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ORIGINAL ARTICLE

Applying an artificial neural network approach for drought tolerance screening among Iranian wheat landraces and cultivars grown under well-watered and rain-fed conditions

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Abstract
In the current study, an α-lattice design was used to investigate 320 Iranian bread wheat cultivars and landraces under non-stressed and rain-fed conditions, according to phenological, morphological and physiological parameters. An artificial neural network (ANN) was trained to evaluate the relative importance of different drought tolerance indices (DTIs) using a multilayer perceptron model. Our findings suggest that the Iranian wheat germplasm harbors large genetic diversity for all the studied traits. Correlation analyses highlighted the important role of seed number per spike, thousand kernel weight, leaf greenness and canopy temperature in predicting grain yield under both non-stressed and rain-fed conditions. Moreover, correlations between stressed-yield (Y_s) and yield index (Y_I, $r=1^{**}$), harmonic mean (HM, $r=0.94^{**}$), geometric mean productivity (GMP, $r=0.86^{**}$), and stress tolerance index (STI, $r=0.86^{**}$) were all large, which was further confirmed by the results of ANN and a principal component analysis. A hierarchical clustering, visualized using a heatmap plot, classified cultivars and landraces into four separate groups, where high-yielding and drought-tolerant genotypes clustered in the same group. The result of ANN indicated that MP and YI had the highest relative importance for screening compatible genotypes for well-watered and rain-fed conditions, respectively. Overall, the selection of genotypes according to agronomic and physiological traits in association with an appropriate DTI can identify favorable wheat genotypes in a field trial to breed for well-watered and water-limited environments. Furthermore, the ANN successfully evaluated the relative importance of different DTIs in wheat.

Keywords Artificial neural network · Drought tolerance indices · Multilayer perceptron · Principal component analysis · *Triticum aestivum*

Introduction

Bread wheat (*Triticum aestivum* L.) is grown on 220 million ha throughout the world with 757.9 million ton of grain was produced in 2017 which then dropped to 734.7 million ton in 2018 (FAO STAT 2018). The cultivation of wheat has been extended to arid and semi-arid areas, although it is most commonly grown in humid, cold and moderate temperate zones (Chakraborty et al. 2008; Fehér et al. 2017; Avila et al. 2019). Wheat provides an important part (about 20%) of the required calories for approximately 1/3 of all humans (Byerlee and de Polanco 1983; Fu et al. 2009). Wheat demand has steadily been increasing as a consequence of population growth, particularly in developing countries, such as in South Asia, Africa and South America (Chatrath et al. 2007; Ray et al. 2013; Mason et al. 2015). Most current breeding programs of wheat are aimed at increasing

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yield potential, but environmental stresses, such as drought, salinity, cold, and fungi pathogens, remain severe challenges for sustainable production (Mondal et al. 2013). Soil–water deficiency has been reported to curb about half of the wheat production, and fluctuations in annual precipitation lead to a direct influence on wheat output worldwide (Parry et al. 2004).

Although many morphological, physiological and molecular traits are involved in plant drought tolerance, they are usually summarized through three major processes, drought escape, dehydration avoidance, and dehydration tolerance (Van Ginkel et al. 1998; Mickelbart et al. 2015). In the areas with terminal water deficit, such as Iran, drought escape is a desirable property and leads to higher performance and lower drought-induced damage during the grain filling period (Isidro et al. 2011; Shavrukov et al. 2017). Previous research has indicated that modern wheat cultivars start flowering at an earlier stage compared to older cultivars (Isidro et al. 2011; Álvaro et al. 2008). Identifying high-yielding and early-maturing genotypes should consequently be a priority in wheat-breeding programs aimed at areas experiencing terminal drought. On the other hand, increasing proline content, relative water content, water-soluble carbohydrates, and reducing in canopy temperature are important physiological traits which are known to accelerate drought tolerance (Foulkes et al. 2007; Zhang et al. 2014; Mwadzingeni et al. 2016; Thapa et al. 2018). Another important physiological trait is the 'stay-green under stress' condition that is associated with a delay in leaf senescence and the maintenance of high chlorophyll content. In wheat, this trait is especially important for the flag leaf as it plays a crucial role in providing carbohydrates during the grain filling period (about 30–50% of grain carbohydrates, Ramya et al. 2016). Chlorophyll pigments degrade under drought stress and this leads to a reduction in final grain yield. Genotypes with high pigment content and stronger 'stay-green' phenotypes are, therefore, better able to utilize available resources under adverse environmental conditions (Rivero et al. 2007; Kalaji et al. 2016; Rehman et al. 2016).

Wheat is characterized by very large genome size, about 17 Gb, and a striking amount of genetic variation in morphological and molecular characteristics (Devos et al. 2009; Battenfield et al. 2016). Although many reports focus on the improvement of drought tolerance using molecular breeding techniques, the success of such approaches in producing truly tolerant wheat genotypes is still low compared to classical breeding methods (Mir et al. 2012; Barakat et al. 2016; El-Hendawy et al. 2017). However, breeding efforts aimed at developing stable and high-performing varieties, especially in arid and semi-arid areas, relies on using high-efficiency methods for screening genetic resources based on suitable traits and indices (Fleury et al. 2010; Budak et al. 2015; Reynolds et al. 2015). Drought tolerance indices (DTIs) have been widely used to identify compatible genotypes by assessing their performance under non-stressed and water-limited conditions (Abdolshahi et al. 2015; Mursalova et al. 2015). For instance, the tolerance index (TOL), stress tolerance index (STI), geometric mean productivity (GMP), and stress susceptibility index (SSI) have all been previously employed for selecting genotypes (Mursalova et al. 2015; Ali and El-Sadek 2016). Mathematical algorithms, such as regression, clustering or principal component analysis can be employed to investigate the efficiency of these various indices and identify the best criterion for assessing genotypes (Hefny et al. 2013; Sahar et al. 2016).

Artificial neural network (ANN) is a novel statistical method that can be trained according to different purposes in biological and agricultural experiments (Kaul et al. 2005; Matsumura et al. 2015; Safa et al. 2015). ANN determines the association of inputs and outputs using a non-linear model based on a transformative mathematical structure. This underlying system is motivated by analogies to animal brains, which has the ability to learn algorithms and proceed with complex traits (Gardner and Dorling 1998; Schmidhuber 2015). An ANN is made up of a series of related nodes, or artificial neurons, with the goal to mimicking the human brain. ANNs are designed to work on predictions, associative memory, optimization, and control with great flexibility and has many advantages across different applications (Safa et al. 2015; Schmidhuber 2015). Alvarez (2009) applied ANN for the prediction of wheat performance affected by soil and climate conditions in Argentina. Similarly, Safa et al. (2015) employed ANN to predict wheat production using data collected from 40 agricultural lands in New Zealand. Although almost all previous research about the use of ANN in agriculture have been concentrated on yield prediction in plants such as soybean (Kaul et al. 2005), corn (Matsumura et al. 2015), and wheat (Pantazi et al. 2016), ANN has recently been used for identifying salinity-tolerant genotypes in Iranian wheat cultivars (Ravari et al. 2016). However, thus far ANN has not been employed for the assessment of drought tolerance in wheat. The first aim of the present study was, therefore, to screen Iranian wheat landraces and cultivars using different DTIs and a collection of agronomic and physiological traits to gain a better insight into how these indices and traits changed across different environments. Second, we applied an ANN approach to investigate different measures of DTIs and to determine the relative importance of each index in a wheat-screening program, to better understand how ANNs can complement the result of traditional linear models.

Materials and methods

Plant materials

For the experiments described in this paper, we used a collection of 320 Iranian wheat genotypes (supplementary table S1), including 102 cultivars (tolerant and susceptible; spring, winter, and facultative cultivars, supplementary table S1), that were released between 1942 and 2014, and 218 landraces gathered from 1931 to 1968 from different locations of Iran (Fig. 1). The seeds were provided by the University of Tehran, Iran and the International Centre for the Improvement of Maize and Wheat (CIMMYT), Mexico.

