

Diversity in D-genome synthetic hexaploid wheat association panel for seedling emergence traits under salinity stress

Zeeshan Khan¹, Javaria Qazi^{1*}, Awais Rasheed^{2,3} and Abdul Mujeeb-Kazi⁴

¹Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan, ²Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China, ³International Maize and Wheat Improvement Center (CIMMYT), c/o CAAS, Beijing 100081, China and ⁴University of Texas A&M, Amarillo, TX 79106, USA

Received 8 March 2016; Accepted 13 May 2016 – First published online 13 June 2016

Abstract

Seedling emergence is the preliminary factor defining wheat adaptability and stability under salt stress. This study was led to assess the salinity tolerance amongst 226 synthetic hexaploid wheats (SHWs) evaluated against two check cultivars, the tolerant ‘S-24’ and the susceptible ‘PBW-343’ at three sodium chloride treatments (0, 100 and 200 mM). Highly significant and positive correlation was observed between germination % and germination index ($r=0.85$), and between seedling height and weight ($r=0.85$). All four traits across three treatments were transformed into the salt tolerance trait index and salt tolerance index (STI). STI had significant positive correlation with all four parameters indicating reliability of this index for ranking the tolerance levels. STI-based 20 best performing genotypes were known as being promising candidates for wheat breeding. Local tolerant check was amongst the top three tolerant accessions. Two SHWs, AUS30288 {Croc_1/*Aegilops squarrosa* (466)} and AUS34444 {Ceta/*Ae. squarrosa* (872)} outperformed S-24 with STI of 61.8 and 55.7, respectively. SHW with same durum parents were included in tolerant and susceptible categories indicating that tolerance is contributed by the *Ae. squarrosa* *syn. tauschii* parent of SHWs. In conclusion, this baseline study revealed that continuous variation in the seedling emergence traits under salt stress is a conduit towards implementing genome-wide association studies. Likewise, new diversity has implications in development of salt tolerance germplasm after genetic dissection permitting unique *Ae. squarrosa* accessional diversity validation to target SHW donors for breeding.

Keywords: *Aegilops tauschii*, germination test, salinity stress, salt tolerance index, synthetic hexaploid wheats

Introduction

Salinity afflicts crop production on more than 800 million hectares worldwide, either because of salinity which affects 397 million hectares or due to the associated sodicity prevailing on 434 million hectares. This forms nearly 7% of the

world’s land (Munns, 2009) with estimated annual losses currently being over USD 12 billion due to salinity (Shabala and Bose, 2012). The crop plants are more prone to salinity as it decreases yield of agricultural crops since most plants are salt-sensitive glycophytes. Effects of salinity on plants comprise of: (1) osmotic stress, (2) disruption of membrane ion transport, (3) direct toxicity of cytoplasmic sodium and chloride at high concentrations, and (4) induced oxidative stress. This ultimately leads to

*Corresponding author. E-mail: javariaq@yahoo.com

low vigour, poor seed germination, lower water uptake capacity that affects plant growth, with various biochemical, physiological and morphological processes being affected.

Under salinity stress we are still lagging behind in terms of genetic improvement in wheat grain yield mainly due to the limited genetic diversity available in wheat for salinity tolerance (Wyn Jones and Gorham, 1991; Dreccer *et al.*, 2004). Likewise, slight consideration is given to the salinity tolerance *per se* in wheat improvement programmes (Rauf *et al.*, 2010). Although greater variation for salinity is present in various Triticeae gene pools (Trethowan and Mujeeb-Kazi, 2008; Mujeeb-Kazi *et al.*, 2013), however preference is given to *Aegilops tauschii* due to its close resemblance with the wheat D-genome and wide genetic proximity (Sohail *et al.*, 2012; Ogonnaya *et al.*, 2013). Synthetic hexaploid wheats (SHWs), derived from crossing durum cultivars with *Ae. tauschii* accessions have been reported to reveal great variation for many important agronomic traits particularly abiotic stresses such as salinity, drought and heat tolerance. There is a wide array of SHWs produced globally (Ogonnaya *et al.*, 2013); however, very limited numbers have been evaluated for salinity tolerance (Dreccer *et al.*, 2007).

