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# Identification of salt-tolerant barley genotypes using multiple-traits index and yield performance at the early growth and maturity stages

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## Abstract

**Background:** Salinity is one of the major limiting abiotic stresses that decrease crop production worldwide. To recommend genotypes for cultivation under saline stress conditions, a comprehensive understanding of the genetic basis and plant responses to this stress is needed. In the present study, a total of 20 barley genotypes were investigated to identify potential salt-tolerant genotypes, both at the early growth stage using a hydroponic system, and in adult plants under field conditions. For these purposes, the multi-trait genotype-ideotype distance index (MGIDI) was used to identify salt-tolerant barley genotypes at the seedling stage, and the weighted average of absolute scores (WAASB) index was used to identify the high-yielding and stable genotypes in adult plant stage. At the early growth stage, barley seedlings were treated with two salinity levels: 0 mM NaCl (as control conditions) and 200 mM NaCl (as stress conditions) for 30 days, and during this period different growth and physiological traits were measured. Besides, the yield performance and stability of the investigated barley genotypes were evaluated across five environments during the 2018–2020 cropping seasons.

**Results:** Salinity stress significantly decreased growth and physiological traits in all seedling plants; however, some salt-tolerant genotypes showed minimal reduction in the measured traits. Multivariate analysis grouped the measured traits and genotypes into different clusters. In the early growth stage, the G12, G14, G6, G7, and G16 were selected as the most salt-tolerant genotypes using MGIDI index. In the multi-environment trials experiment, AMMI analysis showed that grain yields of the tested barley genotypes were influenced by the environment (E), genotype (G), and GE interaction. Based on the weighted average of absolute scores of the genotype index (WAASB) and other stability statistics, G7, G8, G14, and G16 were selected as superior genotypes.

**Conclusion:** Together the MGIDI and WAASB indices revealed that three genotypes—G7, G14 and G16—can be recommended as new genetic resources for improving and stabilizing grain yield in barley programs for the moderate climate and saline regions of Iran. Our results suggest that using the MGIDI index in the early growth stage can accelerate screening nurseries in barley breeding programs. Besides, the WAASB index can be used as a useful stability measurement for identify high-yielding and stable genotypes in multi-environment trials.

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## Background

Among cereal crops, barley (*Hordeum vulgare* L.) is known as one of the main cereals that is tolerant to abiotic stresses in the world in general, and in Iran in particular (Ahakpaz et al. 2021). Barley has more tolerance to salinity stress compared with other cereals such as wheat, corn, and rice, so it is one of the most saline-tolerant crops, it is frequently used as a model to understand resistance mechanisms in plants (Mwando et al. 2020). Among environmental stresses, salinity is a major global factor that limits crop productivity and food security around the world (Rasel et al. 2020). It has been reported that more than 800 million hectares (Mha) of land worldwide are located in saline regions (Dasgupta et al. 2015). Nevertheless, increased soil salinization in many croplands around the world caused by climate changes and human activities is progressively reducing agriculture outputs, despite escalating calls for more food (Ahmadi et al. 2018; Isayenkov and Maathuis 2019). In Iran, about 6.5 Mha of croplands are affected by varying degrees of salinity that dramatically affects crop production. Based on the Stanford Iran 2040 Project's report, many parts of Iran are located in an arid area with more than 70% annual aridity index (Emadi 2018). Vast areas of this country will face serious problems associated with soil salinization in the near future. Moreover, in many parts of Iran, farmers use saline water for irrigation in their agricultural systems. Hence motivating the development of salt tolerant varieties of crop plants to help agricultural productivity.

Ionic hemostasis is one of the main tolerance mechanisms; disruption of this phenomenon is known as a primary response to the increase in sodium ion content ( $\text{Na}^+$ ) in plant cells (Basu et al. 2020). Accordingly, Munns et al. (1995) stated that salinity stress induces two types of stress on plants. First, when salt accumulates around the roots, the water availability will be limited for plant cells, which immediately appears as 'osmotic stress'. The main consequence of this phenomenon is the disruption of water transfer from the soil to the roots, ultimately decreasing the rate of shoot growth. The second phase, known as 'ionic stress' will appear through increasing content of cytosolic chloride ( $\text{Cl}^-$ ) and  $\text{Na}^+$  in the developed leaves. Plants possess several protective mechanisms for maintaining ion homeostasis through  $\text{Na}^+$  exclusion (Munns and Tester 2008). One of the main mechanisms is by regulating the balance between  $\text{Na}^+$  and  $\text{K}^+$  accumulation in different plant tissues.

Indeed, excluding  $\text{Na}^+$  and maintaining high  $\text{K}^+$  concentrations are imperative for maintaining a high inter-cellular  $\text{K}^+:\text{Na}^+$  ratio and regulating ionic homeostasis in plants (Singh et al. 2020). It has been demonstrated that impairing germination and establishment of seedling are the primary effects of ionic toxicity (Purty et al. 2008). Disruption of these key phases of growth and development mainly decreases the water uptake ability, and the subsequent growth hastens with time. Ryu et al. (2014) reported that ionic imbalances negatively affected the flowering process and eventually disturbed seed maturation. Inhibiting cell division and accelerating cell death are other adverse effects of ionic imbalances on plant growth and development under salinity stress (Cheong and Yun 2007). In general, salinity stress interferes with many physiological and biochemical activities, such as restricting uptake of water and nutrients into plant tissues, changing metabolic processes, decreasing stomatal conductance, and limiting the photosynthesis activity—all of which impede plant growth and development (Kumar et al. 2009; Tavakkoli et al. 2011; Basu et al. 2017; Radanielson et al. 2018; Basu et al. 2020).

Genetic gain is an important component in plant breeding, and hence plays a key role in development of breeding programs. Selection based on only a few traits is generally not considered the most appropriate strategy, because there is no assurance of genetic gains in other important traits (Jahufer and Casler 2015). Hence, breeders often try to gather various desirable traits in one new genotype that would lead to high performance (Olivoto and Nardino 2020). For this purpose, several selection indices have been proposed to select superior genotypes (Ceron-Rojas and Crossa 2018). In these approaches, expressing the economic value of such traits and converting them into realistic economic weightings are the main challenges that often limit breeders in selecting the best genotypes (Bizari et al. 2017). To overcome this limitation, Olivoto and Nardino (2020) introduced a new multi-trait index based on factorial analysis and genotype-ideotype distance (MGIDI). This index focuses on the selection of superior genotypes where multiple traits have been measured. Olivoto et al. (2021) and Pour-Aboughadareh and Pocza (2021) used the MGIDI index to select superior strawberry and wild wheat genotypes, respectively. They further showed that this index can simultaneously consider many traits and or indicators as well as also evaluate the strengths and weakness of the tested genotypes.

Although various agronomic characters are assessed in breeding programs, yield performance is always considered as the target trait. Grain yield is a quantitative trait, and is mainly affected by environmental fluctuations. When experimental genotypes are tested in multiple environments (more than one location or year), the main effects of environment (E) and genotype (G) can be determined, along with a two-way interaction between them as a third effect (GEI). The GEI effect is very important for agronomists and breeders, as it reduces the correlations among genotypic and phenotypic values and complicates the selection of superior genotypes across different environment (Pour-Aboughadareh et al. 2019b). In such circumstances, it is necessary to use approaches of adaptability and stability analyses to select superior genotypes across different environments. Investigating genotypes in multi-environment trials (MET) allows breeders and agronomist to identify ideotypes with a specific adaptability to several environments (Olivoto et al. 2019). In order to interpret the GEI effect in METs, numerous statistical approaches and models have been developed. The additive main effect and multiplicative interaction (AMMI) analysis is one of the best models that is widely used for selecting superior genotypes in barley and other crop plants (Khalili and Pour-Aboughadareh 2016; Paderewski et al. 2016; Vaezi et al. 2017, 2018; Baraki et al. 2020; Ahakpaz et al. 2021). Although this analysis has many advantages in explaining GEI, the main drawback of this model in analyzing the structure of the linear mixed-effect model (LMM) stimulated the use of new models. To circumvent these issues, Olivoto et al. (2019) proposed a novel model, WAASB (weighted average of absolute scores) from the singular value decomposition of the matrix of BLUP for GEI effects generated by a LMM, to better characterize ideal genotypes based on both yield performance and stability. This model combines the features of AMMI and BLUP models in a unique index. Recently, Zuffo et al. (2021) and Ahakpaz et al. (2021) used the proposed statistic to select soya bean and barley genotypes, respectively, and highlighted the usefulness of this parameter in choosing high-yield and stable genotypes. The main objective of the current work was to use the multi-trait genotype-ideotype distance index (MGIDI) to evaluate a set of new advanced barley genotypes under control and salinity conditions. Furthermore, the WAASB index was used to identify the high-yielding and stable genotypes.

