TR* is the transpiration ratio, and *iTRave* is the average transpiration ratio of each genotype during the early stages of drought treatment, when the drought-treated pots still had a well-watered moisture range (Devi *et al.* 2009). This period corresponds to 8–10 d in Norin 61, 3–8 d in MSD53, and 19–24 d in MSD345. The *iTRave* values were 0.98, 1.00, and 1.09 for 'Norin 61', MSD53, and MSD345, respectively (**Supplemental Table 3**). Therefore, the NTR of each drought-stressed genotype was centered on 1.0 during the well-watered stage to make NTR values comparable among the three genotypes (**Supplemental Table 4**). The dry-down experiment continued until the NTR of all stressed plants fell below 0.12 (i.e., when the transpiration of drought-stressed plants was <12% of that of well-watered plants). The weight difference between the initial and final weight (at NTR <0.12) of each pot was referred to as total transpirable soil water. Then, the fraction of transpirable soil water (FTSW) was calculated as follows:

$$FTSW = \frac{[\,daily\ weight - final\ weight\,]}{[\,initial\ weight - final\ weight\,]} \quad (2)$$

Finally, to estimate the FTSW$_{Th}$ for the decline in TR for each genotype, segmented linear regression analysis was performed using GraphPad Prism, version 8 (GraphPad Software Inc., San Diego, CA). The following model was applied:

$$NTR1 = a1FTSW + NTR0$$
$$NTR2 = a2(FTSW - FTSW_{Th}) + NTRatThreshold \quad (3)$$

where *NTR0* is the NTR value at which the first line segment intersects the vertical axis; *a1* is the slope of the first line segment, *a2* is the slope of the second line segment, *FTSW$_{Th}$* is the FTSW value at which the two line segments cross and *NTRatThreshold* is the NTR value at the crossing point.

Determination of drought stress response function

To quantitatively predict transpiration rate and growth under drought stress, we applied a widely used root water uptake model (Simunek *et al.* 2006), which uses Van Genuchten's stress response function. In this model, the rate of water uptake, S (s^{-1}), is calculated by multiplying the reduction coefficient for root water uptake α by the potential water uptake rate S_p (s^{-1}) (Feddes and Raats 2004):

$$S = \alpha * S_p \quad (4)$$

The reduction coefficient depends on suction at each depth. We assumed that root density is uniform throughout the root zone; under these conditions, the reduction coefficient equals NTR. We fitted data with a linear function below 4000 cm, where the data are distributed fairly linearly based on the model, and obtained threshold suctions at which the reduction coefficient starts to decrease below unity. Then, we determined the parameter values of Van Genuchten's stress response function (Van Genuchten 1987) as follows:

$$\alpha = NTR = \frac{1}{1 + \left(\frac{h}{h_{50}}\right)^p} \quad (5)$$

where h is suction (cm), and h_{50} and p are fitting parameters; h_{50} is the suction at which water uptake is 50% of its potential rate and is a simple index of plant stress tolerance. The suction for each genotype was calculated using the average daily volumetric water content in the pots and the soil water retention curve (**Fig. 1**). The aim of this modeling was to confirm the earlier results obtained by FTSW$_{Th}$ and to project these results for potential characterization of water conservation traits in wheat.

Results

Heading date

MSD345 and 'Norin 61' had similar heading dates while MSD53 was late heading under control conditions. In our recent study in Wad Medani, Sudan, the heading dates were 57.8 ± 3.7, 62.7 ± 3.1, and 58.2 ± 1.5 days for 'Norin 61', MSD53, and MSD345, respectively (Itam *et al.* 2021a). Therefore, in this study, we assume that the heading date difference is narrow and did not significantly affect the dry-down process.

