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Short Title

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Full Title

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Approval

We authors and our institutions have read and are fully aware of the journal policy.

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Abstract

Changes in the relative genetic performance of genotypes across environments are referred to as genotype × environment interactions (GEIs). GEIs can affect barley breeding improvement for salt-tolerance because it often complicates the evaluation and selection of superior genotypes properly. The present study was seeking to evaluate the GEIs over 60 barley genotypes that were evaluated for yield components and grain-yield in six-salinity environments in North Delta, Egypt. Data were analyzed using the additive main effects and multiplicative interaction (AMMI) and Tai's stability parameters. GEIs effects on yield explained 20.3, 20.1, 14.6 and 33.0% of the total variation besides, the first two principal components account for 67.3, 56.3, 64.3 and 83.7% of the explained variance in the four sets, respectively. The ideal genotype-model are G-4, G-7, G-20, G-34, G-36, and G-39 which, were most stable and high-yield

genotypes across environments (GY > 2.00 t ha⁻¹), and located almost zero and/or close to zero

 projection onto the AEC ordinate. Tai's stability parameters demonstrated that they were more responsive to the environmental changes. The genotypes G-50 and G-53 showed perfect/static stability ($\alpha = -0.95$, -0.91, respectively). In contrast, the genotype; G-36 had $\alpha = 0$ and $\lambda = 1.10$, indicating parallel with the environmental effects followed by G-44. Overall, we found that GEIs for grain yield is a highly significant in all sets, suggesting that responded differently across environments. This interaction may be a result of changes in genotypes' relative performance

across environments, due to their differential responses to various abiotic factors.

Keywords: *Hordeum vulgare*, G × E interaction, AMMI, Tai's stability parameters, salt stress

Introduction

Climate changes affect agricultural productivity globally and may outcome in strong influence on agriculture, particularly on crop yield improvement. Crops are largely certain by climatic situation during growing season, thus even slight deviations from optimal conditions can seriously overhang yield (Lobell et al. 2011; Kole et al. 2015). Therefore, understanding of the environmental factors effect on crop production could reduce the possibilities of significant yield losses and improve the selection of elites cultivars for using in the target aspects and area (Smith and Tirpak 1989). Soil salinity is one of the most important abiotic stresses worldwide, affecting crops production and act as a major obstacle to increasing barley production in growing areas worldwide, particularly in the Mediterranean region (Rodriguez et al. 2008; Sayar et al. 2010; Rharrabti et al. 2013; Elakhdar et al. 2016a). Globally, it is estimated that 19.5% of irrigated land (about 230 million ha) and almost 2.1% of dry land agriculture (about 45 million ha) is affected by salinity (FAO 2015).

Today cultivated barley became the fourth most abundant cereal in term of area and harvested tonnages as well (FAO 2015). Barley is widely adapted to various environmental

 conditions and is more stress tolerant compared with its close relative wheat (Lobell et al. 2011). Barley production in Egypt is affected by increasing dryland salinity, which severely limits growth and reduces yields (Elakhdar et al. 2016a). To maintain high agricultural productivity, the development of cultivars with high-yield potential is the ultimate goal of plant breeders in a crop improvement program. Recently, the barley breeding program in Egypt has placed special focus on developing barley cultivars with improved grain yield under different stress conditions, and resistance to major barley diseases. In addition to high-yield potential, a new cultivar should have stable performance and broad adaptation over a wide range of environments.

In many aspects of plant breeding, studying of genotype-by-environment interaction (GEI) plays an important role for identifying high-yield and stable genotypes. It continues to be a challenging issue among plant breeders and agronomists when conducting crop performance trials across diverse and unpredictable environments (Ceccarelli 1996; Rodriguez et al. 2008; Hongyu et al. 2014). Indeed, most of the economic or quantitative traits are controlled by complex gene networks, which are in turn affected by the environment (Cooper 1996; Haussmann et al. 2004). For this reason, understanding the causes of GEI would help in developing and breeding genotypes, which show satisfactory performance in several environments and adapting as well. The additive main effects and multiplicative interaction (AMMI) model are a combined analysis that incorporates both multiplicative components and the additive of the two-way data structure that clearly distinguishes between the main and interaction effects (Shafii and Price 1998; Hongyu et al. 2014). Therefore, it is essential to assess the genotypes sensitivity during the environmental in terms of simultaneous stable and higher yields genotypes (Yan et al. 2007; Balestre et al. 2009).

For the past decades, the concept of GEI and its stability statistics were being analyzed in different ways. In stability studies, the interaction term is separated into two components; the linear response to environmental effects, which is measured by a statistic (α), and the deviation from the linear response, which is measured by another statistic (α), that reported by (Tai 1971). A perfectly stable variety has (α , α) = (-1, 1) and a variety with average stability is expected to have (α , α) = (0, 1). Tai's analysis also supports a method of obtaining the prediction interval for α = 0 and a confidence interval for α values, so that the genotypes can be distributed graphically in different stability regions of the Tai's plot.

Thus, this paper aims to apply AMMI biplot and Tai's stability statistical models for determination of the magnitude and pattern of genotype × environment interaction GEI effects and performance stability of grain yield under the salt-affected soil on a set of Egyptian spring barley genotypes.

Materials and methods

Germplasm

The plant material consisted of a set of 60 hulled and hull-less barley genotypes (53 advanced lines and seven cultivars) presented in (supplemental Table 1). The genotypes were developed includes progeny selection on ear-to-row method (modified pedigree method) at Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt.

Total protein profiling using SDS-PAGE

To study the genetic variation among the genotypes using SDS-PAGE; seed coats were removed and homogenized using 8 M urea buffer (seed per genotypes). After vigorous vortex, samples were subjected to centrifugation at 12,000 rpm for 10 min at 4 °C and clear supernatants were used for analysis. Protein profiling of samples was performed using SDS-PAGE as

- 1 described by (Laemmli 1970). Equal volumes of proteins supernatants (8 μl) from each sample
- were loaded into 14% gel. Staining of gels was done in 0.025% Coomassie Brilliant Blue 250
- 3 containing 40% methanol and 7% acetic acid.

Experimental layout; design and testing locations

The genotypes were planted into four-sets, consisted 15 genotypes each (the large layouts are not recommended instead of the variation caused by the salinity). The treatments were laid out in a randomized complete block design (RCBD) with three replications. The genotypes were evaluated in three salt-affected soil locations during two cropping season; 2012-2013 and 2013-2014. Each year and location were treated as a separate environment, making six-test environments [(E1 to E6) (E = year \times location combination)], the weather and climate information for the locations were described in (Table 1). Each plot was four rows, 2-m long with 30-cm apart. Data were recorded from the central rows on 15-yield traits as the following; Days to Heading (DH) and Maturity (DM), Flag Leaf Area cm² (LA), Chlorophyll content (Chl as SPAD value), Plant Height (PH), Spike Length (SL), Peduncle length (Ped), Number of Tillers/ m² (NT), Number of Grains/ spike (NG), 1000-Grain weight (GW), Grain Yield GY t ha⁻¹, Biological Yield BY t ha⁻¹, Grain Filling Period (GFP), Grain Filling Rate (GFR) and Harvest Index (HI), However our study focus only on the grain yield in details. Grain yield was measured from a net plot size of 1 m² and was converted into t ha⁻¹. At harvest, soil samples were taken from upper soil layer (0-30cm) to conduct chemical and physical analysis (Table. 2).