## Methods

### Plant material

We used a set of 20 barley genotypes, including 18 breeding lines and two Iranian commercial cultivars. The breeding lines were derived from different crosses among

Iranian landraces and an international parent provided from the International Center for Agricultural Research in the Dry Areas (ICARDA). Additional information on the pedigrees of the selected genotypes is found in Additional file 1: Supplementary Table S1.

### Experimental conditions at the seedling stage

A greenhouse experiment was carried out in 2019–2020 at the Cereal Research Department, Seed and Plant Improvement Institute (SPII), Karaj, Iran. All genotypes were tested in a hydroponic system at optimal growing photoperiod (16 h light, 8 h dark) and temperature (25 °C day, 20 ± 2 °C night) conditions. All planting trays were placed into tanks filled with 20 L of Hoagland nutrient solution (consisting of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (115 g L<sup>-1</sup>), KNO<sub>3</sub> (107 g L<sup>-1</sup>), Ca (NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (236 g L<sup>-1</sup>), MgSO<sub>4</sub>·7H<sub>2</sub>O (246 g L<sup>-1</sup>), Fe-EDTA (5 g L<sup>-1</sup>), H<sub>3</sub>BO<sub>3</sub> (0.38 g L<sup>-1</sup>), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.22 g L<sup>-1</sup>), MnSO<sub>4</sub>·4H<sub>2</sub>O (1.02 g L<sup>-1</sup>), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.08 g L<sup>-1</sup>), and (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>-O<sub>24</sub>·4H<sub>2</sub>O (0.02 g L<sup>-1</sup>) (Hoagland and Arnon 1950). Two separate experiments were performed based on a randomized block design with three replicates. The first included 200 mM NaCl (~20 dS m<sup>-1</sup>) as the stress conditions, and the second (0 mM NaCl) was the control conditions. During germination and seedling growth and development, nutrient solutions in the hydroponic systems were changed every 2 days. Aeration was supplied to each tank with a central air pump and two large airstones, and started 24 h after planting. In the salinity experiment, salinity stress was initiated by adding NaCl in five steps to reach 200 mM at the third-leaf stage, while the control seedlings (non-stressed experiment) only received the nutrient solution. After 21 days of growth and salinity treatment, several physiological traits were measured as described below.

To obtain the fresh weights of roots and shoots (RFW and SFW, respectively), all seedlings of each genotype were harvested and weighted using a digital balance apparatus with an accuracy of ±0.001 g. Then, the roots and shoots were dried in a hot air oven at 70 °C for 48 h, after which the dry weights (RDW and SDW, respectively) were determined. The water relative content (RWC) was measured using the leaf samples of each genotype according to the following equation (Blum 2010):

$$\text{RWC \%} = \frac{[\text{fresh weight} - \text{dry weight}]}{[\text{turgid weight} - \text{dry weight}]} \times 100$$

A handheld chlorophyll meter device (Minolta SPAD-502, Tokyo, Japan) was used to detect the relative chlorophyll content (SPAD index). To measure leaf gas exchange, a clean and healthy leaf from each genotype was selected. The net photosynthetic rate ( $P_N$ ),

stomatal conductance ( $G_s$ ), and transpiration rate ( $T_E$ ) were measured on a developed leaf using an infrared gas analyzer (LICOR, Lincoln, USA). While the traits were being measured, light intensity was fixed from 800 to 1800 lx. All measurements were recorded between 9 AM and 3 PM at a fixed flow rate of  $400 \mu\text{mol s}^{-1}$ . The membrane stability index (MSI) was measured as proposed by Sairam et al. (2002). Leaf samples (0.1 g) were cut and floated in 10 ml of double-distilled water in one of two sets. One set was maintained at  $40^\circ\text{C}$  for 30 min and the other at  $100^\circ\text{C}$  for 15 min in a boiling water bath. The electronic conductivity of each set (C1 and C2, respectively) was measured by a conductivity meter (AQUALYTIC, Germany) and MSI was then measured by the following equation:

$$\text{MSI} = [1 - (\text{C1}/\text{C2})] \times 100$$

To analyze ionic concentrations in plant tissues, the root and shoot samples (10 mg) were digested with 10 ml 0.5 N nitric acid and maintained at  $85^\circ\text{C}$  for 2 h in a boiling water bath. The digested plant material was filtered and analyzed for  $\text{Na}^+$  and  $\text{K}^+$  concentrations ( $\text{mmol g}^{-1}$  dry weight (DW)) using flame photometry (Sherwood Scientific Flame Photometer 420, UK). Furthermore, the  $\text{Na}^+$  and  $\text{K}^+$  translocation from roots to shoots were estimated following the method of Saqib et al. (2005) using the formulae below:

$$\text{Root-to-shoot } \text{Na}^+ \text{ translocation (RTSN)} = [\text{shoot } \text{Na}^+ \text{ content (mmol)}/\text{root } \text{Na}^+ \text{ content (mmol)}]$$

$$\text{Root-to-shoot } \text{K}^+ \text{ translocation (RTSK)} = [\text{shoot } \text{K}^+ \text{ content (mmol)}/\text{root } \text{K}^+ \text{ content (mmol)}]$$

### Field trial experiments

The multi-location trials were performed at three saline regions of Iran (Yazd, Birjand and Esfahan) during the cropping seasons of two consecutive years (2018–2019 and 2019–2020). All regions have a moderate climate and are located in the tropical zones of Iran. In all trials, the salinity level of the soil and irrigation water was tested during the experimental period from seed sowing to harvesting. The mean salinity of the soil and irrigation water was 12 and  $10 \text{ dS m}^{-1}$ , respectively. Sowing and crop managements in all regions were done based on expert advice. At each research station, genotypes were investigated in a randomized complete block design with four replicates. Each plot consisted of six 5-m-long rows with intra-row spacing of 20 cm. Sowing was performed by an experimental plot planter (Wintersteiger, Austria) with a plant density of 400 seeds per square meter. At harvest time, plots were harvested using an experimental combine (Wintersteiger, Austria). Grain yields were measured and data were converted to tonnes per hectare.

### Statistical data analysis

In the greenhouse experiment, a combined analysis of variance (ANOVA) was performed using SAS software ver. 9.1 (SAS 2011) to test the effects of environment [control and salinity], genotype, and their interaction. The relative change (RC) due to salinity stress was computed for each trait according to Pour-Aboughadareh et al. (2020). Principal component analysis (PCA) was computed to detect interrelationships among measured traits using the 'factoextra' package of R software 4.0.3. To group investigated barley genotypes and measure traits, a hierarchical cluster analysis was computed based on the Euclidean distances using 'ggdendro' and 'ggplot2' packages of R software (R Core Team 2020). The multi-trait genotype-ideotype distance index (MGIDI) was used to rank the genotypes based on information of multiple traits as proposed by Olivoto and Nardino (2020). In the first step, each trait ( $\text{rX}_{ij}$ ) was rescaled using the following equation:

$$\text{rX}_{ij} = \frac{\eta_{nj} - \varphi_{nj}}{\eta_{oj} - \varphi_{oj}} \times (\theta_{ij} - \eta_{oj}) + \eta_{nj}$$

where  $\varphi_{oj}$  and  $\eta_{oj}$  are the original minimum and maximum values for the trait  $j$ , respectively;  $\varphi_{nj}$  and  $\eta_{nj}$  are the new minimum and maximum values for trait  $j$  after rescaling, respectively; and  $\theta_{ij}$  is the original value for  $j$ th trait of the  $i$ th genotype. The values for  $\eta_{nj}$  and  $\varphi_{nj}$  are

chosen as follows: for the traits in which positive gains are desired,  $\varphi_{nj}=0$  and  $\eta_{nj}=100$  should be used, while for the traits in which negative gains are desired,  $\varphi_{nj}=100$  and  $\eta_{nj}=0$  should be used (Olivoto and Nardino 2020). In the next step, a factor analysis (FA) was performed to account for the dimensionality reduction of the data and relationship structure. This analysis was performed according to the following model:

$$F = Z \left( A^T R^{-1} \right)^T$$

where  $F$  is a  $g \times f$  matrix with the factorial score;  $Z$  is a  $g \times p$  matrix with the rescaled means;  $A$  is a  $p \times f$  matrix of canonical loading, and  $R$  is a  $p \times p$  correlation matrix between the traits. Furthermore,  $g$ ,  $f$ , and  $p$  indicates the number of genotypes, factor retained, and measured traits, respectively. In the third step, a  $[1 \times p]$  vector was considered as the *ideotype* matrix. In the last step, the Euclidean distance between the scores of the genotypes and the ideal genotypes was computed as the MGIDI index using the following equation:



$$MGIDI = \sum_{j=1}^f \left[ (\gamma_{ij} - \gamma_j)^2 \right]^{0.5}$$

where  $\gamma_{ij}$  is the score of the  $i$ th genotype in the  $j$ th factor ( $i = 1, 2, \dots, t$ ;  $j = 1, 2, \dots, f$ ), where  $t$  and  $f$  are the number of genotypes and factors, respectively; and  $\gamma_j$  is the  $j$ th score of the ideal genotype. The genotype with the lowest MGIDI is closer to the ideal genotype and thus indicates desired values for all the measured traits. The selection differential for all traits was performed considering a selection intensity of ~20%. Data manipulation and the index computation were performed in R software using the ‘metan’ package (Olivoto and Lucio 2020).