Pot weight and TR

The pot weight for MSD345 after wrapping (May 2) was significantly higher than that for 'Norin 61' and MSD53, indicating a lower transpiration rate in MSD345 (**Supplemental Table 2**). The transpiration rates during 24 h from May 2 to May 3 were 37.1 g, 43.9 g, and 19.4 g plant^{-1} day^{-1} for 'Norin 61', MSD53, and MSD345, respectively (**Table 1**). This indicates that the transpiration rates in 'Norin 61' and MSD53 were twice that in MSD345. Overall, the time-course change in TR highlights the differences in transpiration during the dry-down process: MSD345 had an extended duration of normal transpiration before a drastic reduction occurred, whereas, 'Norin 61' and MSD53 had shorter durations (**Fig. 3**).

FTSW$_{Th}$

The mean total extracted water for 'Norin 61', MSD53, and MSD345 was 525 g, 514 g, and 519 g, respectively, with no significant difference among the three genotypes (**Table 1**), indicating that water extraction capacity was similar among the three genotypes. However, the number of days taken to extract the water until NTR decreased below 0.12 (referred to as duration) differed among the genotypes. This difference was consistent with the differences in their transpiration rates (**Table 1**): the duration was

longest for MSD345, which had the lowest transpiration rate, emphasizing the inverse relationship between transpiration rate and duration under drought conditions.

The NTRs remained approximately 1.0 until an FTSW$_{Th}$ was reached, and then decreased linearly (**Fig. 4**) due to decreasing transpiration. The slopes of the linear decrease (S1$_{NTR}$) were: 2.41 for 'Norin 61', 1.70 for MSD53, and 2.65 for MSD345 (**Fig. 4**). The FTSW$_{Th}$ varied among the genotypes: from 0.32 in MSD345 to 0.52 in MSD53 (**Fig. 4**). This variation indicates the differences in water conservation traits among the genotypes; MSD53 had an early response to drought stress compared with MSD345. Overall, the genotypic differences in FTSW$_{Th}$ tend to positively correlate with transpiration rate; MSD345 which had the lowest transpiration rate also had the lowest FTSW$_{Th}$ compared with MSD53 and 'Norin 61' (**Table 1**).

Drought stress response function

The h_{50} values of the three genotypes ranged from 2917 cm (MSD53) to 5051 cm (MSD345), indicating the highest drought resilience of MSD345 (**Fig. 5**). Similarly, MSD345 had a higher threshold suction than MSD53 (**Fig. 5**), confirming the result for FTSW$_{Th}$ obtained from segmental linear regression (**Fig. 4**, **Table 1**).

Discussion

Introgressed wild alleles from *Ae. tauschii* have been recently used to increase genetic diversity and introduce desirable agronomic trait(s) into elite wheat germplasm (Cox *et al.* 2017, Kishii 2019, Ogbonnaya *et al.* 2013). The wheat MSD lines MSD53 and MSD345 were developed using two *Ae. tauschii* accessions adapted to the dry regions of Iran and Georgia, respectively (Gorafi *et al.* 2018, Tsujimoto *et al.* 2015). Both MSD lines are more drought resilient than 'Norin 61' (Itam *et al.* 2020). The main difference between the two MSD lines is in their drought resilience trait or mechanism. The mechanism determines
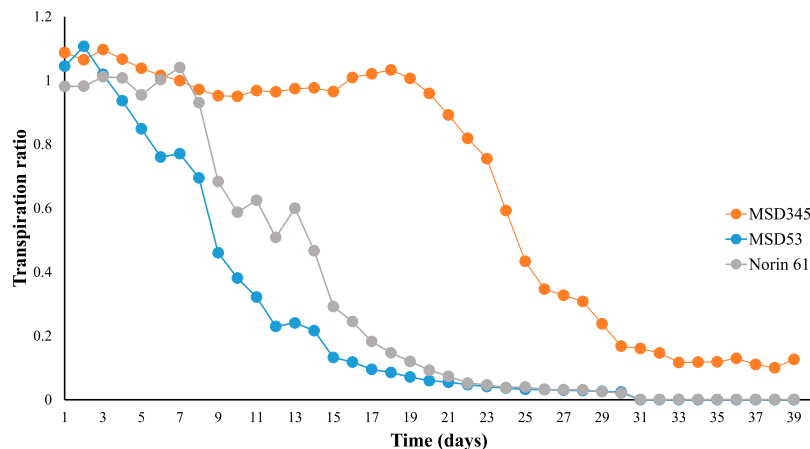