Fast and effective screening methods that can find genetic variation for salinity tolerance in wide array of genetic resources are very important (Munns and James, 2003). It is quite difficult to screen such a large population of genotypes for salinity tolerance because of soil's spatial heterogeneity, physical/chemical properties plus seasonal rainfall irregularities and fluctuations. It was reported after the field study in Syria by utilizing ICARDA's progressive durum wheat breeding lines that tolerance against salinity might be possible due to presence of genetic variation, but the baffling occurrence of stress against drought made it inflexible to recognize genotypes with salinity tolerance (Srivastava and Jana, 1984). Germination speed, emergence and subsequent seedling vigour are very significant factors for good crop establishment and ensure high productivity. Salinity stress reduces the germination percentage, rate of germination and seedling vigour in crops (Yildirim *et al.*, 2002). Seeds may be extra delicate to stress than developed plants, because of exposure to the active environment near the soil surface (Dodd and Donovan, 1999).

The differences in genotypic response in controlled salinity stress conditions can be assessed through germination percentage (G%) and seedling growth under saline conditions. This type of information is vital for proposing appropriate germplasm resources for saline soils. Therefore, this study was conducted to determine the performance of 226 SHWs under saline conditions by measuring germination indices as a proof-of-concept of a critical preliminary screening method to identify promising genetic resources and reliable traits for implementation of future genetic dissection studies.

Material and methods

Plant material

An association panel comprising of 226 SHWs was phenotyped for the current experiment (online Supplementary Table S1). These SHWs were derived from combinations of 196 *Ae. tauschii* accessions and 44 durum wheat cultivars that had been previously characterized for grain morphology (Rasheed *et al.*, 2014), biotic stress resistances (Joukhadar *et al.*, 2013; Mulki *et al.*, 2013) and grain quality (Emebiri *et al.*, 2010). The salt tolerant variety 'S-24' (Ashraf, 2010) and the salt-sensitive cultivar 'PBW-343' were used as standard checks in the germination test. The 1% sodium hypochlorite solution was used to sterilize the seeds for 3 min before the germination study. Seeds were rinsed with sterilized water and were air-dried.

Salt solution and germination test

Two different concentrations of NaCl solution (100 and 200 mM) were made by dissolving analytical grade NaCl (Merck, USA) in distilled water. A distilled water (0 mM) treatment was used for comparison as a control. Saline conditions were used by using aqueous NaCl solutions.

Five seeds per entry were surface sterilized and sown following the method described by Tlig *et al.* (2008). Sterilization was done in 1% sodium hypochlorite solution for 1 min; thoroughly washed 4–5 times with distilled water and air dried before starting the germination experiment. All 226 D-genome SHWs were tested at three treatments: control (distilled water), 100 and 200 mM salt (NaCl) solutions along with the tolerant (S-24) and the susceptible (PBW-343) checks. All genotype seeds were sown in filter paper lined petri plates and allowed to germinate in the dark for 48 h at 25°C. They were then transferred to a growth chamber running at $27 \pm 2^\circ\text{C}$ and 10 h photoperiod with 80–85 $\mu\text{M}/\text{S}/\text{m}^2$ light intensity was maintained for further growth and observations. The experimental design used was a factorial combination of treatments and genotypes organized in a totally randomized design with three replications. A seed was considered germinated when the plumule was longer than half of the length of the seed, and the radicle was equivalent to or longer than the seed length.

Traits evaluated for seedling emergence

Data were recorded on G%, germination index (GI), seedling height (SHt) and seedling weight (SWt). Seed germination was regularly noted daily according to AOSA (1990) till a continuous count was attained. Seed was considered to be germinated if plumule length exceeds half of the seed size and when radicle length surpassed 2 mm. G% was

recorded for each genotype after every 24 h for 7 d by the formula adopted by Rauf (2005).

$$\text{Germination percentage (G\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of sown seed}} \times 100.$$

GI was recorded by counting the seeds germinated daily until they reached a constant. Number of seeds germinated divided by days from first seed germinated.

GI was calculated as described (Baalbaki *et al.*, 2009):

$$\text{GI} = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}.$$

Percentage change over control was computed as under:

$$\text{Treatment over control} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100.$$

SHt was measured by a ruler in centimetres. The height was taken from the portion of the plant above roots to the seedling tip (Fokar *et al.*, 1998). Three randomly selected seedling's length was recorded from each replication. Fresh SWt was also recorded by using a precision weighing balance in milligrams (Fokar *et al.*, 1998).