Experimental condition and field trial site

Accessions were grown during the 2016–2017 cropping season at the agricultural research farm of the University of Tehran, located at N 35′0.80° and E 50′0.95° in Karaj, Iran. An alpha-lattice design was used to investigate genotypes under well-watered (non-stressed, tape irrigation) and rain-fed (drought stress) conditions using two biological replications for each genotype. An alpha-lattice is an incomplete block design with the flexibility to control accidental alterations when testing a large number of genotypes. The pattern of the alpha-lattice design used in the experiment was determined by CycDesigN 3 software. The 320 accessions were distributed among 32 incomplete blocks based on the design map, where each block contained 10 plots for 10 accessions. In both environments, each plot contained four rows (1×1 m^2) spaced at 0.5-m intervals. The lattice map and the number of replications were the same for two conditions and available water resource was the only difference between the two treatments. For the well-watered condition, 40 mm evaporation from an evaporation pan was considered the threshold for the initiation of irrigation. Crop evapotranspiration (ET_c) was then calculated using reference crop evapotranspiration (ET_0) and the crop coefficient (K_C) for each month

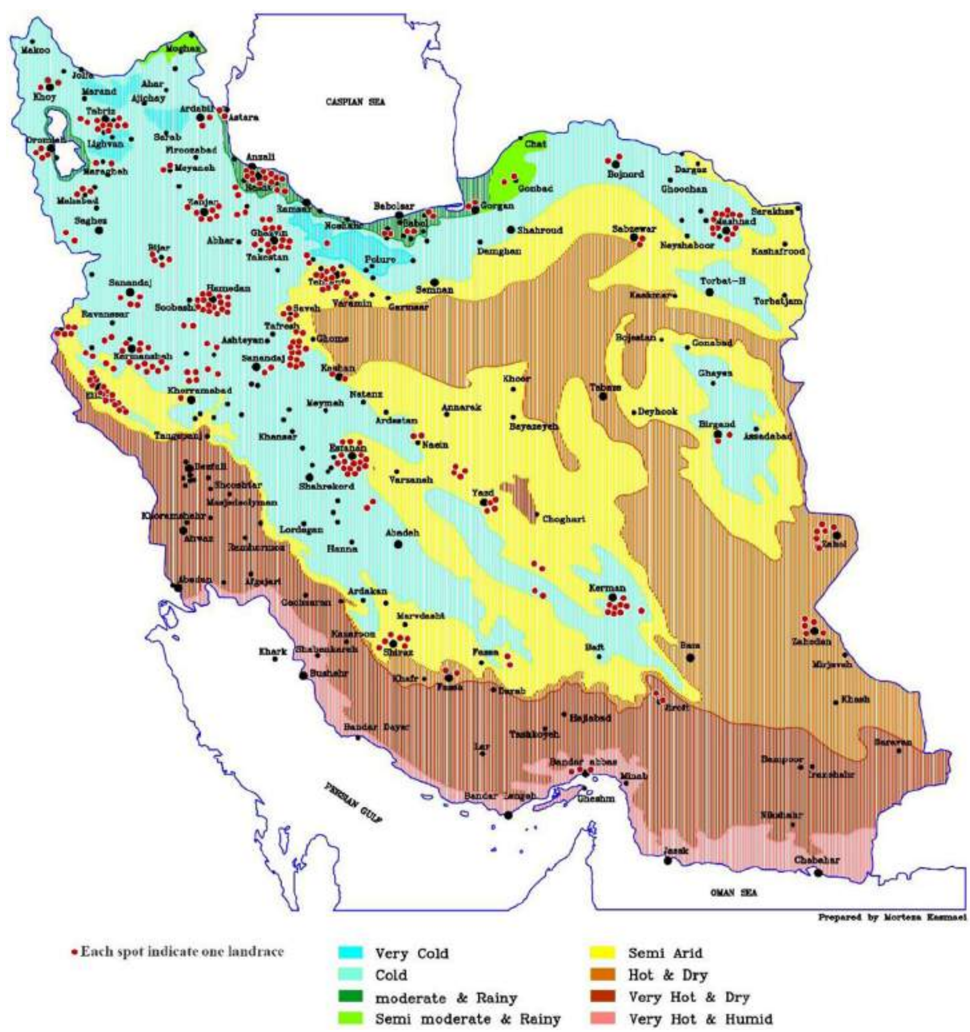

Fig. 1 The geographic distribution of Iranian wheat landraces collected between 1931 and 1968 years

where irrigation was required (Table 1). ET_0 was measured using the following equation (Jensen 1974):

$$ET_0 = K_{pan} \times E_{pan},$$

where K_{pan} is pan coefficient (0.8) and E_{pan} is the depth of evaporation from the pan surface (40 mm).

The crop coefficients, K_C, used in this experiment were extracted from Kang et al. (2003) and then used to calculate ET_c through the following equation:

$$ET_C = K_C \times ET_0.$$

The volume of required water for each ha (m^3/ha) was calculated using the depth of crop evapotranspiration (mm) multiplying by 10. The time of irrigation was calculated using the ratio of allocated water for 1377.5 m^2 (the cultivation area of 320 accessions in two replications) to water discharge (10.8 m^3/h, Table 1). On the other hand, plants grown under rain-fed condition were only exposed to natural precipitation, which served as the sole as water resource. The patterns of average monthly precipitation and maximum, mean, and minimum temperature for the 2016–2017 cropping season are illustrated in supplementary figure S1.

Field trial

Phenological stages, including days to emergence (Zadoks 12), days to anthesis (Zadoks 65), and days to physiological maturity (Zadoks 91) were scored when half of each plot reached the corresponding stage. Leaf greenness was measured using a SPAD502 Plus Chlorophyll Meter at Zadoks 60 and canopy temperature was measured at Zadoks 60 in Jun 2017 between 1 and 2 p.m. using a LIHERO Infrared thermometer. DTIs were calculated based on the grain yield per plant for well-watered (Y_P), rain-fed (Y_S), the total average of grain yield for well-watered ($\bar{Y}_P$), and rain-fed ($\bar{Y}_S$) conditions using the formulas displayed in Table 2.

Phenotypic and genotypic coefficients of variation

The phenotypic and genotypic variation was assessed using the coefficients of variation as described by Singh and Chaudhary (1979):

$$\text{Phenotypic variance } (\sigma^2 ph) = \frac{\left(\sigma^2 e + r\sigma^2 g\right)}{r},$$

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\left(\sigma^2 e + r\sigma^2 g\right) - \sigma^2 e}{r},$$

Table 1 Summary of allocated water to the cultivation area for the well-watered treatment

Month	ET_0 (mm)	K_C	ET_C (mm)	Required water per ha (m^3/ha)	Required water for 1377 m^2 (m^3)	Water discharge (m^3/h)	Period of irrigation (h)
March	40	0.92	36.8	368	50.69	10.8	4.69
April	40	1.33	53.2	532	73.28	10.8	6.79
May	40	1.15	46	460	63.37	10.8	5.87
June	40	0.58	23.2	232	31.96	10.8	2.96

Table 2 Drought tolerance indices used for investigation of Iranian wheat germplasm

Index	Abbreviation	Equation	References
Tolerance index	TOL	$Y_p - Y_s$	Rosielle and Hamblin (1981)
Mean productivity	MP	$\frac{Y_p+Y_s}{2}$	Bouslama and Schapaugh (1984)
Geometric mean productivity	GMP	$\sqrt{Y_p \times Y_s}$	Mardeh et al. (2006)
Stress tolerance index	STI	$\frac{Y_p \times Y_s}{\bar{Y}_p^2}$	Fernandez (1993)
Harmonic mean	HM	$\frac{2(Y_p \times Y_s)}{Y_p+Y_s}$	Rosielle and Hamblin (1981)
Yield stability index	YSI	$\frac{Y_s}{Y_p}$	Bouslama and Schapaugh (1984)
Stress susceptibility index	SSI	$\frac{1-\frac{Y_S}{Y_P}}{1-\frac{\bar{Y}_S}{\bar{Y}_P}}$	Fischer and Maurer (1978)
Yield index	YI	$\frac{Y_s}{\bar{Y}_s}$	Gavuzzi et al. (1997)

Y_P performance under well-watered condition, Y_S performance under stress condition, $\bar{Y}_P$ mean performance of all genotypes under well-watered conditions, and $\bar{Y}_S$ mean performance of all genotypes under stress conditions

Table 3 Mean squares and significance of Iranian wheat accessions from a combined analysis of variance for agronomic traits