Fig. 3. Time-course change in transpiration ratio (TR) for two wheat multiple synthetic derivative lines and their backcross parent, 'Norin 61' under dry-down conditions.
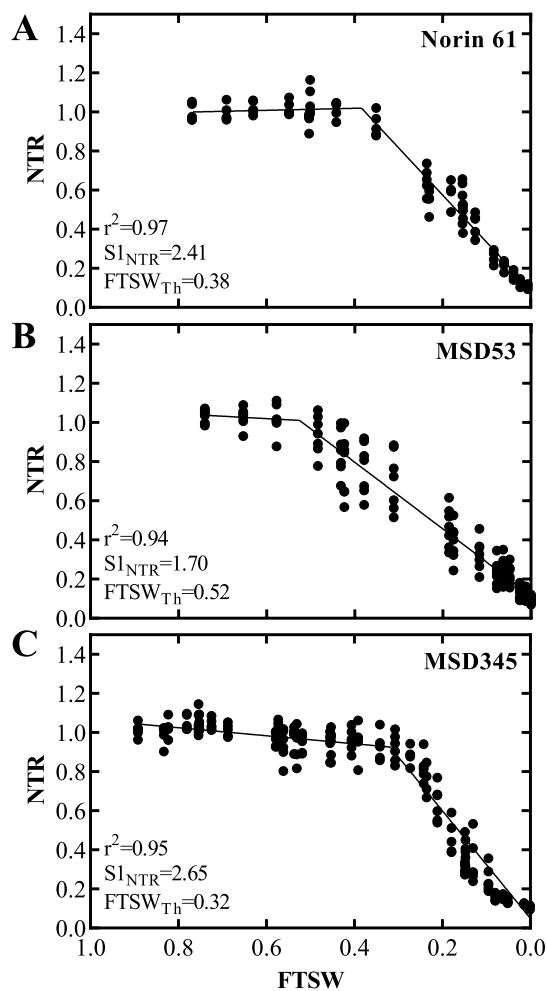
Fig. 4. Normalized transpiration ratio (NTR) plotted against fraction of transpirable soil water (FTSW) for two wheat multiple synthetic derivative lines and their backcross parent, 'Norin 61' under dry-down conditions. $S1_{NTR}$, slope 1; $FTSW_{Th}$, FTSW threshold for decrease in transpiration.

Fig. 5. Stress response function of two wheat multiple synthetic derivative lines and their backcross parent, 'Norin 61', under dry-down conditions. Solid lines are curves fitted with equation 5; dashed lines are linear regressions within 4000 cm.