At germination, salt tolerance trait index (STTI) was calculated according to the formula of Ali *et al.* (2007):

$$\text{STTI} = \frac{\text{Value of trait under salt stress}}{\text{Value of trait under control}} \times 100.$$

Salt tolerance index (STI) was measured as a mean of STTIs. STIs were used to group the 226 SHW genotypes into four salt-tolerant groups. Percentage survival rate was calculated as ratio of survived and germinated genotypes under salt stress to total number of genotypes tested.

Statistical analysis

Descriptive statistics was calculated on data using MS excel and the analysis of variance (ANOVA) of the data was done using STATISTICA 7. Co-efficient of correlation between all parameters was estimated using R-package.

Results

The results revealed significant differences amongst genotypes, treatments and their interaction for all four traits (online Supplementary Table S2). Although salinity affected all traits, results were highly significant for SHt and SWt, as compared with G% and GI (Table 1, online Supplementary Table S2). Similarly, all the traits decreased significantly by increasing the salt concentration from 100 to 200 mM (Table 1). All genotypes showed 100% germination at 0 mM NaCl, except few which showed >90%

germination. Therefore, this treatment was excluded for G% for further analysis. On an average, G% decreased 14.5 and 25.3% at 100 and 200 mM, while GI decreased 11 and 24% at 100 and 200 mM, respectively. SHt decreased 57.1 and 90.3% and SWt decreased 54.2 and 85.8% at 100 and 200 mM, respectively.

In our experiment, we used two check cultivars, which showed expected results. In tolerant standard (S-24), G% was not affected at both 100 and 200 mM, while GI decreased 20.4% at both 100 and 200 mM, SHt decreased 37.7 and 62.3%, and SWt decreased 24.5 and 68.8% at 100 and 200 mM, respectively. S-24 is derived from the reciprocal crossing of two high-salt-tolerant genotypes 'LU26 S' and Indian spring wheat landrace 'KHARCHIA' (Ashraf, 2010). S-24 is known to maintain high K^+/Na^+ ratio in the plant tissue and possesses good characteristics of agronomic importance, including 1000-kernel weight (TKW) of 42 g and grain yield. Therefore, this control was a stringent challenge to the accessions evaluated in our panel and ranked third based on STI.

STTI calculated for all traits provided an estimate for salinity effect on each trait and correlated well with the relevant traits (Fig. 1). STTI of each trait was averaged together to calculate STI. In correlation analysis, the four traits studied were positively and significantly ($P \leq 0.01$) correlated with each other (online Supplementary Table S3). Strongest correlation was between G% and GI and ($r=0.846$) and between SHt and SWt ($r=0.86$). STI has significant positive correlation with SHt ($r=0.75$), SWt ($r=0.69$), G% ($r=0.79$) and GI ($r=0.77$). Additionally, the data indicated that the variation is continuous for SHt and SWt as compared with G% and GI (Fig. 1), indicating SHt and SWt are more reliable traits for genetic analysis.

Discussion

Although, salinity suppressed germination, but SHt and SWt were more prone to salinity stress as compared with germination indices. Therefore, the selection criteria based on germination indices should be complemented with other quantitative traits including SHt and SWt for reliable selection of tolerant accessions. Munns and James (2003) also concluded that seedling-based screening techniques are effective and could identify genetic variations for salinity tolerance in a wide array of genetic resources. The genotypic difference for biomass production or leaf elongation (SHt in our case), was due to decrease in growth rate imposed by the osmotic effect of salt. These traits correlated well with Na^+ exclusion (Munns and James, 2003).

For an accurate screening of large germplasm collections, very fast and specific techniques will be needed. Several immediate experiments and their failure are

Table 1. Descriptive statistics for germination, GI, seedling height and weight, and their STTI at various salt stress conditions

Description	Germination (%)			STTI Germ%		GI			STTI GI	
	0 mM	100 mM	200 mM	100 mM	200 mM	0 mM	100 mM	200 mM	100 mM	200 mM
Mean	100	85.5	74.67	85.5	74.67	11.03	9.82	8.38	90.34	76.53
SD	0	19	27.74	19	27.74	1.66	2.43	3.28	24.83	29.48
SE	0	1.26	1.83	1.26	1.83	0.11	0.16	0.22	1.64	1.95
CV (%)	0	22.22	37.15	22.22	37.15	15.01	24.7	39.2	27.49	38.52
Min	100	0	0	0	0	6	0	0	0	0
Max	100	100	100	100	100	12.25	12.25	12.25	177.11	151.55
S-24	100	100	100	100	100	12.25	9.75	9.75	79.59	100
PBW-343	100	100	60	100	60	12.25	12.25	6.35	100	60