In the METs, the grain yield data of barley genotypes were subjected to the additive main effects and multiplicative interaction (AMMI) analysis as proposed by Zobel et al. (1998) using the ‘metan’ package of R 4.0.3. This method combines features of the AMMI and BLUP methods, computed as proposed by Olivoto et al. (2019). The weighted average of absolute scores of the genotype (WAASB) was used as follows:

$$WAASB = \sum_{n=1}^p |IPCA_{gn} \times EP_n| / \sum_{n=1}^p EP_n$$

where  $IPCA_{gn}$  is the score of the genotype  $g$  in the  $n$ th interaction principal component axis (IPCA), and  $EP_n$  is the amount of variance explained by the  $n$ th IPCA. The genotypes with lower WAASB were identified as stable

genotypes. This index was calculated using the ‘metan’ package of R software.

## Results

### Root and shoot features

The results of the combined analysis of variance (ANOVA) showed that the effect of salinity was highly significant for root and shoot fresh weights (RFW and SFW, respectively) ( $P < 0.0001$ ) as well as root and shoot dry weights (RDW and SDW, respectively) ( $P < 0.001$ ). Significant differences were also observed for RFW, SFW and SDW among the investigated genotypes. However, the interaction effect between salinity stress and genotypes was significant only for SFW (Table 1). RFW drastically decreased (60.43%) in all barley genotypes when exposed to 200 mM NaCl salinity compared to the control treatment (Additional file 1: Table S2). The maximum reduction of RFW under salinity stress was observed in G19 (78.67%), G17 (75.64%), G3 (74.10%), G6 (71.49%), G13 (70.92%), and G2 (70.13%). However, the lowest reduction was displayed by G11 (43.48%), G15 (45.83%), G9 (47.13%), and G14 (47.85%), similar to salt-tolerant G1 (46.49%) and G20 (22.83%) (Additional file 1: Table S2). Salinity stress significantly affected the RDW in the barley seedlings, as seen by a 48.75% reduction compared with control conditions (Additional file 1: Table S2). The minimum reduction of RDW was found in the genotype G20 (4.16%) followed by G14 (26.62%), G3

**Table 1** Combined analysis of variance of the measured traits in 20 barley genotypes under control and salinity stress conditions

Trait	Salinity treatment (S; df = 1)	Replication/S (df = 4)	Genotype (G; df = 19)	S × G (df = 19)	Error (df = 76)
Root fresh weight (RFW)	108.39***	1.09	1.6**	1.05	0.66
Shoot fresh weight (SFW)	5898.39***	35.17	49.63**	23.98*	11.61
Root dry weight (RDW)	0.47**	0.02	0.01	0.005	0.007
Shoot dry weight (SDW)	64.64**	1.85	0.51*	0.27	0.3
Membrane stability index (MSI)	9242.18*	776.24	405.6***	206.04	130.21
Relative water content (RWC)	6276.623**	285.07	117.9**	136.07**	53.17
Relative chlorophyll content (SPAD)	590.96*	42.77	28.06**	19.35	15.3
Photosynthetic rate ( $P_N$ )	110.68	64.71	37.74	30.49	23.63
Stomatal conductance ( $G_s$ )	7731.02*	620.89	213.54	251.32	222.22
Transpiration rate ( $T_e$ )	13.35***	0.01	0.33**	0.39**	0.14
Root Na <sup>+</sup> content (RN)	403,186***	2996.48	6373.21***	12,485.03***	1592.84
Shoot Na <sup>+</sup> content (SN)	331,272.10***	165.4	1691.4***	1741.66***	134.16
Root K <sup>+</sup> content (RK)	2354.89***	4.1	10.37***	8.19***	1.81
Shoot Na <sup>+</sup> content (SN)	1079.4***	4.06	23.76***	21.80***	4.18
Root K <sup>+</sup> /Na <sup>+</sup> ratio (RKN)	0.26***	0.0001	0.003***	0.003***	0.001
Shoot K <sup>+</sup> /Na <sup>+</sup> ratio (SKN)	35.32***	0.11	0.24***	0.24***	0.06
Root-to-shoot Na <sup>+</sup> translocation (RTSN)	5.23***	0.04	0.08***	0.10***	0.01
Root-to-shoot K <sup>+</sup> translocation (RTSK)	2802.18**	51.77	43.53*	44.56*	22.87

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$

(32.02%), G12 (34.77%), G11 (35.80%), and G2 (35.94%), whereas the maximum reduction was found in genotypes G6 (61.06%), G13 (64.57%), and G17 (70.59%). Under salinity stress conditions, the mean SFW and SDW across the 20 investigated genotypes was reduced by 76.63% and 61.02%, respectively, compared with their respective values in control conditions. All tested genotypes showed a high rate of reduction (>50%) for SFW; the maximum reductions were found in genotypes G19 (86.71%), G17 (85.60%), G20 (82.62%), G18 (81.69%), G16 (81.51%), G1 (81.35%), and G7 (75.78%). Only one genotype, G7 (50.35%), showed a minimal reduction in SFW compared with other genotypes (Additional file 1: Table S2). Furthermore, significant reduction of SDW was found in most of the barley genotypes under salinity stress conditions. A slight reduction of SDW was observed in genotypes G4 (41.82%), G7 (46.57%), G20 (48.31%), and G3 (49.04%), while the greatest reduction was recorded in G1, G6, G17, and G18 (Additional file 1: Table S2).

#### Relative water content and membrane stability index

The ANOVA for the relative water content (RWC) and membrane stability index (MSI) showed significant effects of salinity conditions and genotypes. The two-way interaction effect between salinity and genotype was significant for RWC ( $P < 0.001$ ) (Additional file 1: Table S2). The overall means of the 20 barley genotypes for RWC and MSI showed a slight reduction (15.53% and 19.45%, respectively) under salinity conditions (Additional file 1: Table S2). Genotypes G6 (2.46%), G8 (2.65%), G15 (4.77%), G1 (5.26%), G16 (6.10%), and G20 (7.74%) had the lowest reduction of RWC relative to the other genotypes, whereas genotypes G3 (33.18%), G4 (32.30%), G5 (28.63%), and G10 (26.51%) showed the greatest reductions (Additional file 1: Table S2). For the MSI parameter, a slight increase was seen in genotypes G5 (0.51%) and G18 (0.94%), while the lowest reductions were observed in genotypes G10 (5.52%), G3 (8.46%), and G16 (10.55%) (Additional file 1: Table S2).

#### Relative chlorophyll and leaf gas exchanges

Salinity significantly influenced the relative chlorophyll content (SPAD value), net photosynthesis rate ( $P_N$ ), stomatal conductance ( $G_s$ ), and transpiration rate ( $T_E$ ). The ANOVA showed highly significant differences among the tested barley genotypes for SPAD and  $T_E$  ( $P < 0.001$ ). On the other hand, the interaction effect between salinity conditions and genotype was only significant for  $T_E$  ( $P < 0.001$ ) (Table 1). Under salinity stress conditions, genotypes G4 (1.34%), G15 (2.72%), and G6 (4.13%) showed a minimum reduction of SPAD, while genotypes G5 (3.51%) and G10 (4.76%) indicated a reverse pattern for this index—they showed a slight increase

in their leaf chlorophyll content. The highest reduction in SPAD was observed in genotypes G11 (28.85%), G3 (25.60%), G7 (21.57%), and G9 (21.77%) (Additional file 1: Table S3). Although there were no significant differences among the tested genotypes for  $P_N$  and  $G_s$  traits, genotypes G14 (2.99%/77.77%), G19 (3.23%/65.35%), G20 (4.74%/66.04%), G12 (6.07%/40%), G2 (6.21%/54.55%), and G18 (6.48%/23.65%) showed the minimum reduction for these traits. Similarly, the minimum reduction of  $T_E$  under salinity conditions was observed in the genotypes G1 (5.79%), G18 (3.35%), G12 (41.59%), G4 (55.34%), and G19 (55.92%). The highest reduction of  $T_E$  was recorded in genotypes G10 (97.98%), G3 (95.81%), G9 (94.91%), G13 (87.02%), and G6 (86.11%) (Additional file 1: Table S3).