Data Analysis

- 21 The analysis of variance ANOVA for the grain yield values was performed using the general
- 22 linear model (GLM) procedure to partition the total variation into components due to genotype
- 23 (G), environment (E) and GEIs effects. As suggested elsewhere (Kang and Gauch 1996), the

- appropriate linear model for the genotypic value of genotype i grew in the environment (year \times
- 2 location) j and replication r within this j environment was determined by:
- $g_{ijl} = \mu + E_j + r/E_{jl} + G_i + GE_{ij} + e_{ijl}$.
- Where μ is the general mean, \mathbf{E}_{i} , r/\mathbf{E}_{jl} and \mathbf{G}_{i} are the main effect of respective environment,
- 5 replication within environment and genotype, whereas GE_{ij} and e_{ijl} are the GEIs and the
- 6 experimental error. Genotype and environment were considered as a fixed and random effect,
- 7 respectively.

The AMMI model

- 9 Multivariate stability measures additive main effects and multiplicative interaction (AMMI)
- 10 equation by (Gauch 1992) is;

$$Y_{ger} - \alpha - \beta e + \mu = \sum_{n} \lambda_{n} \gamma_{gn} \mathring{o}_{en} + \rho_{ge} + \epsilon_{ger}$$

- Where Y_{ger} is the plot of genotype g in the environment e and replicate r; μ is the grand mean; α
- is the deviation of the genotype g from the grand mean; βe is the deviation of the environment e
- from the grand mean; λ_n is the singular value of PCA axis n; γ_{an} is the genotype eigenvector for
- axis $\mathring{\mathbf{o}}_{en}$ is the environment eigenvector; ρ_{en} is the residual of the GEI and ε ger is the error term.
- 16 The basic model for a GGE bi-plot equation by (Yan et al. 2007) is;
- $\hat{Y}_{ij} \beta e = \lambda_1 \varepsilon_{i1} \eta_{1j} + \lambda_2 \varepsilon_{i2} \eta_{2j} + \varepsilon_{ij} = g_{i1} e_{1j} + g_{i2} e_{2j} + \varepsilon_{ij}$. Where \hat{Y}_{ij} is the expected
- yield of genotype i in environment j is the grand mean of all observations and βe is the main
- effect of environment j. λ_1 and λ_2 are the singular values of first and second largest PC1 and
- PC2, respectively; ε_{i1} and ε_{i2} are the eigenvectors of genotype i for PC1 and PC2, respectively;
- g_{i1} and e_{1j} are called the primary scores for genotype i and environment j, respectively; g_{i2} and

- e_{2j} , the secondary scores for genotype i and environment j, respectively; and ε_{ij} is the residue
- 2 not explained by the primary and secondary effect. $g_{il} = \lambda_l^{fi} \varepsilon_{ij}$ and $e_{li} = \lambda_l^{1-fi} \eta_{1j}$. Where f
- 3 is the partition factor and theoretically it can take any value between 0 and 1.
- 4 AMMI analysis and AMMI2 GE bi-plot was completed using the standard procedures and
- 5 analyzed through GeneStat software (GenStat 2011). AMMI1 graph, histogram, and box-plot
- 6 were performed using the scatter plot Quantum XL analysis runs in Excel spreadsheet.

Tai's model

- 8 Tai (1971) was used as two parameters of stability; Tai's alpha (α) and lambda (λ), mean-
- 9 variance component for a pairwise GEI. The linear response to environmental effects was
- calculated by α_i statistic and the deviation from the linear response was calculated by λ_i statistic.
- 11 A perfectly stable genotype has $(\alpha_i, \lambda_i) = (-1, 1)$ and the genotype with average stability has
- $(\alpha_i, \lambda_i) = (0, 1)$ and the value $(\alpha < 0, \lambda = 1)$ refers to the above average stability.

Results

Genotype mean performance

- The mean grain yield performance varied from 4 t ha⁻¹ for G-43 to 0.63 t ha⁻¹ for G-44 and
- 16 G-59 across the all diversified salt-environments, indicating large variation in yield potential of
- genotypes (Table 3). The highest mean yield for an individual genotype per set in an individual
- 18 environment were (3.13, 3.02, 3.33 t ha⁻¹ for G-8 in E4, E5, and E6)/ set-1, (3.33 t ha⁻¹ for G-18
- 19 in E3, E5, and E6)/ set-2, (3.33 and 3.34 t ha⁻¹ for G-32 in E3 and E5)/ set-3 and (2.81 t ha⁻¹ for
- 20 G-46 in E3 and E6)/ set-4, respectively.
- In addition, the genotypes combined means obtained from the evaluation of barley genotypes for
- 22 yield stability over environments are summarized in (Table 4). The grain yield ranged from a
- 23 high of 2.52, 2.51, 2.37, and 2.00 to a low of 1.79, 1.76, 1.58, and 1.17 t ha⁻¹ in set 1, 2, 3, and 4,

- 1 respectively. The highest genotypes grain yield performance was recorded for G-8, G-22, G-3,
- 2 and G-23 as hulls-type; moreover, other high yielding genotypes were G-32 and G-33 as naked-
- 3 type over the environments.
- 4 The molecular analysis among the genotypes was matched with SDS-PAGE patterns seed
- 5 storage protein (Fig. 1). The seed protein profiles of 60 barley accessions showed diversity in the
- 6 banding pattern. The types of band pattern were depicted on the basis of their molecular weight.
- 7 The different sizes of the protein banding patterns of all the genotypes studied are represented in
- 8 the schematic drawing (Fig. 1 a) (only, some of the 60 genotypes were present). Using the
- 9 hordein bands pattern, the genetic distance was presented as UPGMA (based on Nei's genetic
- distance) dendrogram showing the relationship between various genotypes (Fig. 1 b).

Environment effects and classification

- Based on the grain yield data the environments were classified into two groups (Fig. 2)
- according to Ward cluster analysis. In the cluster analysis, two environments (E5 and E2), are
- corresponding to the salt stress with high and medium yield potential, respectively (Table 3).
- The low-yielding environment per sets were 1.28 t ha⁻¹ E2/ set-1, 1.44 t ha⁻¹ E2/ set-2, 1.55 t
- 16 ha⁻¹ E4/ set-3, and, 1.52 t ha⁻¹ E1/ set-2 (Table 5).

Genotype – By - Environment interaction

- The analysis of variance for grain yield of each genotype group in each environment based
- on GE interaction effects is presented in (Table 6). The treatments, locations, genotypes, and the
- interaction effects GEIs were highly significant ($P \le 0.001$) of the total variance. The location
- 21 (E) effect for the first three-sets was much stronger than the GEI effect, which explains 33.34%,
- 22 22.16% and 49.92% (ranged from 3.59 to 5.31-fold the genotype effect) of the variance
- component (VC %), respectively. While the interaction variance component (VC %) in the set-4

 1 recorded 33.00% in contrast, 11.50% for the location (E). At the same time, the genotype effect

was slightly higher than the location for the set-4 (Table 6).