Description	Seedling height (cm)			STTI SHt		SWt (mg)			STTI SWt	
	0 mM	100 mM	200 mM	100 mM	200 mM	0 mM	100 mM	200 mM	100 mM	200 mM
Mean	15.22	6.53	1.48	42.7	9.61	93.03	42.59	13.17	47.92	14.46
SD	3.08	3.31	1.05	19.65	6.72	21.99	16.11	9.34	21.99	10.76
SE	0.20	0.22	0.07	1.30	0.44	1.45	1.06	0.62	1.45	0.71
CV (%)	20.21	50.62	71.06	46.03	69.95	23.63	37.83	76.28	45.89	74.41
Min	3.33	0	0	0	0	21.33	0	0	0	0
Max	21.83	15.33	5.83	113.46	37.7	152.67	88.33	56.67	164.33	63.92
S-24	10.17	6.33	3.83	62.29	37.7	61.0	46.0	19.0	75.13	30.81
PBW-343	11	4.83	0.166	43.93	1.51	57.0	75.0	3.0	132.16	0.58

STTI, salt tolerance trait index; GI, germination index; SHt, seedling height; SWt, seedling weight.

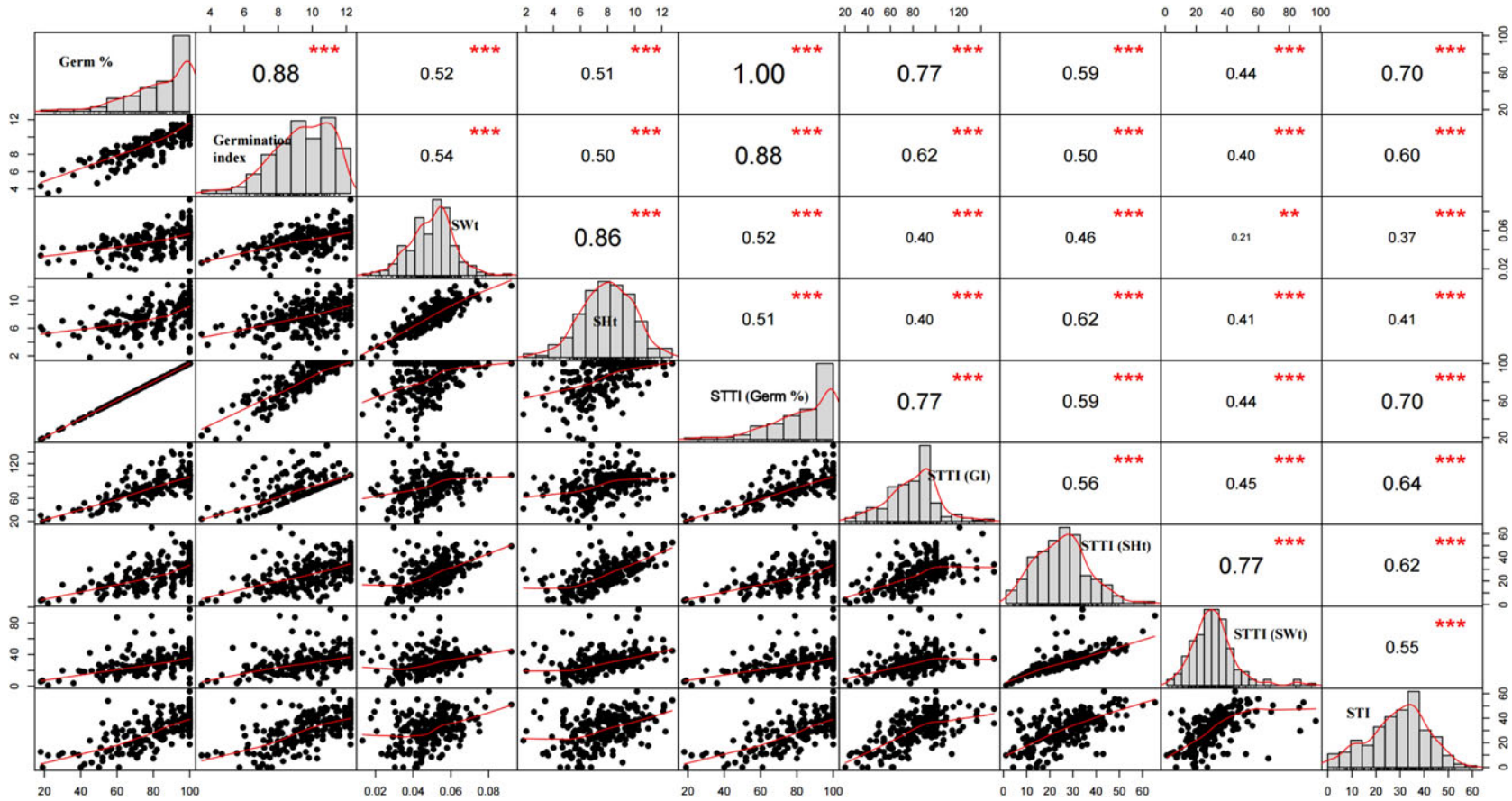


Fig. 1. Frequency distributions, co-efficient of correlations and relationship between all traits and their STTI. The values are based on data averaged over all treatments (see footnote of [Table 1](#) for trait abbreviations).

debatable for salt stress screening, because the preliminary reaction to salt stress is considered as an osmotic effect of the salt, recognizing that the salt is externally present outside the root (Munns, 1993). Germination is an appropriate trial for big populations of genotypes as described previously, but slight or no association was seen between genotypic alterations in germination and later development in saline conditions for species as diverse as barley (Norlyn and Epstein, 1982), bread wheat (Ashraf and McNeilly, 1988) and durum wheat (Almansouri *et al.*, 2001). Many species such as barley and wheat have the potential to germinate at highly saline conditions (over 300 mM NaCl), but the radicle emerging from seed could not develop further at that high salinity level. The difference present among species to germinate under saline conditions can be well elucidated by physicochemical nature of the swelling phenomenon in germination. For example, glycophytes are no more salt tolerant than halophytes at germination, even though haplophytes rapidly express their higher tolerance in the initial stage of hypocotyl enlargement (Malcolm *et al.