#### Ionic concentrations

Based on the ANOVA, highly significant differences for both factors (salinity conditions and genotypes) and their interaction were observed for  $\text{Na}^+$  and  $\text{K}^+$  concentrations in roots (RN and RK, respectively) and shoots (SN and SK, respectively), as well as for their  $\text{K}^+:\text{Na}^+$  ratios (RKN and SKN, respectively) ( $P < 0.0001$ ). Moreover, the main effects and their interaction were significant for root-to-shoot  $\text{Na}^+$  translocation (RTSN) and root-to-shoot  $\text{K}^+$  translocation (RTSK) (Table 1). Salinity stress significantly increased RN and SN compared with control conditions (854.56% and 91.68%, respectively). Two genotypes, G8 and G11, showed a reduction in the  $\text{Na}^+$  content in their roots, while G20 (11.97%), G5 (45.81%) and G13 (55.89%) showed a minimal increase in NR compared to other genotypes. The highest increase in RN was recorded in G1 (379.69%), G10 (292.01%), G4 (250.63%), G6 (245.96%), and G9 (223.78%) (Additional file 1: Table S3). All genotypes displayed a high range of variability for  $\text{Na}^+$  content in their leaves. The maximum relative change for SN was observed in genotypes G20 (2652.68%) followed by G3 (1135.95%), G1 (1110.11%), G17 (772.22%), and G14 (1048.34%). On the other hand, G2, G8, G9, G19, and G16 showed minimal changes in their SN compared to other genotypes (375.76%, 408.08%, 486.35%, 586.91%, and 634.06%, respectively) (Additional file 1: Table S3). The pattern of  $\text{K}^+$  concentration in root and shoot tissues was different from the patterns in  $\text{Na}^+$  concentrations. Salinity stress severely decreased RK (91.23%) across the 20 tested barley genotypes (Additional file 1: Table S3). The maximum reduction of RK was found in G17 (95.63%), G18 (95.14%), G1 (94.38%), G7 (93.59%), and G6 (93.52), whereas G12 (80.07%), G20 (87.04%), G8 (87.61%), G4 (89.34%), and G10 (89.49%) showed a minimum reduction of RK under salinity stress conditions (Additional file 1: Table S3).

Salt stress also negatively affected the SK in the barley seedlings (46.89%) (Additional file 1: Table S4). Although two genotypes, G7 and G18, showed an increasing trend in  $K^+$  content in their leaves, other genotypes showed a reduction pattern in response to salinity stress. The maximum reduction was observed in G2 (73.45%), G6 (63.55%), G4 (60.62%), G18 (60.08%), and G16 (58.43%), while G11, G17, G20, and G13 were reduced by 10.04%, 10.61%, 36%, and 37.14%, hence these were recognized as the best genotypes under salt stress situations (Additional file 1: Table S4). The mean of RKN and SKN of barley seedlings decreased from salinity stress by 96.11% and 94.70% compared with control conditions (Additional file 1: Table S4). Under salinity conditions, genotypes G11 (84.77%), G8 (86.29%), G20 (88.36%), G12 (89.06%), and G13 (94.44%) showed the minimum reduction of RKN, whereas the maximum reduction was reported in G1 (98.86%), G6 (98.24%), G9 (97.83%), G7 (97.82%), and G18 (97.73%). For SKN, genotypes G7 (84.60%), G18 (86.05%), G11 (90.90%), G8 (91.12%), and G17 (91.95%) showed the minimum reduction. On the other hand, genotypes G20, G6, G4, G3, and G1 were identified as the weakest genotypes for maintaining  $K^+$  ions in their leaf tissue; their respective reductions were 97.84%, 96.84%, 96.83%, 96.65%, and 96.18% (Additional file 1: Table S4). Salinity stress conditions significantly increased the transfer of both  $Na^+$  and  $K^+$  ions from roots to shoots. Under salinity conditions, the mean RTSN across all investigated barley genotypes increased by 356.27% compared with control conditions. Genotypes G9 (92.45%), G1 (131.54%), G10 (149.25%), G2 (162.86%), and G6 (192.21%) exhibited the minimum relocation of  $Na^+$ , while G20, G11, G3, G17, and G12 had the highest ability to move  $Na^+$  (increasing percentage of 2227.57%, 1689.28%, 689.31%, 558.38%, and 476.34%, respectively, compared with control conditions) (Additional file 1: Table S4). The mean RTSK increased by 677.46% under salinity stress relative to the control conditions (Additional file 1: Table S4). Among the tested genotypes, G18 (2592.27%), G7 (2124.16%), G17 (2094.08%), G11 (997.78%), and G6 (964.35%) were identified as the genotypes with highest ability to transfer  $K^+$  ions to different organs. The lowest ability to relocate this ion was found in genotypes G20 (166.63%), G8 (259.13%), G10 (372.46%), G15 (388.23%), and G16 (398.02%) (Additional file 1: Table S4).

#### Principal component analysis and hierarchical clustering pattern

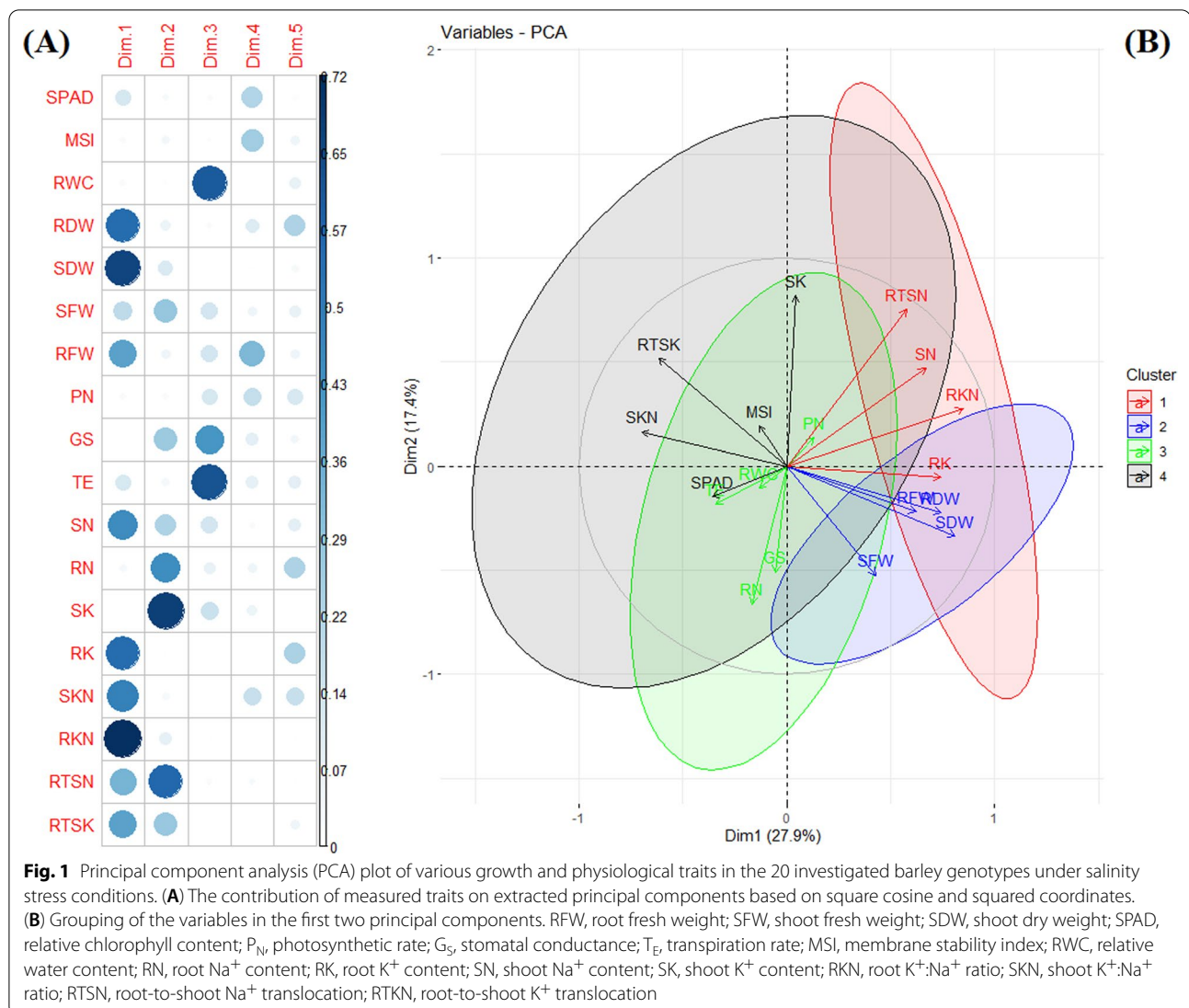
The principal component analysis (PCA) was computed on the experimental dataset including 18 physiological variables and 20 barley genotypes with the aim of enhancing the discrimination power to group the measured

traits based on relationships among genotypes under salinity stress conditions. The results showed that the first six components (PCs) with eigenvalues  $\geq 1$  accounted for 82.01% of the total variation. The first PC accounted for 27.88% of the total variation in the data and was significantly correlated with RDW, SDW, RFW, SN, RK, SKN, RKN, RTSN, and RTSK. PC2 explained 17.42% of the total variation and was mainly influenced by SFW,  $G_s$ , SN, RN, SK, RTSN, and RTSK. PC3 accounted for 13.68% of the total variation and was significantly correlated with RWC,  $G_s$ , and  $T_E$ . PC4 and PC5 accounted for 8.67% and 8.17% of the total physiological variation, respectively. PC4 was mainly correlated with  $P_N$ , RFW, SPAD, and MSI, while PC5 showed a slight correlation with RDW, RN, RK, and SKN. PC6 only explained 6.18% of the total variation and there was no considerable correlation between this PC and the measured traits (Fig. 1A). Since the first two PCs showed the highest percentage of variance, they were used to create a PCA-based biplot. Based on the biplot, all measured traits were grouped into four clusters (I-IV): cluster I included RK, SN, RKN, RTSN, RFW, RDW, and SDW; cluster II was comprised of RK, RFW, RDW, SDW, and SFW; cluster III consisted of  $P_N$ ,  $G_s$ ,  $T_E$ , RWC, RN, SK, SFW, SPAD, and MSI; cluster IV contained all traits except RKN, RK, RFW, RDW, SDW, and SFW (Fig. 1B).