how water is managed under drought stress conditions. MSD345 resists drought damage by lowering its transpiration throughout the growing season, and therefore, senses drought only when the soil water is very low—near severe levels (i.e., low $FTSW_{Th}$ trait). Thus, MSD345 has a prolonged lifespan under drought and can maintain production under very dry conditions (**Fig. 3**, **Supplemental Tables 1**, **4**). Conversely, MSD53 absorbs plenty of water when water is in abundance, but quickly reduces transpiration when the water level begins to decrease (i.e., high $FTSW_{Th}$). Thus, MSD53 maintains production under drought stress by lowering its transpiration rate earlier compared with MSD345. Their drought tolerance efficiencies were 52.3% for 'Norin 61', 66.2% for MSD53, and 81.5% for MSD345, indicating differences in drought resilience under post-anthesis drought stress in field conditions (Itam *et al.* 2020). This implies that, although MSD53 had high grain yield under well-watered and drought conditions (5.09 t ha⁻¹ and 3.38 t ha⁻¹, respectively) (Itam *et al.* 2020), it had higher
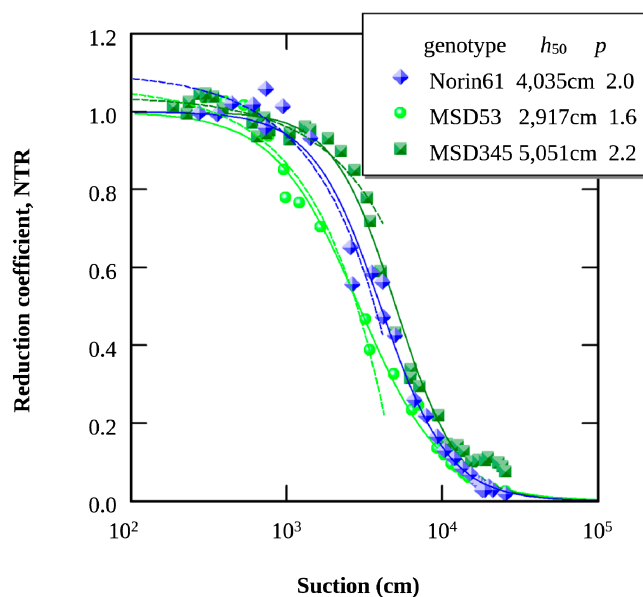
yield reduction compared with MSD345. The differences in drought tolerance efficiency (Itam *et al.* 2020) suggests that genotypes with high transpiration rate (**Table 1**) and high $FTSW_{Th}$ have higher yield reduction compared with those with low transpiration rate and low $FTSW_{Th}$ under drought stress. This negative trend between drought tolerance efficiency and $FTSW_{Th}$ may potentially prove useful in breeding for drought resilience and needs to be validated with a large sample size in future studies.

The trend between transpiration rate and FTSW indicates that plants with low transpiration rates (such as MSD345) have long duration of normal physiological activities before they begin to respond to drought stress (**Table 1**). This trend needs validation in a large-scale study. The low transpiration rate value in MSD345 ensured a limited but sustained water flow, thereby extending the period before the $FTSW_{Th}$ (**Figs. 3**, **4**). Low transpiration rate is common among water-saving wheat lines (Mega *et al.* 2019, Schoppach *et al.* 2017). Under drought, the transpiration rate in the water-saving lines is adjusted to match the water flow rate to the stomata to avoid desiccation (Sinclair 2018), a mechanism linked to aquaporin activity in root cells (Sadok 2017, Sadok and Sinclair 2010). In contrast, MSD53 had an early partial stomatal closure trait at high FTSW, which is linked to effective water use and yield increase (Sinclair 2018). Similar yield increases have been reported in sorghum (Kholová *et al.* 2014), lentil (Guiguitant *et al.* 2017), and soybean, especially in drier growing seasons (Sinclair *et al.* 2010), pointing to the universal nature of this trait in plants. Consistently, the drought stress response function (**Fig. 5**) showed that MSD345 is more drought resilient than MSD53 or 'Norin 61', which

may be attributed to its lowest transpiration rate and $FTSW_{Th}$.