*, 2003). The salt-specific effect takes time to develop. Therefore, we additionally used SHt and SWt parameters and combined all these parameters in STI to use this index as a decisive selection tool. SHt and SWt have more significance because the genotypes, which do not exclude salt effectively from the transpiration stream, salt build up reaches to a toxic level in the leaves that have been transpiring the longest (Munns, 1993). The speed at which they die is relative to the speed at which new leaves are produced and is critical. This is probably because the cell driven expansion processes during germination and during subsequent growing are completely different. Uptake of water and elongation that permits imbibition and development of the radicle is a physicochemical procedure, in comparison with the molecular and biochemical processes that motivate successive cell division and extension. External to the roots the osmotic stress of the salt reduces the development rate of new leaves and rate of production of tillers (Munns, 2002). Consequently, the salt's effect can be calculated in weight and height of plant. The development of a leaf usually responds in linear proportion to the osmotic strength of the soil solution (Rawson *et al.*, 1988), with few species being more sensitive than the rest (Cramer, 2003).

Top 20 tolerant genotypes along with the tolerant check S-24 with their STTI of all four studied parameters were ranked according to best STI (online Supplementary Table S3), which could be promising resources for use in breeding. SHWs were grouped into four categories. The tolerant (21) SHWs are ample for use in breeding, irrespective whether they are superior to the check cultivars. Their uniqueness is in the fact that diverse *Ae. tauschii* accessions are in their combinations. The relationship between both stress treatments (100 and 200 mM) was stronger for SHt

($R^2=0.38$) and SWt ($R^2=0.31$), as compared with G% ($R^2=0.12$) and GI ($R^2=0.25$) (online Supplementary Fig. S1). This again implies that germination indices solely are insufficient to categorize genetic variations for salinity tolerance.