Source	Environment	Environment × replication	Environment × replication × block	Genotype	Genotype × environment	Error	C.V (%)
Traits df	1	2	124	319	319	514	–
DE	10,992.19**	79.21	5.28	14.87**	18.33**	3.29	4.36
DA	164,507.13**	1.66	0.77	60.1**	7.22**	0.68	0.48
DM	161,191.01**	20.84	2.01	121.69**	13.22**	2.56	0.85
SW	406.43**	1.78	0.17	0.55**	0.21**	0.14	17.27
SL	130.64**	7.99	1.6	8.06**	1.82**	1.43	11.62
GY	296.46**	1.67	0.1	0.31**	0.16**	0.09	19.99
SN	26,756.44**	1228.73	69.03	234.7**	65.48**	46.82	16.08
TKW	80,244.73**	72.39	23.8	77.86**	34.77**	18.99	12.6
LG	14,394.29**	98.09	31.84	160.43**	100.89**	32.59	11.31
CT	5057.71**	77.47	8.51	11.44**	7.08 ns	6.76	8.99

DE days to emergence, *DA* days to anthesis, *DM* days to physiological maturity, *SW* spike weight, *SL* spike length, *GY* grain yield per plant, *SN* seed number per spike, *TKW* thousand kernel weight, *LG* leaf greenness, *CT* canopy temperature, *ns* non-significant

*, **Significant at 5, and 1% probability level

$$\text{Phenotypic coefficient of variation (P.C.V)} = \frac{\sqrt{\sigma^2 ph}}{\bar{X}} \times 100,$$

$$\text{Genotypic coefficient of variation (G.C.V)} = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100,$$

where $\sigma^2 e$ is error variance, $\sigma^2 g$ is genotypic variance, r is the number of replications, and $\bar{X}$ is the population mean for a specific trait.

Artificial neural network (ANN)

An artificial neural network analysis was performed on grain yield per plant using data from both well-watered and rain-fed treatments using a multilayer perceptron (MLP) model which contained an input, hidden, and output layer, respectively (Gardner and Dorling 1998). The number of input neurons was set equal to the eight drought tolerance indices, and Y_P and Y_S were used as output layers. The dataset was divided into three sets including training (224, 70% of observations), validation (48, 15% of observations) and testing (48, 15% observations) randomly to reduce overfitting in the training phase. The designed network was trained 12 times during which at each step one layer was added to other hidden layers using the trial-and-error method. Each network trained using as high as a possible number of epochs, minimizing the mean square error (MSE). This training phase continued epoch by epoch to determine the MSE for the validation phase. Finally, the epoch which resulted in the lowest MSE value in the validation phase was selected for further processing. Hyperbolic tangent and identity activation functions were used in hidden and output layers, respectively. Hyperbolic tangent transformed actual observations into values between 1 and − 1 using the following equation:

$$\gamma(c) = \frac{e^c - e^{-c}}{e^c + e^{-c}}.$$

The identity function uses actual observation without any transformation through the following equation:

$$\gamma(c) = c.$$

The Levenberg–Marquardt algorithm was used to train the network (Moré 1978) and two common indices, including the mean square error (MSE) and coefficient of determination (R^2), were used to evaluate the model validations as described by Ravari et al. (2016). The importance of each independent variable in the neural network was determined based on the training and testing observations.

Statistical analysis

All descriptive statistics and the analysis of variance for all studied traits were calculated using the Means and GLM procedures in SAS v.9.4 software. Color correlation of DTIs and principal component analysis (PCA) to assess the distribution of Iranian landraces and cultivars across the biplot diagram were conducted using Past software (Hammer et al. 2001). We also applied a heatmap analysis using the d3heatmap, dendextend, and gplots packages in RStudio to classify all genotypes according to the drought tolerance indices. The ANN analysis was performed according to the MLP model using SPSS v.22 and MATLAB v 9.2.

Results

Agronomic and physiological parameters

Table 3 displays significant differences among genotypes and environments ($P \leq 0.01$). This result highlights the high genetic variability that exists among Iranian wheat landraces and cultivars in terms of both agronomic parameters and their responses to the two environments. There were significant differences in genotype by environment interaction for growth stage parameters. Three categories of traits including phenology, yield-related and physiological parameters showed variation across accessions and environments. Grain yield and spike weight showed the highest coefficient of variation, respectively.

The descriptive statistics analysis suggests that landraces were more variable in terms of phenological traits under both well-watered and rain-fed conditions compared to cultivars (Tables 4, 5). Drought stress leads to a reduction in the number of days required to complete a specific growth stage. For instance, days to anthesis and physiological maturity were both reduced under drought conditions for both cultivars and landraces. The range of each phenological trait under well-watered treatment was greater compared to under rain-fed condition. Significant differences were observed between the two environments for all studied traits based on *t* tests. The Y_P for landraces and cultivars was 1.98 and 1.97 g per plant, respectively, while Y_S was reduced by approximately

Table 4 Descriptive statistics for agronomic traits in Iranian wheat landraces grown under well-watered and rain-fed environments

Trait	Well watered						Rain fed						
	Mean	Range	SD	SE	P.C.V (%)	G.C.V (%)	Mean	Range	SD	SE	P.C.V (%)	G.C.V (%)	*t* test
DE	35.54	31.35–39.36	1.48	0.1	7.33	5.95	41.38	32.95–53.03	4.27	0.29	13.07	12.95	− 18.54**
DA	179.03	160–190	4.75	0.32	3.35	3.33	156.47	144.51–172.48	4.55	0.31	3.62	3.60	111**
DM	204.53	190–217	5.99	0.41	4.00	3.96	181.95	171–195.5	6.07	0.41	4.62	4.57	84.11**
SW	2.76	1.5–4.88	0.54	0.037	28.99	27.11	1.62	0.82–2.59	0.33	0.02	32.08	28.29	35.01**
SL	10.5	5.52–15.39	1.81	0.12	24.84	23.39	9.93	6.03–13.35	1.45	0.1	21.35	19.73	6.51**
GY	1.98	0.97–3.52	0.42	0.03	31.34	29.45	1.02	0.5–1.84	0.26	0.02	40.66	35.35	34.63**
SN	47	26.29–85.01	9.98	0.67	30.57	28.91	38.2	18.84–58.81	8.15	0.55	31.04	28.15	16.50**
TKW	42.63	26.16–60.86	6.51	0.44	22.05	20.73	26.6	18.34–42.02	4.26	0.28	24.74	22.09	39.54**
LG	51.81	37.61–67.83	7.95	0.54	16.39	16.20	45.6	25.64–69.45	8.57	0.58	18.62	18.41	9.27**
CT	27.28	22.26–32.92	1.93	0.13	11.55	9.97	31.4	23.5–38.25	2.03	0.14	10.26	8.87	− 22.87**

DE days to emergence, *DA* days to anthesis, *DM* days to physiological maturity, *SW* spike weight, *SL* spike length, *GY* grain yield per plant, *SN* seed number per spike, *TKW* thousand kernel weight, *LG* leaf greenness, *CT* canopy temperature

**Significant at 1% probability level

Table 5 Descriptive statistics for agronomic traits in Iranian wheat cultivars grown under well-watered and rain-fed environments

Trait	Well watered						Rain fed						
	Mean	Range	SD	SE	P.C.V (%)	G.C.V (%)	Mean	Range	SD	SE	P.C.V (%)	G.C.V (%)	*t* test
DE	35.44	30.43–38.33	1.53	0.15	7.35	5.97	41.34	35–54.51	4.67	0.46	13.08	12.97	− 11.6**
DA	179.58	171.5–188.5	3.74	0.37	3.34	3.32	156.66	148.12–164.06	3.36	0.33	3.62	3.60	91.78**
DM	205.12	192.5–217.5	6.09	0.6	3.99	3.95	182.99	172–196	6.39	0.63	4.59	4.55	63.57**
SW	2.74	1.49–3.83	0.53	0.05	29.20	27.31	1.62	0.83–2.48	0.34	0.03	32.08	28.29	24.09**
SL	10.81	6.29–14.77	1.71	0.17	24.12	22.72	10.03	5.77–13.33	1.43	0.14	21.14	19.54	5.55**
GY	1.97	1.05–2.87	0.41	0.04	31.50	29.60	1.01	0.38–1.66	0.28	0.03	41.06	35.70	24**
SN	47.37	26.78–86.92	10.19	1	30.33	28.68	37.5	19.5–55.2	8.05	0.79	31.62	28.67	11.48**
TKW	42.28	25.7–60.71	6.47	0.64	22.23	20.90	26.86	17.31–43.36	4.93	0.49	24.50	21.87	24.86**
LG	51.65	38–77.98	7.93	0.78	16.44	16.25	46.24	29.6–67.6	7.76	0.77	18.37	18.16	6.78**
CT	27.09	22.18–31.13	1.86	0.18	11.63	10.03	31.19	23–38.1	2.21	0.22	10.33	8.93	− 18.06**

DE days to emergence, *DA* days to anthesis, *DM* days to physiological maturity, *SW* spike weight, *SL* spike length, *GY* grain yield per plant, *SN* seed number per spike, *TKW* thousand kernel weight, *LG* leaf greenness, *CT* canopy temperature

**Significant at 1% probability level

48% for both types of accessions. A summary of grain yield is provided in Supplementary Table S2. The range of seed number per spike in cultivars was slightly greater that landraces under the well-watered regime, whereas landraces showed greater differences under rain-fed condition.