Additive Main Effects and Multiplicative Interaction

The stability of the genotypes was achieved by drawing an average environment coordinate (AEC) on the genotype- focused bi-plot. Based on IPCA scores, some genotypes such as G-6, G-9, G-21, G-42, G-50, and G-58 had relatively high positive interaction with the environment. On the other hand, the genotypes coded G-1, G-5, G-18, G-20, G-35, G-46, and G-59 had high negative interaction with the environment (Table 4). These genotypes were, therefore, among the most responsive genotypes to environments, in contrast, the genotypes; G-4, G-7, G-12, G-20, G-22, G-23, G-31, G-34, G-36, G-39, and G-54 were the most stable genotypes, as it was located almost zero and/or close to zero projection onto the AEC ordinate, expressed general adaptation. From these stable genotypes, six-genotypes coded; G-4, G-7, G-20, G-34, G-36, and G-39 were high-yielding performance (> 2.00 t ha⁻¹) compared with the others. This observation means that these genotypes most stable with respect to performance across environments. The environments that mostly contributed to the total GEI according to on IPCA scores were E5/ set-1, E1/ set-3, E4/ set-3 and E5/ set-4, that mean the differences across all of the environments were mainly summarized by the IPCA1 (Table 5). Genotype (G), environment (E) and genotype-by-environment (GE) interactions were measured by the AMMI model (Table 6). The first two principal component axes of the interaction PCA explain most of the GEI effect: 44.60%, 32.83%, 42.14% and 46.91% captured by the PCA1; 22.69, 23.42, 22.18 and 36.78% by the PCA2, in the four sets, respectively. AMMI analysis of variance indicated that two PCA were high significant (P < 0.01) in the four sets. The AMMI bi-plot analysis for barley grain yield grown in six environments is presented in (Fig. 3). The x-axis shows the main effects, while the y-axis shows the first PCA axis. Partitioning of GEI

by the GGE bi-plot showed that PCA accounted for 67.28, 56.25, 64.32 and 83.69% of the total variation in the grain yield for the four sets, respectively. The environments that mostly contributed to the total GEI were E6 and E4 for the 1st set; E1 and E3 for the 2nd set; E2 and E5 for the 3rd set; and E6 and E5 for the 4th set. Therefore, most results could be graphically presented in an AMMI1 bi-plot (Fig. 3). It was found that the variation of the environment was

higher than that of genotype.

Tai Stability analysis

Tai stability model partitions the GEIs consequence into two parameters: α, that calculates the linear response to environmental effects and λ that calculates the deviation from the linear response in terms of the magnitude of the error variance.

Based on these parameters, a perfectly stable-genotype is one with a deviation from the linear response of +1 and an environmental effect of -1, so that $(\alpha, \lambda) = (-1, 1)$. In this investigation distribution of 60 barley genotypes were appeared different stability regions based on Alpha-Lambda parameters (Fig. 4). The λ values for G-1, G-3, G-6, G-8, G-16, G-17, G-18, G-21, G-46, G-58, and G-59 were significantly different from unity, but none of them showed an α value of -1 except for G-50 and G-53 = -97. Although, the genotypes G-50 and G-53 showed perfect/static stability ($\alpha = -0.95, -0.91$, respectively), but they recorded a medium (1.76, and 1.17 t ha⁻¹) grain yield values in set-4, respectively. Alternatively, Tai's statistics parameters for G-36 showed $\alpha =$ 0 and $\lambda = 1.10$, this result indicated that G-36 of the tested genotypes average stability followed by G-44. The distribution of α and statistic for genotypes G-7, G-19, G-25, G-31, G-37, and G-60 were negative ($\alpha > 0$, $\lambda = 1$) referring that those genotypes were responsive in relatively to the poor environment and below average stability (Table. 4). Significant effects were found in all environments for grain yield, and present in the box plot (Fig. 5). In the simplest box plot, the

central rectangle spans the first quartile to the third quartile. Simple Pearson's correlation coefficients were computed between 15-physiological and morphological traits, under salinity condition, positive correlations between the GY and MD (r = 0.36, P < 0.01), NT (r = 0.51, P < 0.01) and BY (r = 0.64, P < 0.01) were observed (Table 7). Moreover, negative correlations between the Chl (SPAD value) and GY (r = -0.27, P < 0.01). Analyses of histogram's (Fig. 6) showed that salinity had a significant effect on grain yield at all sets across the six environments.

Discussion

 Efforts to anticipate how climate change will affect future food production accessibility will advantage from comprehension the impacts of changes on the crops production to date. In this study, the variance components explained that both location (E) and genotypes \times environments (G \times E) components were very important (Table 6). For the grain yield, location effects were high significant for all sets (P \leq 0.001) and ranged from 3.59 to 5.31-fold the genotype effect, suggesting that barley breeders can either develop specific-genotypes for each environment and/or with selecting genotypes that usually perform to a wide range of environments (Rodriguez et al. 2008).

In this study, AMMI model had been exploited in the variety evaluation of barley (Rodriguez et al. 2008; Yahiaoui et al. 2014), durum wheat (Reza et al. 2015), rice (Liu et al. 2002), faba bean (Temesgen et al. 2015) and maize (Demissew et al. 2016). The interpretation of the GEIs was established on linear regression (Finlay and Wilkinson 1963). A high positive interaction with the environments appeared from some genotypes such as G-6, G-9, G-21, G-42, G-50 and G-58. On contrary, the genotypes coded G-1, G-5, G-18, G-20, G-35, G-46, and G-59 had high negative interaction with the environment (Table 4). The GEIs are important factors in any crop variation, for example, the performance of protein quality in maize may be affected by tropical-

highland temperature in Ethiopia (Demissew et al. 2016), the variation in barley agronomic and quality characteristics (Nurminiemi et al. 2002) and the effect on barley grain yield (Dehghani et

3 al. 2006).

The main effects (G and E) represents in abscissa and it's the PC1 scores ordinate (Zobel et al. 1988). Our results showed that six genotypes coded; G-4, G-7, G-20, G-34, G-36, and G-39 were high-yielding performance (> 2.00 t ha⁻¹) and located almost zero and/or close to zero projection onto the AEC ordinate. This observation means that these genotypes most stable with respect to performance across environments. Therefore, AMMII bi-plot provides a means of imagination the mean performance of G and the stability PC1 of the genotypes, simultaneously (Gauch and Zobel 1997; Yan and Kang 2003; Yan et al. 2007). GGE bi-plot showed the most environments contributed to the total GEIs which were E4, E3, E5, and E6 in the four sets, respectively (Fig. 3). The GGE bi-plot has therefore been used in crop variety trials to effectively identify the best-performing genotype(s) across environments, whereby specific genotypes can be recommended to specific mega-environments and evaluate the yield and stability of genotypes (Rharrabti et al. 2003; Yan and Kang 2003; Gauch 2006 and Rodriguez et al. 2008).

The combined analysis of variance is a sign of the environments (Table 6). This provided a large difference among environmental means in significant yield variations. Moreover, the environments contributed a large effect on yield stability. This might probably be due to differences in the characteristics of salt-affected soils, which differ, not only in their chemical, physical and biological properties as shown in (Table 2) and described previously by Elakhdar et al. (2016b), but also in their geographical distribution (semi-arid and Mediterranean climate) (Table 1). As the location was significant for the grain yield, the ideal genotype-model should

 have high mean and yield stability further, the breeders should develop stable genotypes that perform well over environments (Yan and Kang 2003; Gauch 2006).

On the other hand, the diversity in the grain yield was observed a high significantly associated with MD, NT, and BY (Table 7) across the environments. This funding probably, because of the nature of the salt-soil composition, so that; causes of the growth unstable under saline conditions (Munns 1993; Munns and Teste 2008). Several studies have reported the contribution of the environment affecting yield stability (Yan et al. 2007; Gauch et al. 2008; Balestre et al. 2009; Yang et al. 2009; Malosetti et al. 2013). Garcı'a del Moral et al. (2002) reported that cereals have shown how environmental differences in yield can be affected by variations in spike number and fertility.