SHWs and their advanced derivatives have been well assessed for drought (Lopes and Reynolds, 2010; Tang *et al.*, 2010; Ali *et al.*, 2014; McIntyre *et al.*, 2014) and heat stress (Gororo *et al.*, 2002; Trethowan *et al.*, 2005), however, less information is available for their performance under salt stress. Although it is well known that modern durum wheat cultivars have less tolerance to salinity as compared with bread wheat (Munns *et al.*, 2006), therefore it is most likely that tolerance in these SHWs is contributed by the *Ae. tauschii* parent and not the durum parent. This has been partially confirmed from the pedigree analysis of current SHWs that accessions with same durum parents were observed in tolerant and susceptible groups indicating the sole contribution of *Ae. tauschii* for salt tolerance (online Supplementary Table S2). For example, among the 24 SHWs derived from durum cultivar Croc_1 three were susceptible, one tolerant, ten moderately susceptible and ten moderately tolerant (online Supplementary Table S4). Such trends can be observed for other SHWs derived from same durum parents, i.e. Decoy-1 and Altar-84. Trethowan and Mujeeb-Kazi (2008) had also observed such tolerance contributions of the *Ae. tauschii* accessions. Previously, we observed similar trend for tolerance to boron toxicity in 45 SHWs derived from Decoy-1 (Ilyas *et al.*, 2015), and we could only identify one SHW {Decoy_1/*Ae. squarrosa* (466)} tolerant to both salinity and boron toxicity. Similarly, Yang *et al.* (2014) concluded that morpho-physiological traits remained dramatically augmented and many of these traits of synthetic wheats became more similar to the *Ae. tauschii* parent than to the durum parent, signifying that the salinity stress has improved functionality of the D-genome in the SHW, giving it the property of salt tolerance. This significant capability of instantly rearranging functionality of the sub-genomes in response to different growing environment is obviously an exceptional property for preferential use of SHWs in breeding (Yang *et al.*, 2014). Formerly, evaluation of the D-genome chromosome replacement stocks for the A and B genome chromosomes of *Triticum turgidum* cv. Langdon and D-genome-based synthetic hexaploid germplasm has shown us the chromosomal link of the trait responsible for salinity (Gorham *et al.*, 1987; Shah *et al.*, 1987). These studies confirmed that the D-genome has a trait situated on chromosome 4D with the ability to enhance K^+ : Na^+ perception. This subsequently led to ascribing the trait to the 4DL arm of chromosome 4 with the locus designated as '*kna1*' (Byrt *et al.*, 2007).

Ogbonnaya *et al.* (2013) validated the effective transmission of salt stress tolerance in SHW measured as Na^+

exclusion into a top Australian well-known wheat variety, Yitpi with few of SBLs displaying considerably improved Na⁺ exclusion in comparison with either the SHW or the persistent local wheat variety. This was also confirmed by an independent study where the SBL genotype ranked 3rd out of 150 lines evaluated for salinity tolerance using a hydroponic system at ICARDA. All the parameters studied showed positive correlation with each other that tells us that these traits were strongly linked with salinity tolerance and could be used to evaluate SHW genotypes in salt stress. All the genotypes with high survival rate indicated that they have resilience to deal with salinity stress inhibiting the wheat establishment at early germination stage. Overall, SHWs showed the potential to provide good plant stand establishment and biomass production at early seedling stage, which are essential to get high production under high-salt stress.

Conclusion and future prospects

SHWs have shown sufficient diversity for seedling emergence traits under salt stress and have the potential to be used for genetic dissection of loci underpinning salt tolerance. Recognizing that SHWs are to be important resources for inducing salinity tolerance through breeding we could also envision that the SHWs will not only allow us to exploit the D-genome-tolerant salinity trait, but also enable us to exploit the variability of the A and B genomes of the durum parent and thus widen the span of variability that would enhance overall productivity with simultaneous infusion of the 'intraspecific' breeding option integrated with the 'interspecific'. We will be applying genetic dissection studies using association mapping on these SHWs using the DARt marker data available (Rasheed *et al.*, 2014) on this panel, which will identify the potential genomic regions associated with adaptability and stability of seedling vigour under salt stress conditions.

Supplementary material

The supplementary material for this article can be found at <http://dx.doi.org/10.1017/S1479262116000198>

Acknowledgements

The facilitation extended by Abdul Aziz, Zahid Mahmood and Muhammad Jamil at Wheat Wide Crosses Program NARC (Pakistan) over the study phases is gratefully recognized and appreciated.

Author contribution

JQ and AM-K designed the research; ZK conducted the research; ZK and AR analysed the data and wrote the paper, JQ and AM-K checked and edited the manuscript. All authors have read and approved the final manuscript.