Although the mean of leaf greenness and canopy temperature were higher in landraces, cultivars illustrated a relatively greater variation in these physiological traits (Tables 4, 5). The minimum canopy temperature of landraces and cultivars was 23.5 and 23.0, respectively, under rain-fed condition. For accessions grown under well-watered conditions, leaf greenness and seed number showed the maximum differences among cultivars and landraces, respectively. We observed high phenotypic and genotypic variation for grain yield and seed number in both landraces and cultivars (Tables 4, 5). The smallest phenotypic and genetic variation was measured for days to anthesis and days to physiological maturity. Overall, for all the studied traits, genetic variation constituted the major part of the total phenotypic variance.

Correlations among the studied traits

Correlations among all traits are displayed in Table 6 for well-watered (above diagonal) and rain-fed conditions (below diagonal). There was a significant correlation between days to physiological maturity and grain yield ($r=0.119$*) under well-watered condition. The highest correlation coefficients were observed between grain yield, spike weight and seed number ($r=0.972$** and 0.707**, respectively). Similarly, in the rain-fed environment, the maximum correlation coefficients were observed among grain yield, spike weight and seed number ($r=0.931$**, 0.756**, respectively). A positive correlation between days to anthesis and days to physiological maturity was observed under rain-fed condition ($r=0.515$**). Despite a low and positive association between grain yield and days to emergence, the correlation between grain yield and days to anthesis and maturity were significantly negative. In contrast, there was a high and positive relationship between spike weight and seed number ($r=0.795$**). Interestingly, we observed a positive and significant correlation between grain yield and chlorophyll content in both environments ($r=0.52$**, and 0.3**), while grain yield and canopy temperature were negatively correlated ($r=-0.216$** and −0.24**).

Drought tolerance indices

We observed a significant positive correlation between Y_P and all drought tolerance indices, except YSI which showed a negative relationship (Fig. 2). The Y_S was negatively correlated with TOL, and SSI and positively correlated with MP, GMP, STI, HM, YSI, and YI. Overall, the correlations between Y_P, Y_S and GMP, STI, and HM indices were all large. Selecting genotypes according to these indices will, therefore, favor genotypes showing high performance in both well-watered and rain-fed environments.

Principal component analysis (PCA) for drought tolerance indices

We performed a principal component analysis (PCA) to evaluate the response of all accessions to both environments to assist with the environment-based selection of superior genotypes (Fig. 3). Even if cultivars have been bred for well-watered or rain-fed conditions, surprisingly enough some of them grouped into the same area of the biplot (Fig. 3a). The two main principal components (PC1 and PC2) explained

Table 6 Correlation coefficient between agronomic traits for Iranian wheat genotypes in the well-watered (above diagonal) and rain-fed (below diagonal) environment

	DE	DA	DM	SW	SL	GY	SN	TKW	LG	CT
DE	1	0.019	−0.021	0.024	−0.026	0.035	−0.064	0.138*	0.095	0.037
DA	0.103	1	0.609**	−0.059	0.470**	−0.108	−0.160**	0.050	−0.105	−0.097
DM	−.0002	0.515**	1	0.134*	0.382**	0.119*	0.004	0.128*	0.048	−0.084
SW	0.048	−0.34**	−0.22**	1	0.006	0.972**	0.698**	0.369**	0.501**	−0.222**
SL	−0.14*	0.279**	0.285**	−0.026	1	−0.065	−0.101	0.020	−0.085	−0.003
GY	0.088	−0.36**	−0.26**	0.931**	−0.124*	1	0.707**	0.395**	0.52**	−0.216**
SN	−0.068	−0.35**	−0.22**	0.795**	−0.092	0.756**	1	−0.34**	0.302**	−0.111*
TKW	0.237**	−0.142*	−0.15**	0.445**	−0.095	0.586**	−0.061	1	0.283**	−0.124*
LG	−0.038	−0.035	0.073	0.374**	0.101	0.299**	0.28**	0.1	1	−0.197**
CT	0.161**	−0.24	−0.046	−0.237	−0.17**	−0.24**	−0.19**	−0.09	−0.19**	1

DE days to emergence, *DA* days to anthesis, *DM* days to physiological maturity, *SW* spike weight, *SL* spike length, *GY* grain yield per plant, *SN* seed number per spike, *TKW* thousand kernel weight, *LG* leaf greenness, *CT* canopy temperature

*, **Correlation is significant at the 0.05 and 0.01 level, respectively (2-tailed)

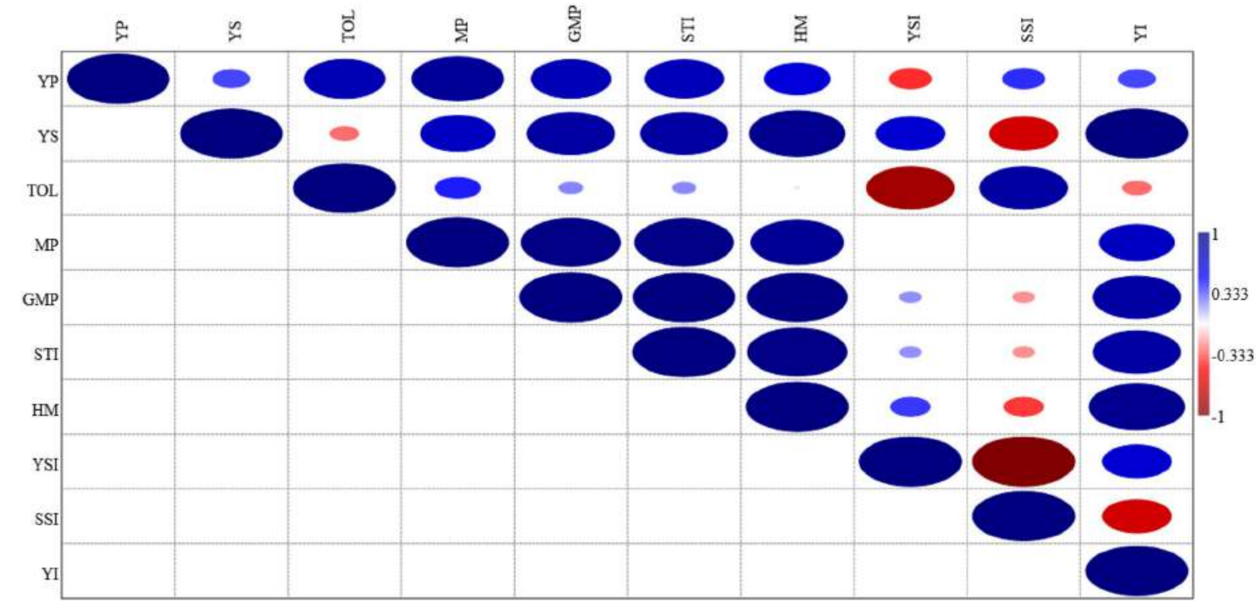

Fig. 2 The color correlation between Y_P, Y_S and various drought tolerance indices for Iranian wheat landraces and cultivars. Y_P well-watered grain yield, Y_S drought-stressed grain yield, *TOL* tolerance index, *MP* mean product, GMP geometric mean product, *STI* stress tolerance index, *HM* harmonic mean, *YSI* yield stability index, *SSI* stress susceptibility index, *YI* yield index

58.4% and 40.8% of the total variance, respectively. The first principal component was positively associated with Y_P, MP, GMP, STI, YI, HM, and Y_S suggesting that PC1 can be used for identifying high yielding cultivars for both normal irrigation systems and rain-fed conditions. The second principal component was positively correlated with SSI and TOL. Cultivars Koohdasht, Naz, Dena, and Moghan3 showed high grain yield in both environments.