Tai's stability model

Tai's figure areas I, II and III indicate regions of average, above and below average stability, respectively. The genotypes G-5, G-9, G-40, G-41, G-47, G-53 and G-55 showed λ values, significantly different from unity; moreover, the distribution of the 60 genotypes on α - λ space showing different stability regions was indicated in (Fig. 4). It should be noted that the larger the λ value, the more difficult it is to show a significant difference between the α estimate and α = 0 and hence the α statistic becomes less meaningful in interpreting the linear response of a cultivar over varying environments (Tai 1971; Thillainathan et al. 2001).

Conclusions

This finding indicated that using AMMI and Tai's analysis would favor the simultaneous development of stable and high yielding barley genotypes across salt location. AMMI analysis appeared to be able to extract a large part of the interaction and is thus more efficient in analyzing $G \times E$ interaction pattern. The genotypes; G-4, G-7, G-20, G-34, G-36, and G-39 were

the most stable genotypes with high-yield (GY > 2.00 t ha⁻¹) across environments. Tai's analysis provides a method of obtaining the prediction interval for $\alpha=0$ and a confidence interval for λ values so that genotypes can be distributed graphically in different stability regions. Two genotypes G-50 and G-53 showed perfect/static stability ($\alpha=-0.95$, -0.91, respectively). In contrast, the genotype; G-36 had $\alpha=0$ and $\lambda=1.10$, indicating parallel with the environmental effects followed by G-44.

A very strong GEI for grain yield is observed, suggesting that may be a result of changes in genotypes' relative performance across environments, due to their differential responses to various abiotic factors which are sited in a typical Mediterranean area. However, both mean yield and stability should be considered simultaneously to exploit the useful effects of G × E

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interaction and to make the selection of favorable barley genotypes more precise.

1 Table's Legends

- **Table 1.** Description of the testing location during the two seasons
- **Table 2.** The soil properties; chemical, physical, and textural characterize for the testing location.
- **Table 3.** The performance of grain yield (tha⁻¹) for 60 barley genotypes tested in six
- 5 environments.
- **Table 4.** AMMI adjusted mean grain yield (t ha⁻¹), IPCA scores, and Tai's stability parameters of
- 7 genotypes of 60 spring barley evaluated across six-environments in Egypt.
- **Table 5.** The environments mean grain yield (t ha⁻¹), and IPCA scores of 60 spring barley
- 9 evaluated across six-environments in Egypt.
- **Table 6.** Analysis of variance for grain yield of 60 barley genotypes tested across six salt- affected
- 11 soil environments.
- **Table 7.** Pearson correlation matrix for salt-related traits across six environments.

Figures Legends

- 2 Fig.1. The SDS-PAGE fractionated proteins showed distinction in the number and molecular
- 3 weight of these polypeptides. The major components of all genotypes were in the hordein
- 4 fractionation and the arrow track indicates D-hordein >100 kDa and the brackets indicate, C-
- 5 hordeins 55-75 kDa and 35-46 kDa B-hordeins (a), A dendrogram showing the relationship of
- 6 the genotypes based on protein (*hordein*) bands (b) Nei (1978).
- 7 Fig.2. Ward's dendrogram presenting the classification of 6-testing environments based on
- 8 environment mean yield. E, year-location combinations during 2012-14: E1: Elhossinia
- 9 2012/2013; E2: Seirw 2012/2013; E3: Sakha 2012/2013; E4: Elhossinia 2013/2014; E5: Seirw
- 10 2013/2014; E6: Sakha 2013/2014.
- 11 Fig.3. Bi-plot showing a comparison of all genotypes with the ideal genotype constructed based on
- 12 environment-centered and genotype focused singular-value partitioning. The x-axis shows the main
- 13 effects, while the y-axis shows the first PCA axis. See codes of environments and genotypes in table
- 14 3.
- **Fig.4.**
- Distribution of the 60 barley genotypes on Alpha-Lambda areas I, II and III indicate regions of
- average, above and below average stability according to (Tai, 1971) method.
- **Fig.5.** Box plots showing the individual scores and the means, the circle 'o' stands for outliers.
- 19 The upper and lower lines outside the box stand for max and min adjacent value, respectively.
- The upper and lower hinge of the box stands for 75% and 25% percentile, respectively.

- **Fig.6.** Histogram showing the contribution effects of 6-environments for grain yields obtained by
- 2 a resembling procedure, between the first principal component (eigenvector) of both matrices of
- 3 co-variance for grain yield.

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Table 1. Description of the testing location during the two seasons

locations	Annual	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Average temperature/ C									
1	20.1	18.4	14.7	13.0	13.6	15.4	19.0	22.2	25.2
2	19.2	15.4	20.2	13.5	14.0	15.7	18.6	21.6	24.7
3	21.0	20.0	16.0	14.0	14.0	16.0	19.0	21.0	25.0
Average precipitation/ mm									
1	71.0	8.4	14.3	19.5	12.6	6.3	4.8	2.0	
2	13.9	21.9	117.6	28.6	18.1	17.4	6.2	1.4	0.3
3	40.0								
Average number of days with pr	ecipitation/ da	ıys							
1	32.0	3.5	6.1	7.9	5.8	4.1	1.7	0.6	0.1
2	4.7	6.9	37.5	8.9	6.6	4.6	2.1	0.8	0.2
3									
Average length of day/ 12.6									
1	10.8	10.6		11.5	12.4	12.4	14.2	14.6	14.4
2	11.0	10.6	12.6	10.8	11.5	12.4	12.4	14.2	14.6
3									
Average relative humidity/ %									
1	68.4	72.0	71.8	71.6	70.4	68.2	62.6	61.0	62.7
2	73.1	73.4	71.2	73.2	72.1	70.7	67.5	67.5	68.0
3									
Average dew point/ C									
1	14.1	13.3	9.7	8.0	8.3	9.6	11.7	14.3	17.6
2	14.3	10.7	14.8	8.8	9.1	10.4	12.5	15.3	18.4
3	15.0	14.0	11.0	8.0	8.0	10.0	13.0	15.0	18.0
Average wind speed/ km/h									
1	10.7	9.4	10.1	10.8	11.9	12.6	12.2	11.9	10.8
2	11.5	11.9	12.9	12.6	14.0	15.5	14.8	14.0	13.3
3	22.0	25.0	30.0	19.0	25.0	20.0	22.0	20.0	19.0

Loc-1: Sakha, Kafrelsheikh prefecture (Elevation: 9 meters -Latitude: 31 07N -Longitude: 030 56E); Loc-2: Seirw, Damietta prefecture (Elevation: 12 meters- Latitude: 31 25N - Longitude: 031 49E) and Loc-3: Elhossinia, Isma'iliyah prefecture (Elevation: 13 meters-Latitude: 30 36N-Longitude: 032 15E).

Source: World weather & climate information (https://weather-and-climate.com/)

Table 2. The soil properties; chemical, physical, and textural characterize for the testing location.