References

- Ali A, Arshad M, Naqvi SS, Ahmad M, Sher H, Fatima S, Kazi AG, Rasheed A and Mujeeb-Kazi A (2014) Exploitation of synthetic-derived wheats through osmotic stress responses for drought tolerance improvement. *Acta Physiologiae Plantarum* 36: 2453–2465.
- Ali Z, Salam A, Azhar FM and Khan IA (2007) Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. *South African Journal of Botany* 73: 70–75.
- Almansouri M, Kinet J-M and Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil* 231: 245–256.
- AOSA (1990) Rules for testing seeds. *Seed Technology Journal* 12: 101–112.
- Ashraf M (2010) Registration of 'S-24' spring wheat with improved salt tolerance. *Journal of Plant Registrations* 4: 34–37.
- Ashraf M and McNeilly T (1988) Variability in salt tolerance of nine spring wheat cultivars. *Journal of Agronomy and Crop Science* 160: 14–21.
- Baalbaki R, Elias S, Marcos-Filho J and McDonald MB (2009) *Seed Vigor Testing Handbook*. Ithaca: Association of Official Seed Analysts, 341p.
- Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M and Munns R (2007) HKT1; 5-like cation transporters linked to Na⁺ exclusion loci in wheat, Nax2 and Kna1. *Plant Physiology* 143: 1918–1928.
- Cramer GR (2003) Differential effects of salinity on leaf elongation kinetics of three grass species. *Plant and Soil* 253: 233–244.
- Dodd GL and Donovan LA (1999) Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *American Journal of Botany* 86: 1146–1153.
- Dreccer MF, Ogbonnaya FC and Borgognone G (2004) Sodium exclusion in primary synthetic wheats. In: Proc. XI Wheat Breeding Assembly, pp. 118–121.
- Dreccer MF, Borgognone MG, Ogbonnaya FC, Trethowan R and Winter B (2007) CIMMYT-selected derived synthetic bread wheats for rainfed environments: yield evaluation in Mexico and Australia. *Field Crops Research* 100: 218–228.
- Emebiri LC, Oliver JR, Mrva K and Mares D (2010) Association mapping of late maturity α -amylase (LMA) activity in a collection of synthetic hexaploid wheat. *Molecular Breeding* 26: 39–49.
- Fokar M, Nguyen HT and Blum A (1998) Heat tolerance in spring wheat. I. Estimating cellular thermotolerance and its heritability. *Euphytica* 104: 1–8.
- Gorham J, Hardy C, Wyn Jones RG, Joppa LR and Law CN (1987) Chromosomal location of a K⁺/Na⁺ discrimination character in the D genome of wheat. *Theoretical and Applied Genetics* 74: 584–588.
- Gororo NN, Eagles HA, Eastwood RF, Nicolas ME and Flood RG (2002) Use of *Triticum tauschii* to improve yield of wheat in low-yielding environments. *Euphytica* 123: 241–254.