The PCA for the Iranian landraces shows that two first principal components explained 53.0% and 46.2% of the total variance, respectively (Fig. 3b). PC1 was positively correlated with Y_P, MP, GMP, STI, Y_S, YI and HM, while PC2 was positively associated with YSI and negatively associated with and SSI and TOL. Screening-based PC1 identifies high-performing and drought-tolerant genotypes, such as accessions 627410, 624582, 626747, and 625047 which are superior landraces with the capacity to being employed in rain-fed environments. However, there are other genotypes which showed varying responses to drought and that grouped into other parts of the biplot. Plant breeders could, therefore, utilize these genotypes in future breeding programs depending on the actual breeding goals.

Cluster analysis

We also created a heatmap plot based on the Ward (1963) method to classify accessions according to the various drought tolerance indices (Fig. 4). In this hierarchical grouping, distances between groups were calculated according to the amount of increase in the sum of squares observed when they are merged in the model, so that within-group variance remain low until each binary merge point. Based on this analysis, Iranian cultivars can be categorized into four groups (Fig. 4a). Uruom, Naz, Koohdasht, Pishgam, Neishabour, Chamran 2, Mahdavi, Dena, Moghan 3, and Akbari are all high-performing cultivars under both environments and were classified as group A. An additional 23, 29 and 40 cultivars were classified into group B, C, and D, respectively. The majority of genotypes that were classified into group A were new cultivars that have been released since 2000 year. The genotypes that clustered into group B were old and low-performing cultivars under rain-fed condition. A similar analysis conducted on the 218 wheat landraces also grouped them into four major clusters, with 35, 73, 51, and 59 landraces for the four groups, respectively (Fig. 4b). Group A contained 35 genotypes that show high grain yield in both environments. When these landraces were evaluated, we found that most of them were originally collected from five provinces, including Sistan-Balouchestan, Kermanshah, Kordestan, Kerman, and Yazd. Other accessions of this group were also collected from Khorasan, Esfahan, Gilan, Mazandaran, Azarbayjan-Shargi, Hamadan, Ilam, Hormozgan, Markazi, Khouzestan, Tehran, and Zanjan. A hierarchical cluster analysis on DTIs obtained from the cultivars and landraces grouped them into three clusters (cluster I, II, and III; Fig. 4a, b). For the cultivars, GMP, STI, and HM grouped in cluster I, Y_S, YI, and YSI grouped in cluster II, TOL, SSI, MP, and Y_P were separated into cluster III. On

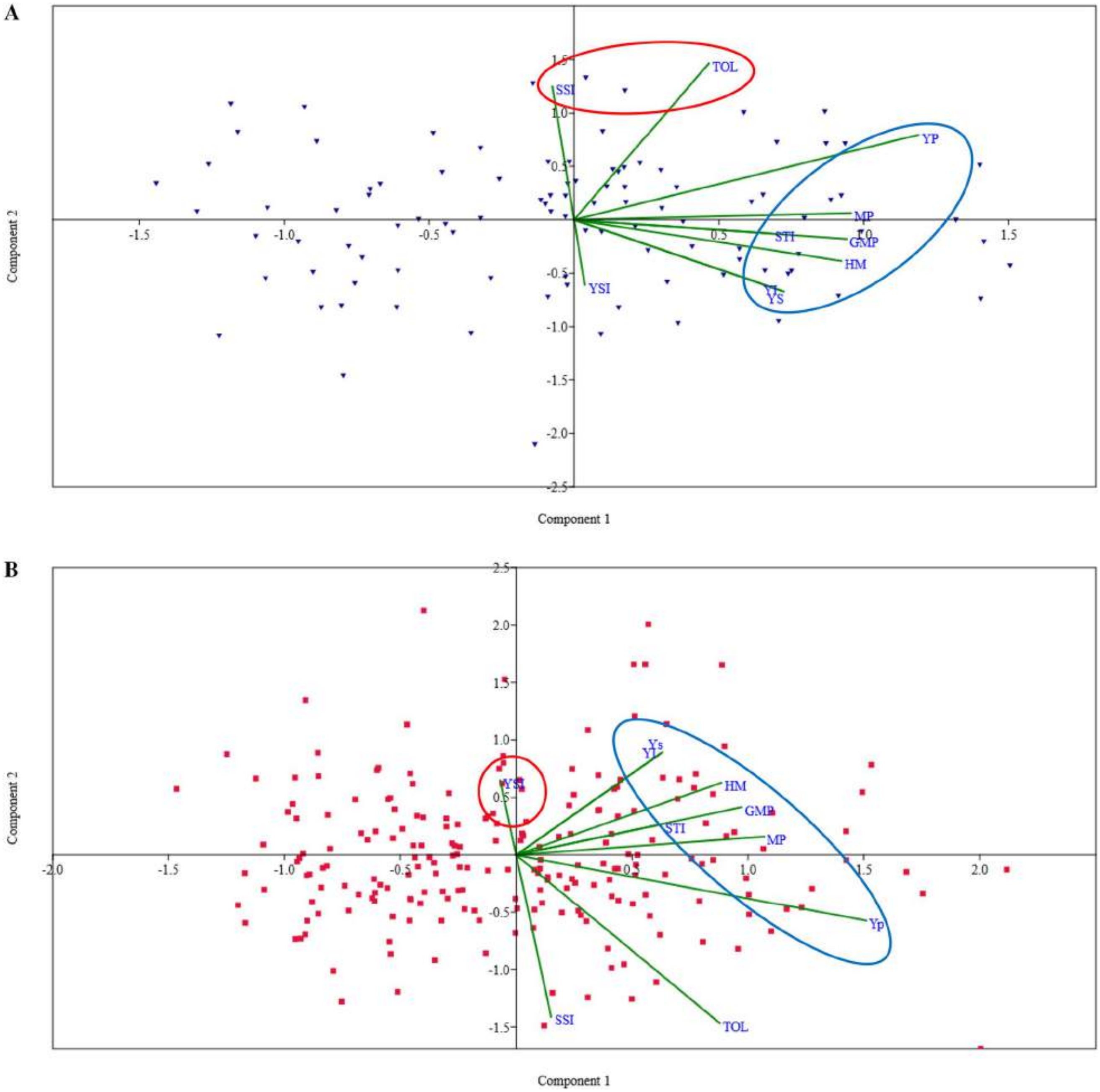

Fig. 3 Principal component analysis of Iranian wheat germplasm exposed to well-watered irrigation and rain-fed environments using PC1 and 2. **a** Biplot for 102 cultivars and **b** biplot for 218 landraces

the other hand, for the landraces GMP, STI, HM, MP, and Y_P formed cluster I, Y_S, YI, and YSI grouped in cluster II, TOL and SSI were separated into cluster III.

Artificial neural network

We trained an artificial neural network using the eight DTIs derived from the experiments in the two environments as an input layer. This is indicated by the input neurons in Fig. 5. Grain yield under well-watered and rain-fed conditions was considered as output layers. Eight neurons were obtained through hyperbolic tangent activation of the hidden layer. The number of epochs, learning initial, momentum, interval offset were 1000, 0.4, 0.9, and 0.5, respectively. The momentum inhibits inconsistency resulting from a high learning rate and should fall in the range 0 and 1. The speed of network training, determined by the learning rate, that was 0.9 (range is 0–1) in the