The two-side dendrogram obtained from the cluster analysis showed that all investigated barley genotypes and measured traits were grouped into different sub-clusters (Fig. 2). Genotype G20 showed positive linkage with several traits such as  $P_N$ , SK, SN, RTSN, RFW, RDW, SDW, RK, RKN, and RWC, and therefore it formed a distinct cluster separate from all other genotypes. The cluster analysis showed that all investigated genotypes separated into different groups. Genotypes G18, G8, G15, and G17 showed a similar pattern for some traits, hence they were grouped into the same sub-cluster. The remaining genotypes fell into a distinct cluster, however some of them were placed in a separate sub-cluster due to their similar linkages with various physiological traits. For instance, G5, G9, G11, G13, G14, and G19 showed the same linkage for most of the traits and hence formed a distinct sub-cluster within a sub-cluster.

#### Selection of salt-tolerant genotypes

The multi-trait genotype-ideotype distance (MGIDI) index was calculated to identify the salt-tolerant genotypes when considering all measured traits. A highly significant genotypic effect was found for 10 measured traits including MSI, RWC, SFW,  $G_s$ , PK, SK, RKN, SN, RN, and RTSN (Table 2). The broad-sense heritability ( $h^2$ ) ranged from 0.56 (RK) to 0.92 (SN). High values of heritability were estimated for all filtered traits,



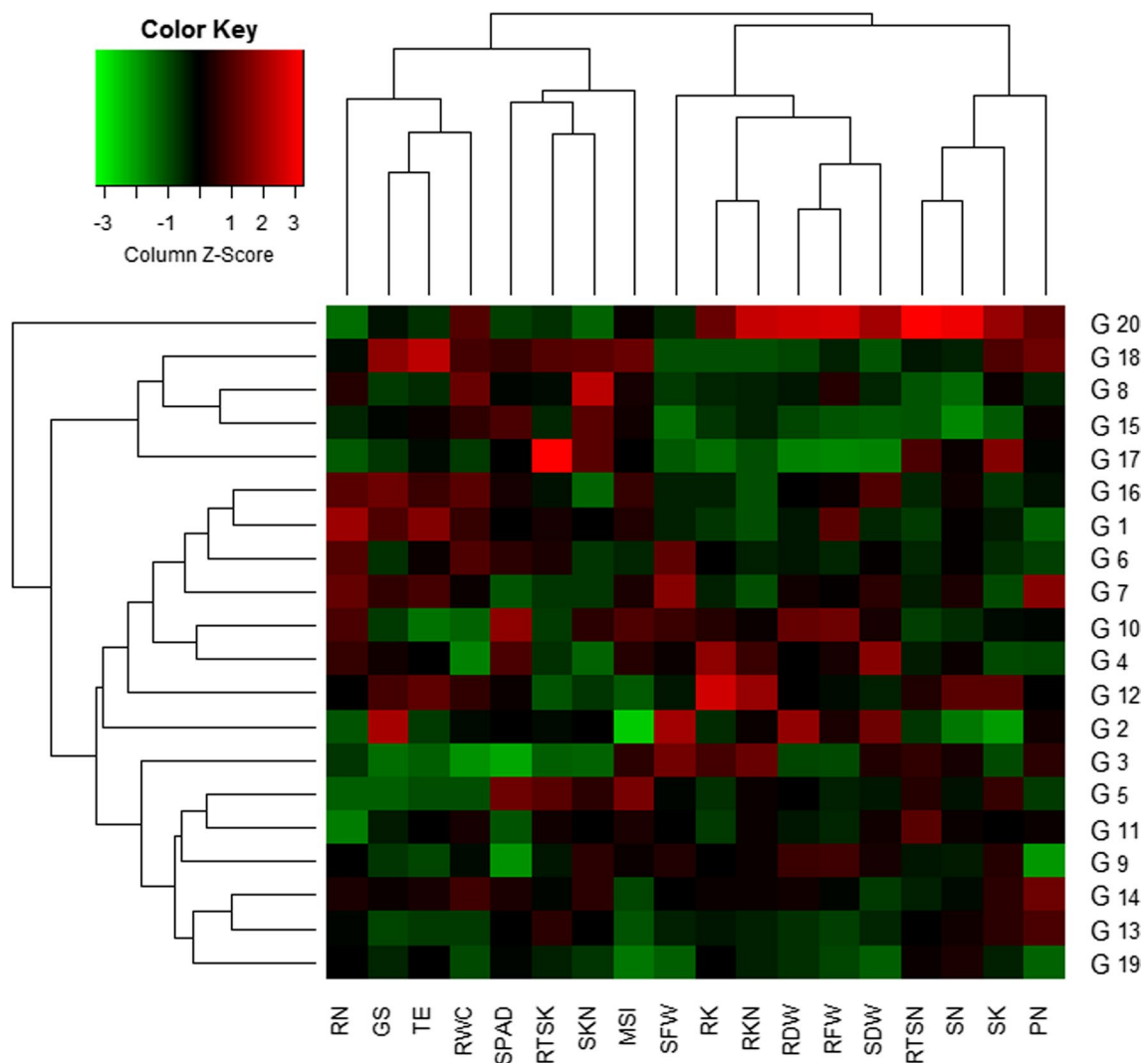
suggesting strong likelihood of selection gains for these traits. The PCA showed that the first four components with eigenvalues  $\geq 1$  accounted for 79.80% of the total variation among traits (data not shown). Among the selected traits, RN, SN, and RK showed the highest genetic gains (19.60%, 10.60%, and 6.92%, respectively). However, only MSI showed an undesirable selection gain ( $-2.81\%$ ). In general, the MGIDI index provided higher total gains, i.e. 42.91% for traits that increased, and of  $-10.62\%$  for traits that decreased. The genotypes identified using the MGIDI index were G12, G14, G6, and G7 (Fig. 3A). The strengths and weaknesses of the genotypes showed that the first factor (FA1) had the highest contribution for genotypes G6, G7, and G14, while FA2 had the highest contribution for genotype G12. FA3 indicated the highest contribution for

genotypes G6 and G7, and FA4 represented the highest contribution for genotypes G6 and G12 (Fig. 3B).

#### Grain yield performance and stability

The AMMI analysis of variance revealed that main effects due to the environment (E), genotype (G), and GE interaction (GEI) were significant (Table 3), and accounted for 26%, 12%, and 22% of the total variation, respectively. This analysis further divided the GEI sum of squares into four interaction principal components (IPCA1–IPCA4) and a residual term. All IPCAs were significant and explained 41.3%, 32.2%, 17.3%, and 9.1% of the total variation due to the GEI, respectively. The mean yield varied between 3.24 tonnes  $h^{-1}$  at Yazd in the first year (YZD2) to 4.73 tonnes  $h^{-1}$  at Birjand in the second year (BRJ2) (Table 3). On the other hand, there was a crossover ranking of the investigated genotypes