The genotypic differences between the MSD lines indicated the presence of alternative water conservation traits within the wheat MSD population (Gorafi *et al.* 2018). Similar alternative traits have been reported in sorghum genotypes under dry-down conditions (Gholipoor *et al.* 2012) and in wheat genotypes under different evaporative demands and soil water deficit (Sadok *et al.* 2019, Schoppach and Sadok 2012, Tamang *et al.* 2019). The alternative traits in the two MSD lines may be attributed to different genomic contributions from their individual synthetic parents. The synthetic parents contain genomic fragments from *Ae. tauschii*, and demonstrate the diversity of drought resilience mechanisms in *Ae. tauschii*. Such genomic fragments have been linked to drought resilience in the MSD lines (Itam *et al.* 2021b). Worthy of note is the possible effect of the durum wheat cultivar ('Langdon') used for developing the synthetic parents. Itam *et al.* (2021b) reported the presence of unique introgressed segments on chromosomes 4B and 6B potentially linked to drought resilience in the MSD lines. Similarly, in a genome-wide association study of the MSD lines under heat and combined heat–drought conditions, Itam *et al.* (2021a) identified positive alleles for grain yield on chromosomes 6B and 7A which were derived from 'Langdon'. The presence of such genomic segments or alleles on the A and B genomes highlights the importance of the synthetic derivative approach for mining useful genes from durum wheat, in addition to those from *Ae. tauschii*.

The present study conditions simulated dryland environments by ensuring that the drought stress is accompanied by heat stress. Therefore, it is conceivable that the heat effect influenced the observed plant response. Genetic variation among the MSD lines under heat stress (Elbashir *et al.* 2017, Itam *et al.* 2021a), combined heat–drought stress, and drought response (Itam *et al.* 2021a) have been previously reported. The genomic regions controlling the variations were identified; some were pleiotropic for all three stresses, whereas others were unique for individual stresses (Itam *et al.* 2021a). Similar results have been reported in other bread wheat populations (Liu *et al.* 2019, Qaseem *et al.* 2019, Schmidt *et al.* 2020), pointing to a complex relationship between drought and heat stress in wheat. Therefore, as heat and drought stresses occur concurrently under natural conditions, designing appropriate study conditions offers better opportunities for wheat improvement, and this study may serve as a reference for the characterization of water conservation traits in wheat.

As expected, the differences in water conservation traits affected yield, which is lower in MSD345 than in MSD53 under both well-watered and drought conditions (Itam *et al.* 2020), pointing to a possible trade-off between yield and drought resilience based on water conservation. Although in this study, the plant yield and dry biomass were not determined, the yield results from Itam *et al.* (2020) indicates that the effective water-use trait results in higher grain yield compared with the water-saving trait.

The choice between these MSD lines for further breeding will depend on breeders' target. For example, plants harboring the low transpiration rate and $FTSW_{Th}$ traits conserve water within the soil and are, therefore, not suitable for regions with high soil evaporation. Such plants may have lower vegetative biomass and consequently lower yield. In our Sudan study, the low transpiration rate and $FTSW_{Th}$ traits, resulted in plants not fully utilizing the available water. Similarly, Sciarresi *et al.* (2019) reported that the limited-transpiration trait only marginally increased wheat yield (by about $0.12\,t\,ha^{-1}$) in comparison with the increases (by $0.60\,t\,ha^{-1}$) due to enhanced root exploration traits in semiarid climate. In contrast, high transpiration rate early in the growing season may support vegetative growth, leading to high biomass, and subsequently, when drought occurs during grain filling, the high $FTSW_{Th}$ may be able to support grain yield. Plants with high $FTSW_{Th}$ may also produce more straw for livestock feed. Furthermore, the high transpiration rate and $FTSW_{Th}$ traits were ultimately beneficial under drought stress in Sudan (Itam *et al.* 2020). This finding agrees with Sinclair (2017) on the potential of the high $FTSW_{Th}$ trait for yield increase and the concept of effective water use (reviewed in Blum 2009, Sinclair 2018), but contradicts a recent simulation study in Tunisia (Sadok *et al.* 2019), which reported that high $FTSW_{Th}$ is wasteful and did not result in yield gains in wheat under drought conditions. Therefore, to develop wheat lines with effective water use for specific locations, factors such as plant phenology and temporal phenomena need to be carefully considered (Sinclair 2018). Overall, the alternative water conservation traits identified and characterized in these lines using $FTSW_{Th}$ and drought stress response function, offer new options for wheat breeding for water-deficit conditions.

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