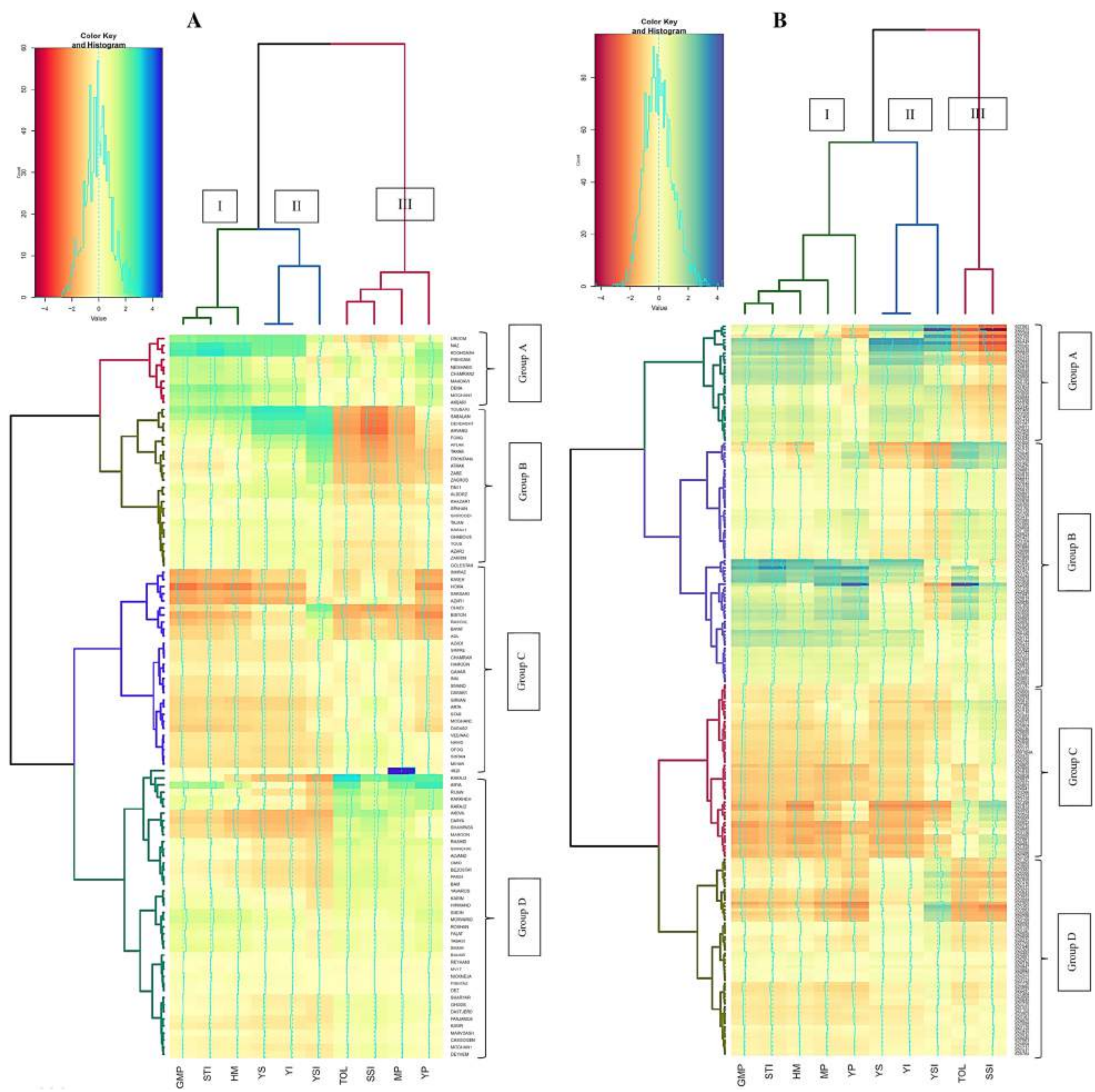

Fig. 4 Heatmap and hierarchical clustering of Iranian accessions based on drought tolerance indices. **a** Cluster analysis for 102 Iranian wheat cultivars and **b** cluster analysis for 218 Iranian landraces

present study, actually higher learning rate will result in faster network training.

The values of network performance after the training phase peaked at 2.57 when 12 neurons were selected in the hidden layer. However, the maximum R^2 and minimum MSE were obtained when selecting 8 neurons as elements in the hidden layer (Table 7). Ultimately a network containing one hidden layer with eight hidden neurons was selected for further investigations. The test of 8 neurons in the hidden layer resulted in values of 0.99, 0.96, and 0.97 for R^2 for the training, validation and testing phase, respectively. As depicted in Fig. 6, the curve of the training phase was located under the validation curve which is expected for correct network training. The optimum value of MSE was approximately 1.6074e−10 at epoch 617. Moreover, high R^2 values in the testing and training phase (0.97 and 0.99, respectively) suggested good network training.

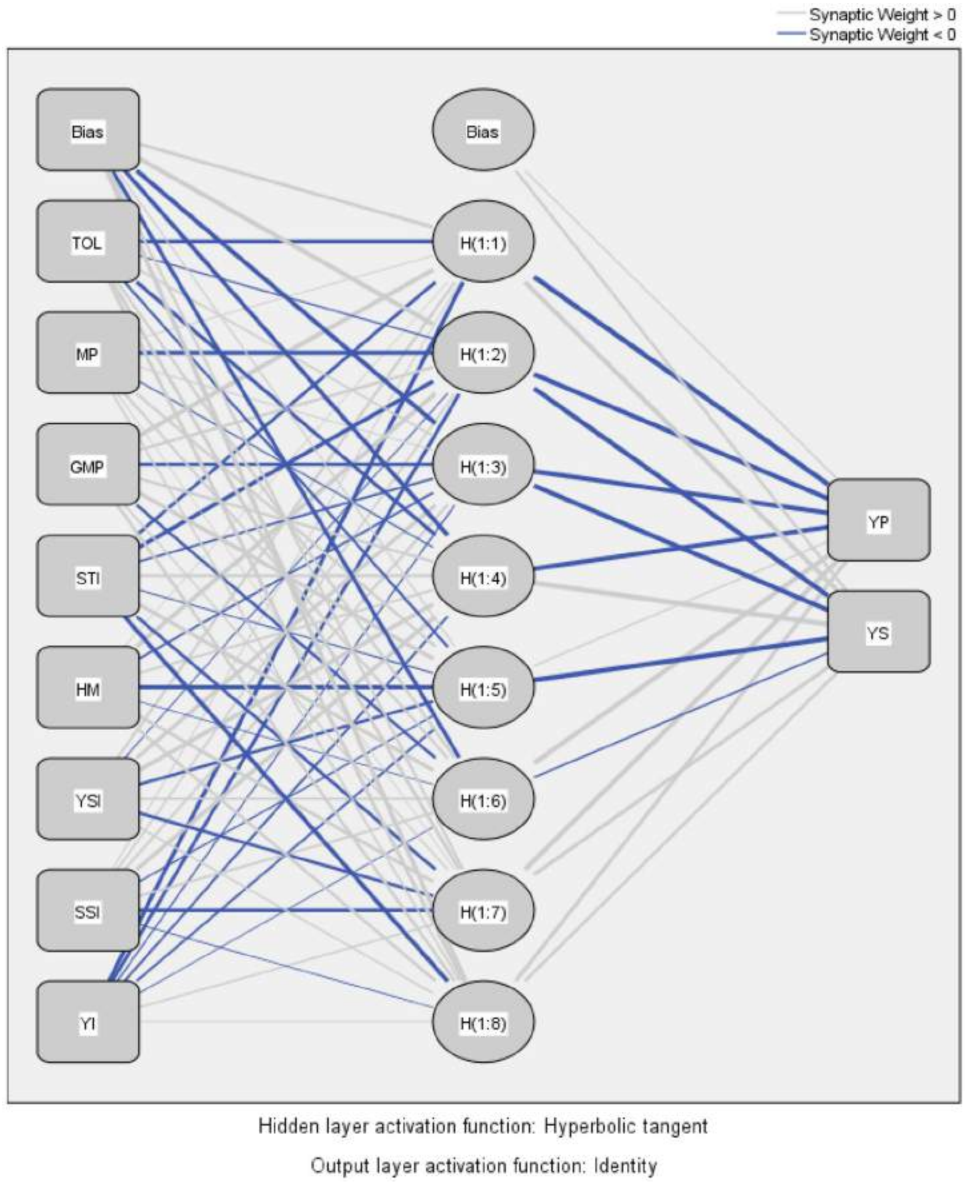

Fig. 5 The structure of the multilayer perceptron neural networks model used for DTIs and grain yield under well-watered and rain-fed conditions

Table 7 Mean square error and coefficient of determination as a function of different number of neurons in the training, testing and validating phases

Number of neurons in the hidden layer	Training		Validation		Testing	
	R^2	MSE	R^2	MSE	R^2	MSE
2	0.91	5.51e−9	0.79	6.42e−9	0.82	6.72e−9
3	0.91	1.89e−9	0.84	1.95e−9	0.84	4.41e−9
4	0.93	3.1e−10	0.88	2.58e−9	0.86	2.95e−10
5	0.94	1.59e−10	0.89	1.58e−10	0.89	1.47−e10
6	0.94	1.23e−7	0.92	2.17e−6	0.92	2.58e−7
7	0.96	1.22e−10	0.95	1.17e−9	0.91	1.06e−9
8	0.99	4.95e−12	0.96	1.61e−10	0.97	1.35e−10
9	0.99	7.46e−11	0.94	5.97e−9	0.91	1.78e−8
10	0.97	8.26e−11	0.89	2.29e−9	0.89	6.15e−10
11	0.98	1.01e−10	0.86	1.62e−9	0.88	1.49e−8
12	0.97	7.19e−11	0.82	1.73e−9	0.87	3.12e−7