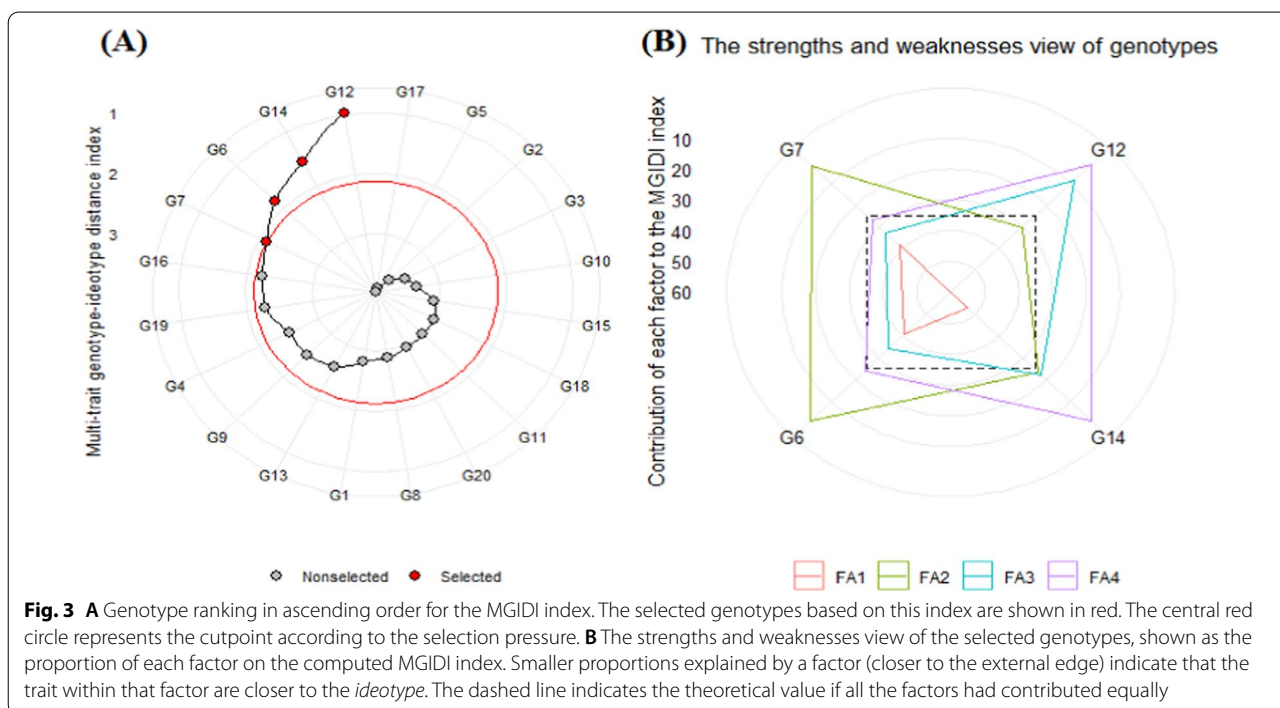
**Fig. 2** Graphical display of the relationships among the 20 investigated barley genotypes and 18 measured growth and physiological traits under salinity stress conditions. The different colors and intensities were adjusted based on associations among genotypes and traits. Colors are representative of a relative scale (−3 to +3) derived after data standardization. The darker green indicates lower values, while the darker red indicates higher values. RFW, root fresh weight; SFW, shoot fresh weight; SDW, shoot dry weight; SPAD, relative chlorophyll content;  $P_N$ , photosynthetic rate;  $G_s$ , stomatal conductance;  $T_E$ , transpiration rate; MSI, membrane stability index; RWC, relative water content; RN, root  $Na^+$  content; RK, root  $K^+$  content; SN, shoot  $Na^+$  content; SK, shoot  $K^+$  content; RKN, root  $K^+ : Na^+$  ratio; SKN, shoot  $K^+ : Na^+$  ratio; RTSN, root-to-shoot  $Na^+$  translocation; RTKN, root-to-shoot  $K^+$  translocation

across the test environments. The WAASB statistic was used to better characterize ideal genotypes based on both yield performance and stability. For this purpose, a biplot was rendered based on the WAASB and mean grain yields. The first quadrant included the YZD1 environment and genotypes G9, G17, G19, and G20. These genotypes and environment showed lower grain yield compared with the average grain yields, hence they play the largest role in GEI. Genotypes G1 and G2 along with environments ESF1, BRJ1, and BRJ2 were placed in the second quadrant. Similar to the previous

section, these genotypes have an acceptable performance and the environments play a big role in GEI. The environments in this quadrant provide above-average production, hence they deserve special attention in discriminating the high-yielding genotypes. G3, G8, G10, G11, and G13 genotypes had a grain yield lower than average yields; along with the YZD2 environment, these were in the third quadrant. Environment YZD2 with low performance showed the lowest discrimination power in GE interaction. However, the WAASB values

**Table 2** Predicted genetic gain for the effective traits in the MGIDI index under salinity stress conditions

Factor	Trait	Goal	$h^2$	Selection gain (%)
FA1	Root $K^+$ content	Increase	0.56	8.12
FA1	Root $K^+ : Na^+$ ratio	Increase	0.65	3.08
FA1	Shoot $Na^+$ content	Decrease	0.93	9.06
FA1	Root-to-shoot $Na^+$ translocation	Decrease	0.87	− 7.81
FA2	Membrane stability index	Increase	0.63	− 2.81
FA2	Shoot fresh weight	Increase	0.77	0.64
FA2	Shoot $K^+$ content	Increase	0.61	0.03
FA3	Relative water content	Increase	0.63	2.23
FA3	Stomatal conductance	Increase	0.61	0.15
FA4	Root $Na^+$ content	Decrease	0.73	19.60
Total (Increase)				42.91
Total (Decrease)				− 10.62



**Fig. 3** **A** Genotype ranking in ascending order for the MGIDI index. The selected genotypes based on this index are shown in red. The central red circle represents the cutpoint according to the selection pressure. **B** The strengths and weaknesses view of the selected genotypes, shown as the proportion of each factor on the computed MGIDI index. Smaller proportions explained by a factor (closer to the external edge) indicate that the trait within that factor are closer to the *ideotype*. The dashed line indicates the theoretical value if all the factors had contributed equally

for these genotypes and environment was minimal. The fourth part of the biplot comprised the remaining genotypes. Hence, G4, G5, G6, G7, G12, G14, G15, G16, and G18 genotypes with low WAASB values and high performance were identified as the most stable genotypes (Fig. 4). As a general result, G7, G8, G12, G14, and G16 with the lowest values of WAASB index were identified as the superior genotypes. Furthermore, this result was confirmed with minimum values of *ASV*, *EV*,

*SPIC*, and *Za* stability statistics for the selected genotypes (Table 4).

## Discussion

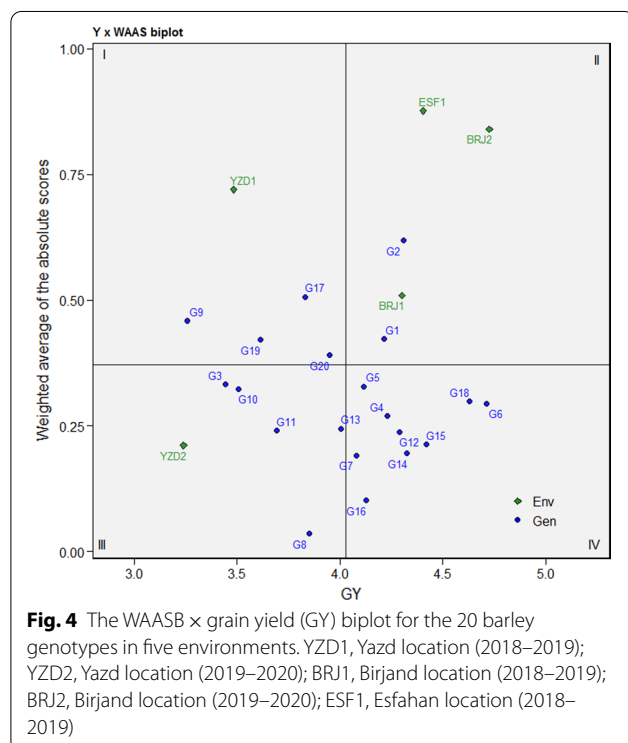
In the current study, various physiological traits were assessed in 20 advanced genotypes of barley at the early growth stage to investigate their relative tolerance under severe salinity conditions. In addition, yield performance and its stability were assessed across different saline regions of Iran. As expected, we observed a highly

**Table 3** AMMI analysis of variance for grain yield of the 20 investigated barley genotypes

Source of variation	df	MS	F-value	(G + E + GE)%	GE%
Environment (E)	4	24.31	23.12***	26	
Replication/(E)	10	1.05	3.45***	12	
Genotype (G)	19	2.27	7.44***	22	
GE	76	1.08	3.52***		
PC1	22	1.53	5.03***	41.3	41.3
PC2	20	1.32	4.31***	32.2	73.6
PC3	18	0.79	2.58***	17.3	90.9
PC4	16	0.66	1.52 <sup>ns</sup>	9.1	100
Residuals	190	0.31			
Environment	YZD1	YZD2	BRJ1	BRJ2	ESF1
Mean GY ( $\pm$ SD)	3.48 (0.08)	3.24 (0.06)	4.30 (0.09)	4.73 (0.12)	4.40 (0.013)

ns, non-significant; \*\*\*,  $P < 0.001$

YZD1, Yazd location (2018–2019); YZD2, Yazd location (2019–2020); BRJ1, Birjand location (2018–2019); BRJ2, Birjand location (2019–2020); ESF1, Esfahan location (2018–2019)



significant effect of salinity stress on various growth and physiological traits, wherein all genotypes were severely affected by the salinity treatment when compared with control conditions (Table 1). However, some genotypes with minimal reduction in growth and physiological features showed a relatively good ability to cope with salinity effects (Tables 2, 3 and 4). Several studies in wheat and barley reported similar results as well (Tavakkoli et al. 2010; Ahmadi et al. 2018, 2020; Rajeswari et al. 2019).