	Soil properties																				
Location	season	Hd	EC dsm ⁻¹	CaCO ₃ %	% dS	SAR %	T/ba	Ca^{++}	$ m Mg^{+}$	Na^{++}	\mathbf{K}^{+}	T/ba	SO_4	-CI-	HCO_3	CO ₃	s	Sand %	Silt %	Clay%	Texture class
LOC-1	1 st	7.83	10.90	3.95	52.90	8.90	S m	20.70	40.30	47.05	0.95	Ē	38.87	67.00	3.13	-	rtie	23.80	24.90	51.30	clayey
	2^{nd}	8.41	9.50	2.32	51.70	8.40				53.50			72.50	60.00	3.00	-	obe	15.10	38.50	46.40	clayey
LOC-2	1 st	7.97	10.50	10.50	51.35	16.20	cati	28.98	48.75	46.37	0.90	ani	44.20	58.00	2.81	-					clayey
	2^{nd}	8.12	11.55	2.18	50.20	15.20	ole	57.80	38.20	105.10	0.85	ole	108.40	90.00	4.00	-	[ca]	22.35	28.90	48.75	clayey
LOC-3	1 st	7.89	6.80	6.80	52.30	18.15	Jul	6.90	16.90	44.30	0.80	Juk	31.77	44.00	3.13	-	lys.	22.35	28.90	48.75	clayey
	2^{nd}	7.75	22.44	2.55	51.50	17.45	Sc	176.80	130.40	216.10	2.10	Sc	241.90	280.00	3.50	-	H I	15.10	38.50	46.40	clayey

Loc-1: Sakha, Kafrelsheikh prefecture; Loc-2: Seirw, Damietta prefecture Loc-3: Elhossinia, Isma'iliyah prefecture. (Elakhdar et al. 2016b).

Table 3. The performance of grain yield (tha⁻¹) for 60 barley genotypes tested in six environments.

	Set-1							Set-	-2			
Genotype	E1	E2 E3	E4	E5	E6	Genotype	E1	E2	E3	E4	E5	E6
G-1	2.29	1.56 2.1	3 3.58	2.29	2.19	G-16	2.40	1.48	2.65	1.88	1.98	3.23
G-2	2.04	1.04 2.3	3 2.71	3.06	2.50	G-17	2.19	1.27	1.88	1.25	2.81	1.77
G-3	2.33	1.56 2.7	3 2.71	2.71	2.60	G-18	1.73	1.67	3.33	2.29	3.33	3.33
G-4	2.19	1.67 2.0	8 2.29	2.50	3.54	G-19	1.58	1.04	2.60	1.67	2.29	1.56
G-5	1.60	1.77 2.4	0 1.40	2.08	1.51	G-20	1.71	1.35	1.88	2.08	2.92	2.19
G-6	2.29	1.10 2.7	1 1.56	3.06	2.08	G-21	2.29	1.56	1.04	2.81	2.19	2.19
G-7	2.23	1.25 2.0	8 1.92	2.29	2.67	G-22	2.40	1.42	2.60	2.92	2.71	3.13
G-8	2.08	1.04 2.5	0 3.13	3.02	3.33	G-23	1.63	1.21	2.02	2.71	2.92	2.19
G-9	1.48	1.25 2.0	4 1.54	3.65	2.92	G-24	2.31	0.83	2.08	2.04	2.19	2.34
G-10	2.08	1.09 2.0	8 1.77	2.60	2.29	G-25	2.19	1.46	2.04	2.77	2.40	2.15
G-11	1.90	1.35 2.3	5 2.81	2.71	2.92	G-26	1.27	1.00	1.81	1.35	3.02	1.98
G-12	1.63	1.10 1.8	8 1.88	2.19	3.13	G-27	1.98	1.35	2.81	3.02	2.60	1.77
G-13	1.35	0.73 2.0	8 1.71	2.50	2.40	G-28	1.83	1.94	2.92	2.92	1.98	2.50
G-14	1.04	1.15 2.0	2 1.81	2.50	2.40	G-29	2.40	1.77	1.83	3.23	3.33	2.50
G-15	2.04	1.46 2.7	1 1.77	2.44	2.81	G-30	2.81	2.19	2.02	3.17	2.60	3.17

E1: Elhossinia 2012/2013; E2: Seirw 2012/2013; E3: Sakha 2012/2013; E4: Elhossinia 2013/2014; E5: Seirw 2013/2014; E6: Sakha 2013/2014.

Cont table 3.

	Set-3	3				Set-4							
Genotype	E1	E2	E3	E4	E5	E6	Genotype	E1	E2	E3	E4	E5	E6
G-31	1.15	1.04	2.04	1.10	2.40	1.98	G-46	1.21	1.04	2.81	2.19	1.17	2.81
G-32	1.69	1.85	3.33	1.38	3.44	2.50	G-47	1.48	1.85	2.60	1.48	1.88	2.71
G-33	1.83	2.19	2.69	2.15	3.19	1.98	G-48	1.42	2.19	1.83	1.63	2.33	2.00
G-34	1.88	1.88	2.98	1.71	3.33	1.88	G-49	1.56	1.88	1.98	1.58	2.10	2.06
G-35	1.17	1.98	2.54	0.83	3.75	1.77	G-50	1.58	1.98	1.88	1.63	2.25	1.23
G-36	1.90	1.44	3.02	1.63	3.02	1.88	G-51	2.13	1.44	2.29	2.23	1.69	2.02
G-37	1.83	1.77	1.98	2.00	3.44	2.08	G-52	1.17	1.77	2.08	1.21	1.83	2.21
G-38	1.52	1.46	2.50	2.15	2.92	2.08	G-53	1.54	1.46	1.56	1.54	1.46	1.42
G-39	1.77	1.44	2.40	1.56	3.23	1.77	G-54	1.44	1.44	2.08	1.44	1.42	1.94
G-40	1.48	1.05	1.60	1.50	2.40	1.46	G-55	1.25	1.05	1.38	1.25	1.13	1.00
G-41	1.46	1.42	1.98	0.83	3.44	1.46	G-56	1.88	1.42	1.98	1.88	1.94	1.63
G-42	2.50	1.50	1.81	1.83	3.23	1.98	G-57	1.58	1.50	1.88	1.79	1.75	1.96
G-43	1.88	1.98	1.81	1.38	<u>4.00</u>	2.29	G-58	1.56	1.98	1.88	1.65	2.56	1.73
G-44	1.33	<u>0.63</u>	2.08	1.54	3.02	1.23	G-59	1.38	<u>0.63</u>	2.23	1.56	0.73	2.21
G-45	1.71	1.88	2.81	1.71	2.81	1.46	G-60	1.58	1.88	1.98	1.67	1.94	2.21

E1: Elhossinia 2012/2013; E2: Seirw 2012/2013; E3: Sakha 2012/2013; E4: Elhossinia 2013/2014; E5: Seirw 2013/2014; E6: Sakha 2013/2014.

Table 4. AMMI adjusted mean grain yield (t ha⁻¹), and IPCA scores of genotypes of 60 spring barley evaluated across six-environments in Egypt.