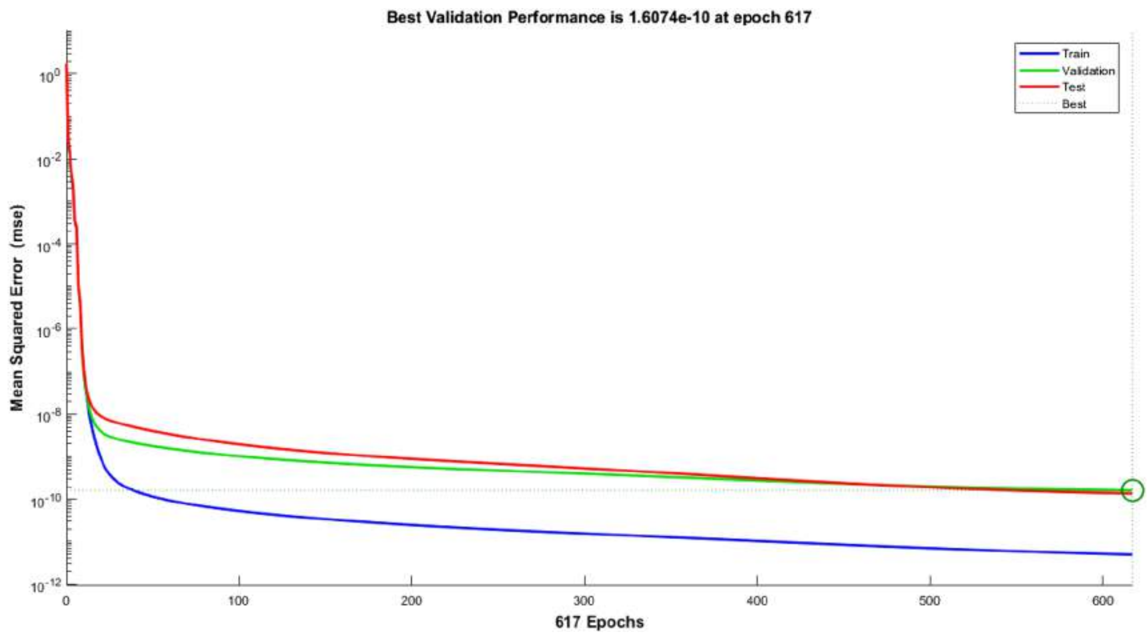

Fig. 6 Mean square error across the training steps

The ANN result demonstrated that MP (100) and GMP (70.4) each played significant roles in identifying high-yielding genotypes under well-watered condition (Fig. 7a). There was a significant positive correlation between these indices and grain performance under the well-watered condition. Selection based on these criteria may, therefore, result in high-yielding genotypes. In contrast, TOL and SSI had the lowest importance of selecting high-yielding genotypes. YI (100) and GMP (88.7) were most important for isolating suitable genotypes under water-limited condition (Fig. 7b). Figure 7 shows that selection according to MP, GMP, YI, YSI, and STI will result in wheat material that is appropriate for both environments.

Discussion

Agronomic and physiological parameters

The selection of high-yielding and tolerant wheat genotypes is of utmost importance for plant breeders and this is a complicated task to perform when genotypes respond differently across environments and when distinct methods may give varying results. The improvement of drought-tolerant varieties is challenging due to a large number of genes responsible for drought tolerance combined with a strong possibility for genotype by environment interactions. However, over the last decade, high throughput-screening technologies, such as next-generation sequencing and high-throughput phenotyping, gene silencing, genome editing, and overexpression techniques, has yielded insights into drought tolerance in plants (Saad et al. 2013; Shan et al. 2013). However, since selection for yield has proceeded without knowledge of the underlying physiological mechanisms, the number of drought-tolerant wheat varieties is fewer than those which have been improved for use under normal environmental conditions (Hussain et al. 2015). Application DTIs and using this information for selection of material allow researchers to integrate information across multiple environments (El-Hendawy et al. 2017).

Our result shows significant differences among Iranian wheat cultivars and landraces in how they respond to drought and illustrates a large potential for using them in future wheat-breeding programs. The largest variation we observed was for seed number per spike under normal irrigation, suggesting that this trait is highly sensitive to available resources. On the other hand, there were minimal differences in grain yield per plant. However, landraces grown under both well-watered and rain-fed conditions show greater potential for grain performance. Even though drought conditions reduce the number of days required to complete each phenological growth stage, both landraces and cultivars reached maturity at a similar time. However, landraces showed larger variation in days to anthesis and days to physiological maturity. Early maturity has been considered a drought escape mechanism which is important particularly under terminal water-limited condition (Hill and Li 2016; Shavrukov et al. 2017). Lopes et al. (2018) suggested that phenological stages of winter wheat are strongly associated with temperature and precipitation regimes in

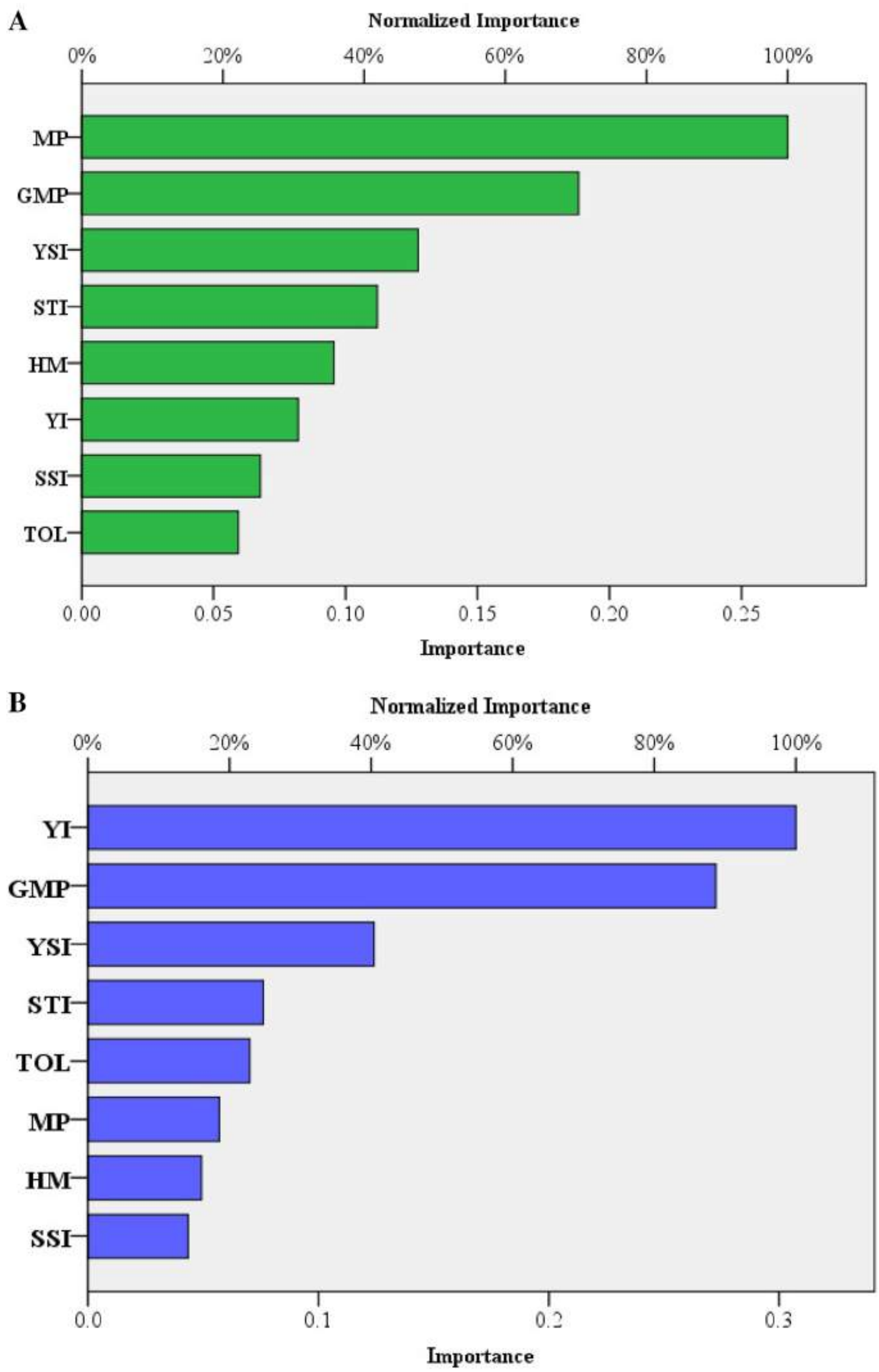

Fig. 7 The relative importance of various DTIs in determining desirable genotypes. **a** The relative importance for DTIs for selecting high Y_P and **b** the relative importance of DTIs for selecting high Y_S

Turkey and Iran. However, well-yielding and early maturing genotypes will need to be assessed in more regions to test their performance across different environments. Nevertheless, they can also be utilized in crossing systems to generate more genetic diversity in term of traits associated with drought tolerance. Kirigwi et al. (2007) reported high variability among recombinant inbred lines for grain yield and yield components under drought stress condition. The positive effect of spike weight, seed number, thousand kernel weight and leaf greenness on grain yield highlight their role in contributing to overall performance. In addition, there is an interesting relationship between canopy temperature and grain yield, which show a significantly negative correlation.