When roots become severely damaged, shoot growth is restricted due to the disturbance of water and nutrient uptake through xylem loading in the root. Furthermore, osmotic stress immediately reduces the leaf area. As such, the shoots are more sensitive to salinity stress compared with roots (Rasel et al. 2020). In this study, our findings indicated that root and shoot growth are severely decreased in all genotypes, although G3 was least affected by stress conditions (Additional file 1: Table S2). Islam et al. (2009) stated that such minimal reduction of root and shoot biomass is likely a result of the plant adopting some physiological and biochemical mechanisms to cope with the stress. Salinity-induced reduction of root and shoot biomass has also been observed in wheat (Ahmadi et al. 2020), Sorghum (Rajabi Dehnavi et al. 2020), barley (Ali and Abbas 2003), purging nut (Abrar et al. 2020), and rice (Rasel et al. 2020).

Relative water content (RWC) has been suggested as a physiological parameter indicative of salinity tolerance in plants (Saeed et al. 2019; Pour-Aboughadareh et al. 2019a, 2020). In this study, we observed a 15.53% reduction in RWC in salinity stress conditions when compared with control conditions, although no significant difference was observed among genotypes in control conditions (Additional file 1: Table S2). This result may be explained by the effect of higher osmolyte concentration indicated by the maintenance of higher RWC. Notably, this result is inconsistent with other reports showing that salinity stress can significantly reduce the intercellular water status in plants (Qin et al. 2010). The membrane stability index (MSI) is another physiological indicator widely used to estimate potential salinity tolerance in different crops (ElBasyoni et al. 2017; Abrar et al. 2020; Ebrahim et al. 2019). It has been reported that

**Table 4** Mean grain yield ( $\text{t ha}^{-1}$ ) and several stability statistics of the 20 investigated barley genotypes across three saline regions of Iran

Genotype code	Grain yield ( $\text{t ha}^{-1}$ )						Stability statistics				
	BRJ1	BRJ2	ESF1	YZD1	YZD2	Mean	ASV	EV	SPIC	Za	WAASB
G1	4.68	3.62	5.31	4.21	3.23	4.21	1.11	0.075	0.96	0.212	0.423
G3	4.01	3.18	4.41	2.71	2.91	3.44	0.71	0.041	0.91	0.172	0.333
G4	4.41	4.29	5.23	3.48	3.73	4.23	0.56	0.022	0.66	0.137	0.269
G5	4.77	4.08	5.10	3.50	3.11	4.11	0.65	0.043	0.94	0.172	0.329
G6	3.77	5.61	5.52	4.43	4.22	4.71	0.38	0.115	1.2	0.168	0.294
G7	4.38	5.16	4.00	3.56	3.28	4.07	0.39	0.011	0.47	0.097	0.191
G8	4.21	4.58	4.17	3.48	2.81	3.85	0.07	0.006	0.11	0.018	0.035
G9	4.14	3.95	2.30	2.93	2.95	3.26	0.9	0.085	1.27	0.24	0.459
G10	3.95	4.86	3.11	3.01	2.61	3.51	0.67	0.032	0.79	0.164	0.324
G11	4.28	3.95	4.52	2.93	2.76	3.69	0.39	0.026	0.74	0.128	0.241
G12	4.05	5.08	4.21	4.18	3.91	4.28	0.28	0.049	0.87	0.133	0.238
G13	4.36	4.97	3.80	2.93	3.95	4.00	0.52	0.019	0.62	0.125	0.245
G14	4.60	5.18	5.27	3.50	3.07	4.32	0.38	0.021	0.61	0.105	0.196
G15	4.14	5.48	5.05	3.84	3.56	4.42	0.33	0.025	0.71	0.116	0.213
G16	4.29	4.75	4.63	3.97	2.99	4.13	0.19	0.005	0.31	0.053	0.110
G17	3.83	5.50	4.99	2.35	2.47	3.83	0.96	0.101	1.44	0.266	0.506
G18	4.45	5.80	5.35	3.45	4.07	4.63	0.58	0.033	0.81	0.155	0.299
G19	4.96	4.62	3.28	2.71	2.48	3.61	0.56	0.109	1.43	0.23	0.421
G2	4.67	4.04	4.23	5.29	3.30	4.31	1.07	0.128	1.77	0.325	0.618
G20	3.99	5.73	3.56	3.16	3.31	3.95	0.95	0.058	0.96	0.198	0.391

YZD1, Yazd location (2018–2019); YZD2, Yazd location (2019–2020); BRJ1, Birjand location (2018–2019); BRJ2, Birjand location (2019–2020); ESF1, Esfahan location (2018–2019)

ASV, additive main effects and multiplicative interaction stability value; EV, averages of the squared eigenvector values; SPIC, sums of the absolute value of the IPCA scores; Za, absolute value of the relative contribution of interaction principal component axes to the interaction; WAASB, weighted average of absolute scores from the singular value decomposition of the matrix of best linear unbiased predictions for the genotype  $\times$  environment interaction effects generated by an linear mixed effect model

MSI is more effective in screening tolerant genotypes at the early growth stage (Farooq and Azam 2006); it is often affected by lipid peroxidation caused by oxidative stress, which results in the production of malondialdehyde (MDA) (Ahmed et al. 2013). Furthermore, MSI is a quantitative and moderately heritable trait that is highly genetically correlated with grain yield (Hemantaranjan 2014). In the present study, MSI changed significantly when plants were exposed to salinity stresses relative to control conditions (Additional file 1: Table S2). Our results validate that these traits can contribute to salt tolerance, as they were less affected by salinity stress (Abrar et al. 2020). Moreover, these physiological parameters were strongly correlated with each other (Fig. 1B). As a result, the G16 genotype showed the lowest reduction in terms of RWC and MSI traits.

Chlorophyll is a green-colored pigment that is a vital component of the photosynthetic apparatus. Its role in capturing light energy, energy transduction, and membrane stabilization has been investigated extensively (Porcar-Castell et al. 2014; Shah et al. 2020; Shin et al.

2020; Abrar et al. 2020). The relative chlorophyll content is likely to decline under salt treatment compared with control conditions. In the present study, chlorophyll content showed different patterns between stress and control treatments. In general, salinity stress caused this trait to decline by 11.62% when compared with the control conditions (Additional file 1: Table S3). This result was similar to the study by Rangani et al. (2016) that also revealed a decrease in chlorophyll contents under high salinity levels, ascribed to the fragmentation of chlorophyll structure. In contrast, salinity stress slightly increased chlorophyll content in some barley seedlings. This finding is in agreement with the results reported in cotton (Higbie et al. 2010), sunflower (Heidary et al. 2014), *Thellungiella sals* (Goussi et al. 2018), and lettuce (Shin et al. 2020). It has been reported that increasing salt solutions in leaf tissue decreases leaf area index, which leads to an increase in specific leaf weight (Sohan et al. 1999). Indeed, reduction in leaf area under salinity stress was correlated with increasing leaf thickness, which in turn led to increase in chlorophyll content (Papp et al. 1983). On the other



hand, Misra et al. (1997) stated that salinity stress could increase the number of chloroplasts, ultimately increasing the chlorophyll content. However, Husain et al. (2003) reported a positive correlation between the SPAD value and concentration of  $\text{Na}^+$  in leaves. In other words, genotypes that maintain low  $\text{Na}^+$  in their leaves showed high relative chlorophyll, whereas genotypes that experienced a sudden reduction in greenness (i.e. low chlorophyll) had high  $\text{Na}^+$  content in their leaves. Hence, we can conclude that there is a clear association between chlorophyll content and  $\text{N}^+$  concentration. This result was also confirmed by PCA analysis, which showed a positive and significant correlation between root  $\text{N}^+$  concentration and SPAD value (Fig. 1B).