Set-1						Set-2					
Genotype	Gm	IPCA1	IPCA2	α	λ	Genotyp	e Gm	IPCA1	IPCA2	α	λ
G-1	2.34	-0.96	-0.38	-0.38	3.79	G-16	2.37	-0.14	0.19	-0.37	2.72
G-2	2.28	-0.21	0.02	0.23	<u>0.96</u>	G-17	2.04	0.20	0.21	-0.25	2.14
G-3	2.44	-0.20	-0.27	-0.09	2.18	G-18	2.13	-0.90	0.02	0.50	2.60
G-4	2.38	<u>-0.07</u>	0.39	0.74	1.46	G-19	2.21	-0.18	0.56	-0.10	1.54
G-5	<u>1.79</u>	0.30	-0.82	-0.58	0.47	G-20	2.04	<u>0.05</u>	-0.82	0.14	0.51
G-6	2.14	0.49	-0.42	-0.23	4.42	G-21	1.93	0.71	0.27	-0.42	2.76
G-7	2.07	0.00	-0.05	-0.18	0.75	G-22	<u>2.51</u>	<u>-0.02</u>	0.25	0.35	0.51
G-8	<u>2.52</u>	-0.39	0.45	0.74	2.08	G-23	2.09	<u>-0.01</u>	-0.44	0.48	0.48
G-9	2.15	0.66	0.46	1.05	1.52	G-24	2.17	0.12	0.28	0.17	0.81
G-10	1.99	0.15	-0.15	-0.21	0.32	G-25	2.23	0.23	-0.02	-0.16	0.38
G-11	2.34	-0.31	0.16	0.60	1.81	G-26	<u>1.76</u>	<u>0.00</u>	0.27	0.37	1.88
G-12	1.97	<u>-0.01</u>	0.42	0.10	1.57	G-27	2.19	-0.44	-0.18	0.13	1.85
G-13	1.80	0.15	0.15	-0.52	1.44	G-28	<u>2.44</u>	-0.42	-0.11	-0.59	1.80
G-14	1.82	0.13	0.14	-0.51	0.55	G-29	2.24	0.36	-0.19	0.24	1.62
G-15	2.21	0.27	-0.12	-0.74	1.39	G-30	<u>2.47</u>	0.45	-0.29	-0.48	1.20

E1: Elhossinia 2012/2013; E2: Seirw 2012/2013; E3: Sakha 2012/2013; E4: Elhossinia 2013/2014; E5: Seirw 2013/2014; E6: Sakha 2013/2014. Deviations are in units of (t ha⁻¹), IPCA scores are in units of (t ha⁻¹), α , λ , Tai's alpha and lambda mean-variance component for a pair-wise G×E interaction.

Table 4. Cont.

Set-3						Set-4					
Genotype	Gm	IPCA1	IPCA2	α	λ	Genotype	Gm	IPCA1	IPCA2	α	λ
G-31	1.62	0.09	-0.16	<u>-0.18</u>	0.97	G-46	1.87	-0.92	0.11	0.95	2.70
G-32	<u>2.37</u>	-0.41	-0.46	0.23	1.90	G-47	2.00	-0.25	0.54	0.96	0.64
G-33	<u>2.34</u>	0.16	-0.14	-0.26	0.44	G-48	1.90	0.43	0.32	-0.68	1.96
G-34	2.27	<u>-0.09</u>	-0.28	0.04	0.49	G-49	1.86	0.20	0.19	-0.25	0.54
G-35	2.01	-0.83	0.00	0.53	1.85	G-50	1.76	0.58	-0.14	<u>-0.95</u>	2.02
G-36	2.15	0.08	-0.42	<u>0.00</u>	<u>1.10</u>	G-51	1.97	-0.19	-0.52	-0.39	1.66
G-37	2.18	0.18	0.39	<u>-0.12</u>	0.94	G-52	1.71	0.02	0.53	0.82	1.11
G-38	2.10	0.37	-0.19	<u>-0.23</u>	<u>0.95</u>	G-53	1.50	0.17	-0.29	<u>-0.95</u>	0.30
G-39	2.03	0.03	0.01	0.05	0.07	G-54	1.63	-0.15	0.05	0.41	0.23
G-40	<u>1.58</u>	0.45	0.14	-0.37	0.40	G-55	<u>1.17</u>	0.16	-0.37	-0.91	0.18
G-41	1.76	-0.43	0.27	0.36	0.67	G-56	1.79	0.14	-0.41	-0.82	0.74
G-42	2.14	0.42	0.54	-0.27	2.19	G-57	1.72	<u>-0.04</u>	-0.13	-0.30	0.17
G-43	2.22	-0.37	0.72	0.24	2.74	G-58	1.89	0.53	0.11	-0.91	2.23
G-44	1.64	0.23	-0.02	<u>0.20</u>	<u>1.22</u>	G-59	1.46	-0.77	-0.17	0.97	2.80
G-45	2.06	<u>0.10</u>	-0.41	-0.21	1.27	G-60	1.88	0.09	0.18	-0.16	0.35

E1: Elhossinia 2012/2013; E2: Seirw 2012/2013; E3: Sakha 2012/2013; E4: Elhossinia 2013/2014; E5: Seirw 2013/2014; E6: Sakha 2013/2014. Deviations are in units of (t ha⁻¹), IPCA scores are in units of (t ha⁻¹), α , λ , Tai's alpha and lambda mean-variance component for a pair-wise G×E interaction.

Table 5. The environments mean grain yield (t ha⁻¹), and IPCA scores of 60 spring barley evaluated across six-environments in Egypt.

Set-1			Set-2				Set-3					Set-4			
Environment	Em	IPCA1	IPCA2												
E1	1.91	-0.08	-0.44	E1	2.05	0.60	0.07	E1	1.67	0.46	0.36	E1	1.52	0.10	-0.71
E2	<u>1.28</u>	0.20	-0.48	E2	<u>1.44</u>	0.13	-0.35	E2	1.57	-0.39	0.07	E2	1.57	0.73	0.38
E3	2.28	0.36	-0.49	E3	2.24	-1.26	0.15	E3	2.37	-0.28	-1.13	E3	<u>2.03</u>	-0.65	0.12
E4	2.17	-1.24	0.06	E4	2.40	0.16	-0.92	E4	<u>1.55</u>	0.96	-0.03	E4	1.65	-0.26	-0.64
E5	2.64	0.65	0.28	E5	<u>2.63</u>	0.44	0.81	E5	<u>3.17</u>	-0.70	0.60	E5	1.74	0.89	0.24
E6	2.62	0.12	1.08	E6	2.38	-0.07	0.26	E6	1.85	-0.05	0.13	E6	1.94	-0.81	0.61

Table 6. Analysis of variance for grain yield of 60 barley genotypes tested across six salt- affected soil environments.

		Set-1		Set-2		Set-3		Set-4	
SOV	d f	MS	VC %	MS	VC %	MS	VC %		VC %
Rep.	12	0.40**	2.76	1.00**	6.85	0.39**	2.56	0.21**	3.10
Treatments ⁺	89	1.21**	61.74+	0.95**	48.35+	1.51**	73.93+	0.55**	59.92
Location (E)	5	11.70**	33.34	7.75**	22.16	18.24**	49.92	1.89**	11.50
Genotypes (G)	14	1.02**	8.13	0.77**	6.17	1.22**	9.39	0.90 **	15.40
$(G \times E)$	70	0.50**	20.27	0.50**	20.01	0.38**	14.61	0.38**	33.00
AMMI analysis									
PC1	18	0.76**	44.60	0.79**	32.83	0.56**	42.14	1.02**	46.91
PC2	16	0.68**	22.69	0.55**	23.42	0.59**	22.18	0.42**	36.78
Residuals	36	0.30**		0.33**		0.196		0.05	

MS; mean of squares, d f; degrees of freedom.