- Ilyas M, Mahmood M, Ali A, Babar M, Rasheed A and Mujeeb-Kazi A (2015) Characterization of D-genome diversity for tolerance to boron toxicity in synthetic hexaploid wheat and *in silico* analysis of candidate genes. *Acta Physiologiae Plantarum* 37: 1–13.
- Joukhadar R, El-Bouhssini M, Jighly A and Ogbonnaya FC (2013) Genome-wide association mapping for five major pest resistances in wheat. *Molecular Breeding* 32: 943–960.
- Lopes MS and Reynolds MP (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Functional Plant Biology* 37: 147–156.
- Malcolm CV, Lindley VA, O'Leary JW, Runciman HV and Barrett-Lennard EG (2003) Germination and establishment of halophyte shrubs in saline environments. *Plant and Soil* 253: 171–185.
- McIntyre CL, Rattey A, Kilian A, Dreccer MF and Shorter R (2014) Preferential retention of chromosome regions in derived synthetic wheat lines: a source of novel alleles for wheat improvement. *Crop and Pasture Science* 65: 125–138.
- Mujeeb-Kazi A, Kazi AG, Dundas I, Rasheed A, Ogbonnaya FC, Kishi M, Bonnett D, Wang R-C, Xu S, Chen P, Mahmood T, Bux H and Farrukh S (2013) Genetic diversity for wheat improvement as a conduit to food security. *Advances in Agronomy* 122: 179–257.
- Mulki MA, Jighly A, Ye G, Emebiri LC, Moody D, Ansari O and Ogbonnaya FC (2013) Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. *Molecular Breeding* 31: 299–311.
- Munns R (1993) Physiological processes limiting plant growth in saline soil: some dogmas and hypotheses. *Plant Cell Environment* 16: 15–24.
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell and Environment* 25: 239–250.
- Munns R (2009) Strategies for crop improvement in saline soils. In: Ashraf M, Ozturk M and Athar HR (eds) *Salinity and Water Stress*. Netherlands: Springer, pp. 99–110.
- Munns R and James RA (2003) Screening methods for salt tolerance: a case study with tetraploid wheat. *Plant and Soil* 253: 201–218.
- Munns R, Richard AJ and Lauchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany* 57: 1025–1043.
- Norlyn JD and Epstein E (1982) Barley production: irrigation with seawater on coastal soil. In: Pietro AS (ed.) *Biosaline Research: A Look to the Future*. New York: Plenum, pp. 525–529.
- Ogbonnaya FC, Abdalla O, Mujeeb-Kazi A, Kazi AG, Steven X, Gosman N, Lagudah ES, Bonnett D, Sorells ME and Tsujimoto H (2013) Synthetic hexaploids: harnessing species of primary gene pool for wheat improvement. *Plant Breeding Review* 37: 35–122.
- Rasheed A, Xia X, Ogbonnaya FC, Mahmood T, Zhang Z, Mujeeb-Kazi A and He Z (2014) Genome-wide association for grain morphology in synthetic hexaploid wheats using digital imaging analysis. *BMC Plant Biology* 14: 128.
- Rauf M (2005) *Screening of wheat genotypes for better germination ability under low moisture levels in the laboratory*. MSc (Hons) Dissertation, University of Arid Agriculture, Rawalpindi, Pakistan.
- Rauf S, Adil MS, Naveed A and Munir H (2010) Response of wheat species to the contrasting saline regimes. *Pakistan Journal of Botany* 42: 3039–3045.
- Rawson HM, Richards RA and Munns R (1988) An examination of selection criteria for salt-tolerance in wheat, barley and triticale genotypes. *Australian Journal of Agricultural Research* 39: 759–772.
- Shabala S and Bose J (2012) Application of non-invasive micro-electrode flux measurements in plant stress physiology. In: Volkov AG (ed.) *Plant Electrophysiology*. Berlin, Heidelberg: Springer, pp. 91–126.
- Shah SMH, Gorham J, Forster BP and Jones RW (1987) Salt tolerance in the *Triticeae*: the contribution of the D genome to cation selectivity in hexaploid wheat. *Journal of Experimental Botany* 38: 254–269.
- Sohail Q, Shehzad T and Kilian A (2012) Development of diversity array technology (DART) markers for the assessment of population structure and diversity in *Aegilops tauschii*. *Breeding Science* 62: 38–45.
- Srivastava JP and Jana S (1984) Screening wheat and barley germplasm for salt tolerance. In: Staples RC and Toenniessen GH (eds) *Salinity Tolerance in Plants: Strategies for Plant Improvement*. New York: John Wiley & Sons, pp. 273–283.
- Tang Y, Yang W, Wu Y, Li C, Li J, Zou Y, Chen F and Mares D (2010) Effect of high molecular weight glutenin allele, Glu-B1d, from synthetic hexaploid wheat on wheat quality parameters and dry, white Chinese noodle-making quality. *Crop and Pasture Science* 61: 310–320.
- Tlig T, Gorai M and Neffati M (2008) Germination responses of *Diploaxis barra* to temperature and salinity. *Flora-Morphology, Distribution, Functional Ecology of Plants* 203: 421–428.
- Trethowan RM and Mujeeb-Kazi A (2008) Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. *Crop Science* 48: 1255–1265.
- Trethowan RM, Reynolds M, Sayre K and Ortiz-Monasterio I (2005) Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. *Annals of Applied Biology* 146: 405.
- Wyn Jones RG and Gorham J (1991) Physiological effects of salinity: scope for genetic improvement. In: Acevedo E, Fereres E, Giménez C and Srivastava J (eds) *Improvement and Management of Winter Cereals Under Temperature, Drought and Salinity Stresses*. Proceeding of the International Symposium, October 1987, Cordoba, Spain. Córdoba, Spain: Proceedings of the ICARDA-INIA Symposium, pp. 177–201.
- Yang C, Zhao L, Zhang H, Yang Z, Wang H, Wen S, Zhang C, Rustgi S, von Wettstein D and Liu B (2014) Evolution of physiological responses to salt stress in hexaploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* 111: 11882–11887.
- Yildirim E, Dursun A, Guvenc I and Kumlay A (2002) The effects of different salt, biostimulant and temperature levels on seed germination of some vegetable species. II Balkan Symposium on Vegetables and Potatoes, *Acta Horticulturae* 579: 249–253.