The leaf gas exchange analysis showed that salt stress affected stomatal conductance ( $G_s$ ) and transpiration rate ( $T_E$ ) (Additional file 1: Table S3). Throughout the experiment, the barley seedlings showed a minimum reduction in photosynthesis rate ( $P_N$ ). Although salinity stress reduced  $P_N$  compared to the control conditions, the difference between treatments was not significant; this was also the case among the investigated genotypes. It has been reported that stomatal behavior directly affects the  $P_N$  (Yang et al. 2008). During the progress of salinity stress, large amounts of salts accumulate in older leaves. Such increasing salt levels results in premature leaf greenness, which leads to a limitation in the photosynthesis rate. Consequently, the  $P_N$  is reduced, ultimately leading to a lower biomass (Flexas et al. 2007). In support of these earlier findings, we also observed a positive association among  $P_N$ ,  $G_s$ , and SFW traits (Fig. 1B). Moreover, several genotypes (G2, G12, G19, and G20) showed the least reduction of  $G_s$  and  $P_N$ . Of these, G12 and G19, along with G1, G4, and G18 showed a minimal reduction of  $T_E$ . As  $T_E$  is correlated with the normal assimilation of  $\text{CO}_2$ , it has an important role in the photosynthesis process (Kamran et al. 2020). Hence, the capability of plants to minimize the reduction of  $T_E$  could be related to their ability to cope with stress conditions.

Plants tend to maintain high  $\text{K}^+$  concentration instead of  $\text{Na}^+$  in their roots and stems. Numerous studies have shown that plants tend to decrease the toxic effects of  $\text{Na}^+$  in their tissues by obtaining sufficient  $\text{K}^+$  contents and excreting more  $\text{Na}^+$ . Potassium is a key macro-nutrient that activates more than 50 enzymes. It has been shown to contribute to the biosynthesis of chlorophyll pigments (Shabala and Cuin 2003). From the chemical viewpoint, both  $\text{Na}^+$  and  $\text{K}^+$  have the same level of hydration energy and ionic radius. Hence, under saline conditions,  $\text{Na}^+$  ions can easily enter into the cell through  $\text{K}^+$  channels located at cell membranes. This, in turn, leads to the higher cytoplasmic content of  $\text{Na}^+$  and reduces the  $\text{K}^+:\text{Na}^+$  ratio, which affects plant growth and

development. Hence, one of the plant's defense strategies to minimize the negative effects of excessive  $\text{Na}^+$  or  $\text{K}^+$  loss is to maintain a high  $\text{K}^+:\text{Na}^+$  ratio in the cytoplasm (Adolf et al. 2013). In this study, we found that both root and shoot  $\text{K}^+$  contents (RK and SK, respectively) decreased under salt treatment (Additional file 1: Table S4). Furthermore, the root and shoot  $\text{K}^+:\text{Na}^+$  ratios also showed a decreasing trend under salinity conditions. Among the investigated barley genotypes, G8 and G11 had the lowest reduction in RKN and SKN. We propose that these genotypes can serve as superior salinity-tolerant genotypes due to their strong capability in maintaining  $\text{K}^+$  in their root and shoot tissues.

Experienced breeders often try to gather various desirable traits in one new genotype that would lead to high performance. However, when multiple traits are measured, selection of ideotype genotypes often is difficult. In this regard, several multivariate approaches (such as principal component analysis (PCA), factor analysis (FA), and cluster analysis (CA)) as well as various selection indices (including Smith (1936) and Hazel (1943), the base index (Williams 1962), the index of Pesek and Baker (1969), and Mulamba and Mock (1978)) have become widely used to group the measured traits and to select the best genotypes, respectively. In the current study, we used a two-way heat map clustering pattern and PCA to group the genotypes and measured traits (Figs. 1, 2), but this did not lead to the identification of tolerant genotypes. To facilitate the selection of genotypes with multiple traits, Olivoto and Nardino (2020) recently proposed a novel method (multi-trait genotype-ideotype distance index: MGIDI) for genotype selection based on information on multiple traits. Accordingly, when the barley genotypes were ranked based on information on measured multiple traits (Fig. 3A), the MGIDI index selected genotypes G12, G14, G6, and G7 as the salt-tolerant genotypes. Apart from these genotypes, G16 was very close to the cut-off point, suggesting that this genotype can present interesting features. Hence, close attention should also be paid to genotypes that are very close to the cut-off point (Olivoto and Nardino 2020). As another result from this analysis, we found that some physiological traits including MSI, RWC, SFW, GS, PK, SK, RKN, SN, RN, and RTSN have a high broad-sense heritability, indicating that they deserve attention in future studies aimed at screening tolerant genotypes at the early growth stage (Table 2). Olivoto et al. (2021) recently used the MGIDI index for choosing ideal strawberry genotypes; indeed, the use of this index in investigating plant crops is expected to rapidly expand.

In the current study, we tested barley genotypes across five different locations in saline regions of Iran. The AMMI analysis showed a highly significant GE interaction for grain yield (Table 3), which is in agreement with

almost all similar studies (Khalili and Pour-Aboughadareh 2016; Vaezi et al. 2017, 2019; Ahakpaz et al. 2021). Since the response of barley genotypes was variable in each environment, we used the WAASB index as a quantitative stability statistic to identify stable genotypes (Table 4). Based on the results, the lowest value of WAASB was estimated for G7, G8, G14, and G16. These genotypes also showed the lowest values of *ASV*, *EV*, *SPIC*, and *Za* stability statistics (Table 4). For a better interpretation of stability and productivity, a biplot based on WAASB and grain yield was rendered (Fig. 4). The main advantage of this biplot over the AMMI biplot is that all IPCA axes are used, hence allowing the GEI patterns not retained in IPCA1 to be considered in the genotypes' ranking (Olivoto et al. 2019). Indeed, this biplot may be helpful in identifying highly productive and broadly adapted genotypes. Among the selected genotypes, only one—G8—was associated with one environment (YZD2), whereas the remaining genotypes did not depend on any specific environment.

Selection approaches in METs are of great interest to barley breeders, particularly because strong GE interaction can reduce yield potential of genotypes across environments (Zuffo et al. 2021). Presently, agricultural systems are faced with demands for more feed and food due to frequent and severe climate changes. One of the approaches to solve this challenge is to obtain desirable genotypes through research that allows a comprehensive understanding of the genetic and plant developmental variation in response to environmental stresses (Bailey-Serres et al. 2019). Therefore, studying the responses of various genotypes under stress conditions and evaluating their yield stability are two important objectives that should be considered. Integrating these results has provided a context for technological solutions to these studies.

## Conclusion

Variation in physiological traits of seedlings is critical for determining the scale of salinity tolerance in plants. The early growth stage may better reveal the physiological and biochemical characteristics of different genotypes. In the present study, we showed genetic variability in several physiological traits among a set of barley advanced lines under salinity stress conditions. The MGIDI index indicated that G6, G7, G12, G14, and G16 were the most desirable among the 20 investigated genotypes. Using MET experiments to explore yield performance, we showed that the WAASB index can be powerful in identifying stable and high-yield genotypes. Using this index, specific genotypes—G7, G8, G14, and G16—were selected as stable genotypes and hence can

be recommended for cultivation under salinity conditions. Moreover, we presented a positive relationships between results obtained from MGIDI and WAASB indices, such that both procedures selected the same desirable genotypes (G7, G14, and G16) as highly tolerant and stable across different saline regions. Overall, our results provide new insights into the screening nurseries in breeding programs that can be applied to understanding and identifying salt tolerance in barley. Hence, these methods can serve as powerful tools to improve recommendation strategies.

## Abbreviations

RFW: Root fresh weight; SFW: Shoot fresh weight; SDW: Shoot dry weight; SPAD: Relative chlorophyll content; PN: Photosynthetic rate; GS: Stomatal conductance; TE: Transpiration rate; MSI: Membrane stability index; RWC: Relative water content; RN: Root Na+ content; RK: Root K+ content; SN: Shoot Na+ content; SK: Shoot K+ content; RKN: Root K+:Na+ ratio; SKN: Shoot K+:Na+ ratio; RTSN: Root-to-shoot Na+ translocation; RTKN: Root-to-shoot K+ translocation; AMMI: Additive main effect and multiplicative interaction; MGIDI: Multi-trait genotype-ideotype distance index; PCA: Principal component analysis; MET: Multi-environment trials.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-021-00576-0>.

**Additional file 1: Table S1.** The passport of the tested genotypes. **Table S2** Performance and percent reduction of barley genotypes considering root and shoot growth features, water relative content, and membrane stability index at the seedling stage under control and salinity conditions. **Table S3** Performance and percent reduction of barley genotypes considering traits related to photosynthesis process, and concentration of Na+ in the root and shoot at the seedling stage under control and salinity conditions. **Table S4** Performance and percent reduction of barley genotypes considering traits related to ionic concentrations in the root and shoot at the seedling stage under control and salinity conditions.

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## Authors' contributions

AP conceived and designed the experiment. HNC provided the seeds of breeding genotypes. The experiment was performed by AP and AA (the fifth author). AP, AA, SS, MM and AA (the sixth author) collected the experimental data. AP performed the analysis and wrote the manuscript. All authors read and approved the final manuscript.

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## Declarations

### Ethics approval and consent to participate

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### Competing interests

The authors declare no conflicts of interests.

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