^{*} and ** indicate significant (0.01< P <0.05) or highly significant (P <0.01), respectively. VC %; variance component%

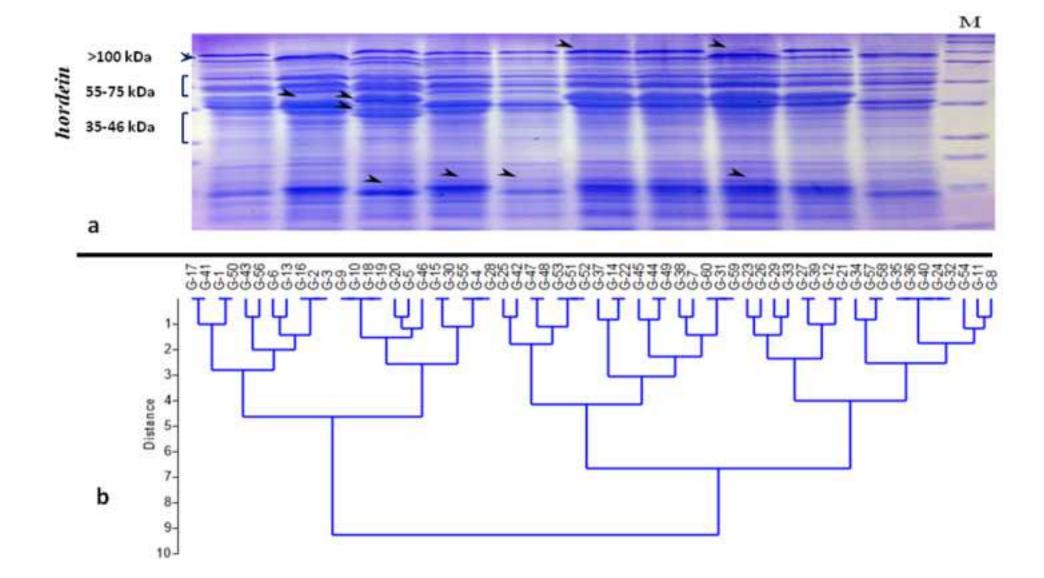
⁺Treatments (Genotypes + locations + interaction).

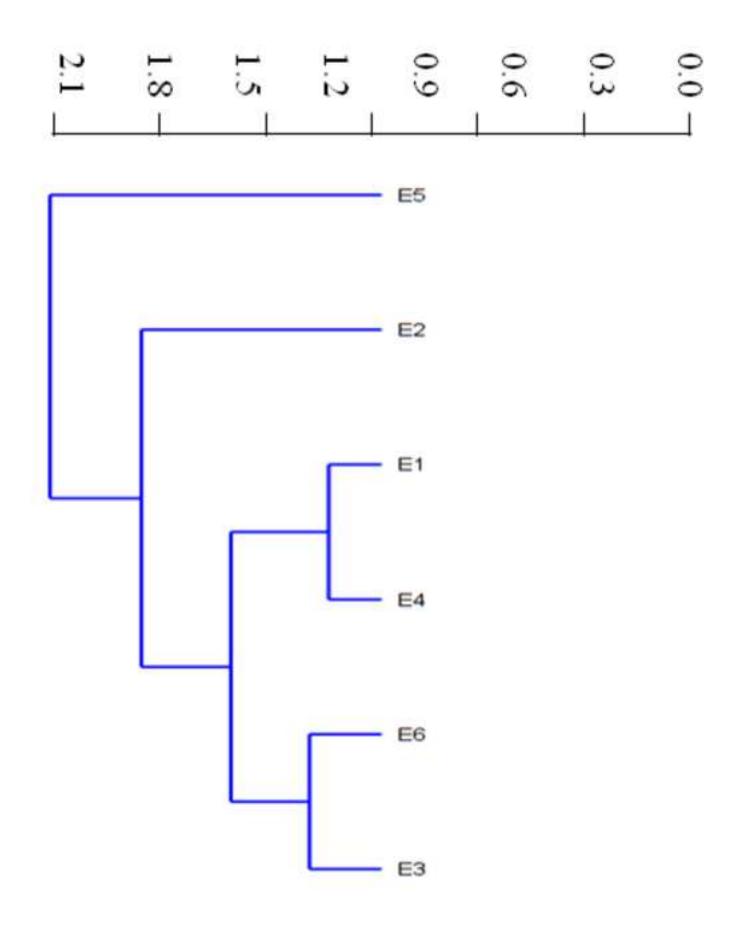
Table 7. Pearson matrix illustrates the correlation between the salt-related traits across six-environments.

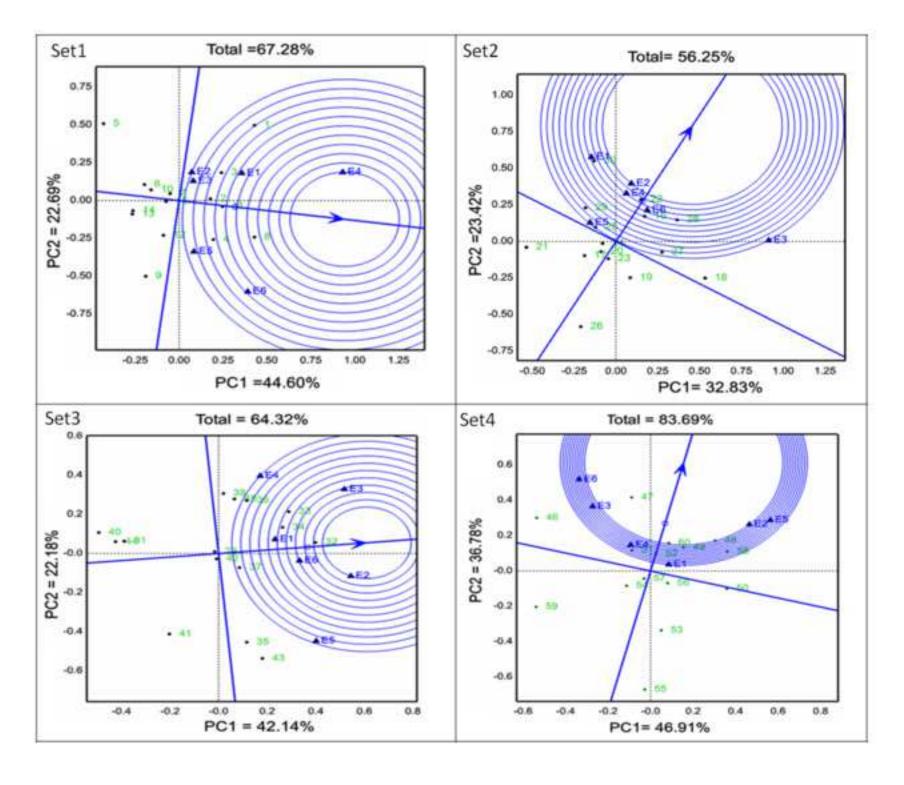
Traits	HD	Chl	LA	MD	PH	SL	Ped	NT	NG	BY	GY	GW	GFP	GFR	HI
HD															
Chl	-0.27**														
LA		-0.28**													
MD		-0.64**													
PH															
SL					ļ .										
Ped				-0.37**	0.50**										
NT		-0.28**		0.38**	-0.44**		-0.26**								
NG					0.26**										
BY				0.37**		0.29**	-0.28**	0.30**							
GY		-0.27**		0.36**				0.51**		0.64**					
GW		-0.43**	0.43**	0.31**					-0.44**		0.47**				
GFP	-0.27**	-0.44**		0.56**	-0.37**		-0.27**	0.50**			0.44**	0.43**			
GFR							0.30**	-0.29**	-0.28**			0.52**	-0.50**		
HI	-0.36**							0.27**			0.56**	0.36**			