Interestingly, leaf greenness was positively associated with spike weight, grain yield, seed number, and thousand kernel weight under normal irrigation system and with spike weight, grain yield, and seed number under rain-fed condition. Degradation of chlorophyll has been considered a result of drought stress which ultimately leads to a reduction of leaf area and photosynthetic rate (del Pozo et al. 2016). Lopes and Reynolds (2012) suggested that genotypes which stay green under drought stress condition would be able to increase their productivity using further water and nutrients resources. Genetic variation has been reported for this trait among different wheat genotypes (Lopes and Reynolds 2012; del Pozo et al. 2016). The Iranian genotypes showed considerable diversity in both leaf greenness and canopy temperature. High variation in leaf greenness under rain-fed conditions is related to the ability of genotypes to adapt to adverse conditions, where high-yielding genotypes had higher SPAD values. On the other hand, canopy temperature was negatively associated with grain yield and some genotypes showed very low temperature under the rain-fed condition, even though there were not enough leaf area and tillers to cover the ground surface. These genotypes also reduce their shoot temperature under drought as well as they did under normal irrigation system. An integrated application of agronomic and physiological traits may thus accelerate breeding programmes aimed at grain yield under severe environmental conditions (del Pozo et al. 2016; Fleury et al. 2010). The significant relationship we established between grain yield and these two physiological traits showed that these traits are suitable screening criteria for identifying desirable genotypes.

Drought tolerance indices

Application of DTIs for the screening of desirable genotypes allows us to assess how grain yield is affected under normal and drought conditions. However, earlier research has demonstrated that STI, GMP, MP, and HM are critical indices for screening for high-yielding genotypes in both well-watered and water-limited environments (Fernandez 1993; Mardeh et al. 2006; Mohammadi 2016). Climate and soil conditions of the target environments along with a well-defined selection plan are crucial factors in well-designed breeding programs. Previous research has also shown that STI is an appropriate index for discriminating high-yield genotypes grown under both environmental conditions where high-yielding and drought-tolerant genotypes have greater STI values (Rizza et al. 2004; Drikvand et al. 2012; El-Hendawy et al. 2017). Our findings indicate that MP, GMP, STI, YI, and HM were all correlated with potential grain yield and stressed grain yield. Ravari et al. (2016) have found similar results for salinity stress, where YSI, GMP, STI, and HM all were well correlated to the dependent variable.

Principal component analysis

The result of the PCA analyses of the Iranian cultivars and landraces yielded similar results, where the first component was associated with Y_P, Y_S MP, STI and GMP, and high-yielding and drought-tolerant cultivars could be identified by their association with this component. Older cultivars, such as Arya, Karaj1, Karaj2, and Karaj3, showed great performances under normal irrigation system but also suffered large reductions in yield when grown under rain-fed condition. The first principle component can also be used to screen for high-yielding and drought-tolerant landraces. Genotypes 627410, 624582, 626747, and 625047 are recommended as promising materials to include in a wheat-breeding programs aimed at rain-fed environments as they showed high performance across both environments. Similar results have been reported by Farshadfar et al. (2013) and Eivazi et al. (2013) for DTIs. Based on our PCA results, GMP and HM were most appropriate indices for screening among landraces.

Cluster analysis

The result of cluster analysis showed that these cultivars and landraces clustered into four separate groups. The first cluster of cultivars contains a group of new and old cultivars include Uruom, Naz, and Koohdasht which all show high performance in both environments. The most drought-tolerant genotypes were all found in this group of genotypes. Group D contained the largest number of cultivars (40 genotypes) and where most had an average yield. The smallest number observed was for group B (23 genotypes) that all had uniformly low grain yield in both environments. On the other hand, GMP, STI, and HM grouped in the same cluster and clarify the distribution of cultivars across the PCA biplot. We achieved a similar number of clusters also for landraces (four groups) and for DTIs (three clusters). The most drought-tolerant and high-yielding genotypes that clustered into the first group were mostly collected from the Sistan-Balouchestan, Kermanshah and Kordestan provinces. Earlier research has suggested that PCA and heatmap analyses can be successfully utilized to identify drought-tolerant and high-yield genotypes in tomato (Aghaie et al. 2018), chickpea (Jha et al. 2016) and switchgrass (Liu et al. 2015). The cluster analysis highlights the MP, GMP, and Y_P indices as useful for identifying drought tolerance genotypes suitable for planting in a water-limited environment.

ANN

Artificial neural networks were initially developed and used for signal-based processing of data originating from the human brain (Ajith 2005; Yegnanarayana 2009). ANNs are useful tools that allow for multivariate analyses of large volumes of information (Zhang and Zhang 2018). In the current study, eight DTIs representing neurons in the input layer and plant responses based on grain yield under well-watered and rain-fed conditions were considered as neurons in the output layers. A trial-and-error-based approach showed that a hidden layer including eight neurons had the lowest MSE at 617th epoch. Overfitting of the model was controlled by early stopping method where the data are divided into training, validation, and testing subsets and where the error of the validation part is evaluated after each training iteration. The early stopping approach makes the network training phase faster and more accurate (Çelik et al. 2016). The error of the validation and training phase tend to be reduced which is normal until validation errors begin to rise again. As indicated in Fig. 6, the upper location of validation error than the training error curve highlighted well-training of the network. In the training step, observations were used to explore the values of weights and biases of the network. The validation set is used to prevent overfitting by determining the stopping time of training runs. This means that in the validation phase, observations are used to assess the weights and biases obtained in the prior step (Rahimikhoob 2010; Çelik et al. 2016). The next step is validating these weights and biases which is done using the testing set. The purpose of the testing phase is investigating network performance on new information and to ascertain the training stopping efficiency (Coulibaly et al. 2000; Kişi 2007; Kumar et al. 2015). Our ANN analysis highlighted the MP, GMP, YI, and YSI indices for identifying high-performing genotypes. The ANN results were largely consistent with the results from PCA and cluster analyses, confirming that the training of our ANN is correctly implemented. Despite this coordination, ANN provides a more accurate model for the selection of genotypes, based on the relative importance the different criteria. That means ANN allows us to determine the exact proportion of these indices in justifying grain yield as the most important target trait. Ravari et al. (2016) also used ANNs to assess how different indices could help with a screening of salinity tolerance and suggested that the YSI, MP, GMP, and STI indices served as the most appropriate to use for predicting tolerant wheat varieties.

In conclusion, we observed high genetic diversity among both cultivars and landraces collected from across Iran. These diverse germplasms showed significant differences in phenology, yield, yield-related traits, and physiological parameters. Our result suggests that selection for seed number per spike usually results in high-yielding genotypes, although days to anthesis, physiological maturity, canopy temperature, and leaf greenness are all traits that should be considered as well in any breeding program. Our multivariate analysis, which includes PCA, cluster analysis and ANN, identify MP, GMP, YI, and YSI as indices that are capable to discriminate high-yielding and drought-tolerant wheat material for different environments. Interestingly, ANN implemented on DTIs and plant responses provides a fast, accurate and practical approach to predict desirable genotypes, highlighting the value of ANNs for biological and agricultural experiments.

Author contribution statement MRB: corresponding author and assistance in data analysis. YR: worked as a researcher in the filed phenotyping and analysis of the dataset. AT: data analysis. HA: data analysis. PKI: data analysis and assistance in writing the manuscript.

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Compliance with ethical standards

Conflict of interest This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All the authors have approved the manuscript and agree with the submission. The authors declare no conflicts of interest.

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