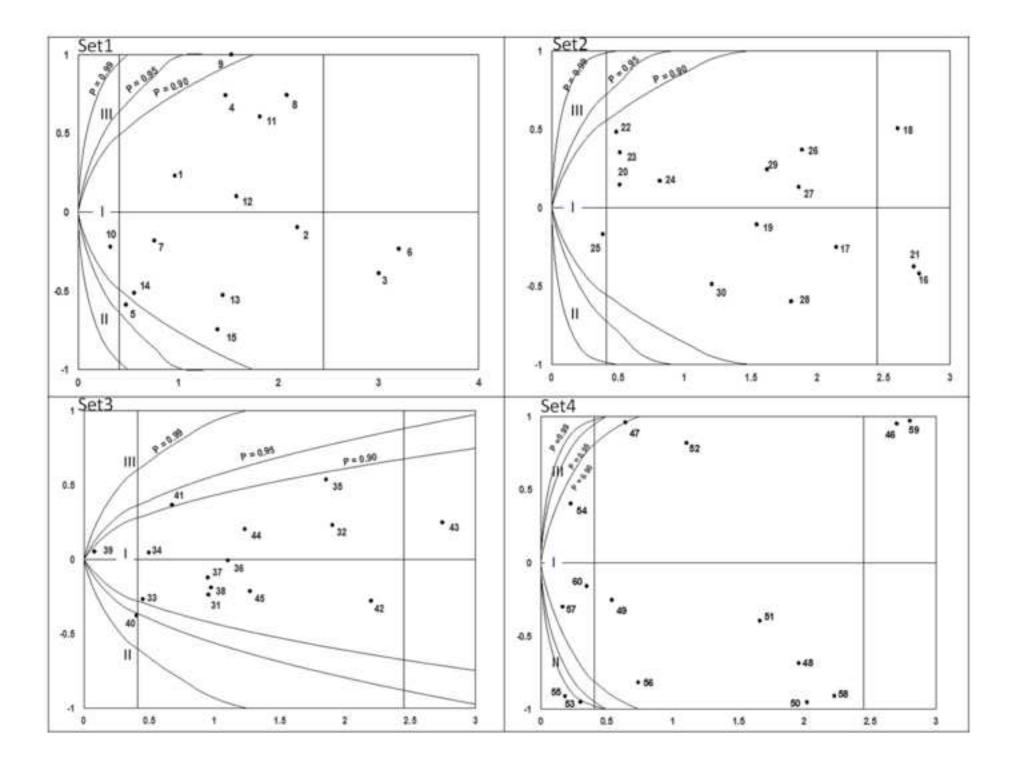
^{**;} highly significant at the level of P < 0.001 and 0.05.

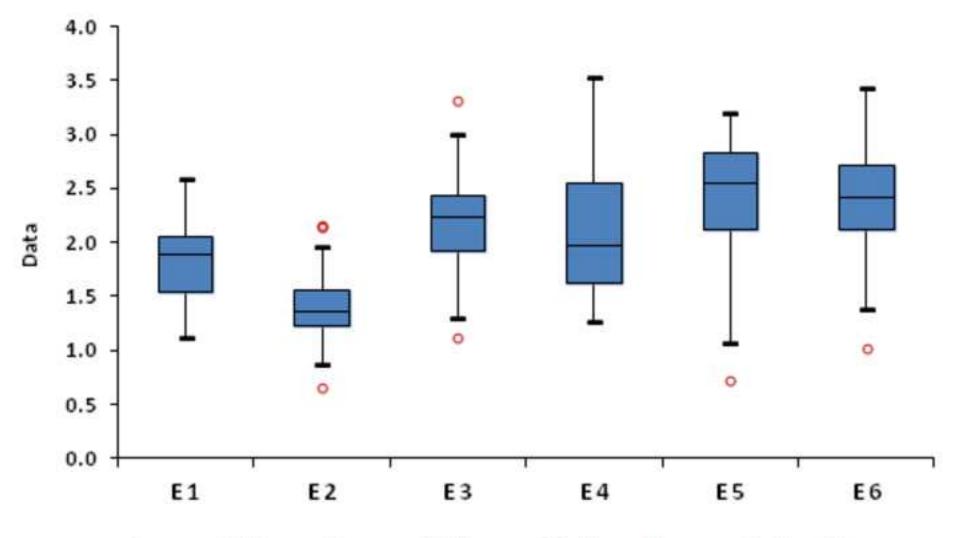
Days to Heading (DH) and Maturity (DM), Chlorophyll content (Chl as SPAD value), Flag Leaf Area cm² (LA), Plant Height (PH), Spike Length (SL), Peduncle length (Ped), Number of Tillers/ m² (NT), Number of Grains/ spike (NG), thousand-Grain weight (GW), Grain Yield GY t h^{-1} , Biological Yield BY t h^{-1} , Grain Filling Period (GFP), Grain Filling Rate (GFR) and Harvest Index (HI).



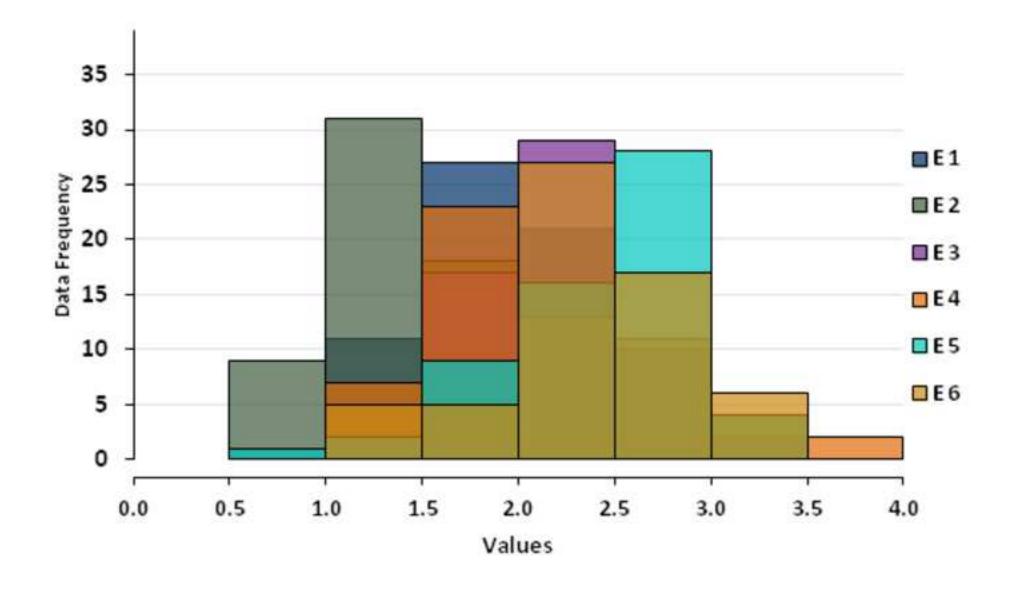








Lower whisker; - Upper whisker; - Median; o suspected outliers



supplementary Table

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Authors responses

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