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## Research Article

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**Pattern of genetic inheritance of grain zinc and iron content, agronomic and biochemical traits in bread wheat using mixed linear models under salinity stress**

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## **Abstract**

Wheat is the most important staple food that acts as a primary source of dietary calories, protein and most of the bioavailable micronutrients such as iron (Fe) and zinc (Zn) for the world's population. Understanding genetic control of micronutrients uptake is necessary for development of good quality wheat genotypes. To study the nature of inheritance of Zn and Fe efficient uptake under none-saline and saline conditions, two Iranian facultative wheat variety; Navid (salt sensitive, Fe and Zn-deficient) and Roshan (salt tolerant, Fe and Zn-efficient); were crossed to generate six basic generations for generation mean analysis. All the genotypes of these six generations were evaluated for grain Zn and Fe content, agronomic and biochemical traits under none-saline and saline conditions. For all the studied traits, the non-additive components were greater than the additive component. Additive effects were negative and significant for all traits under non-saline and saline conditions, except for 100-SW in control, MDA in 100 mM salinity level, EL and LeaNaC in all salinity levels. Additive gene actions were important for grain Zn and Fe content; while for rest of traits both fixable and non-fixable genetic effects were important. Duplicate dominant type of epistasis was involved in inheritance of all the traits. Broad-sense heritability values (>0.6) for most traits under non-saline and saline conditions were high, whereas the narrow-sense heritability values for most of the studied traits were low to moderate. These Zn and Fe efficient uptake indices could be used to select Zn/Fe-efficient wheat genotypes from segregating populations.

**Keywords:** Abiotic stress, Genetic control, Grain Zn and Fe content, Zn/Fe efficient uptake, Mixed linear model

## **Introduction**

Among the major abiotic stresses, salinity is one of the most adverse environmental factors limiting the growth, survival and production of wheat in Iran and worldwide. Salinity stress generates hyperionic and hyperosmotic stress in wheat, which cause hormonal imbalance, nutrient disturbances and negative impact on yield (Munns and Tester 2008; Daneshbakhsh et al. 2013). Iran is a chief producer of wheat in Asia (FAO 2017), challenging for about 34 million ha of salt-affected land area which includes 4.1 million ha of irrigated land (FAO 2000) leading to an annual economic loss of almost US\$ 1 billion (Qadir et al. 2008).

By reducing the micronutrients, the effects of salinity stress can be accelerated in cereal crops (Faran et al. 2019). In other words, high salt concentrations in the soil reduce the root growth leading to poor nutrient uptake, especially Zn and Fe, due to strong competition between Zn and Fe and salt actions which may cause severe

deficiencies of these elements in the plants (Rehman et al. 2017). Under salt stress, the production of reactive oxygen species (ROS) has increased which causes structural and functional damage on lipids, proteins, nucleic acids and water relations that consequently suppress the photosynthesis, Zn and Fe uptake in plants resulting the poor yield (Munns and Tester 2008; Rehman et al. 2017).

To surmount the aforementioned problem and ensure higher crop production, different approaches such as agronomic biofortification (foliar spraying, soil application and/or seed priming) and genetic advancement of crops have been suggested to develop genotypes which can maintain sufficient micronutrients under saline soils (Peleg et al. 2009; Pfeiffer and McClafferty 2007). Among the potential solutions, the breeding of wheat varieties especially landraces and wild relatives of common wheat has been proposed as one of the most cost-effective and environmentally safe approaches to alleviate the malnutrition (Peleg et al. 2009). The agronomic solutions are being exhausted in case of minimizing the impact of salinity stress, and consequent Zn and Fe deficiency. Therefore, developing salt tolerant genotypes of crops accompanied with higher Zn and Fe acquisition efficiency would be may be beneficial to minimize micronutrient deficiency gaps in farming communities. Breeding for both tolerance to salt and micronutrients (particularly Zn and Fe) deficiency, in spite of its importance, has even rarely considered in wheat breeding programs.

A primary requirement of such a breeding effort are exploring about genetic variation of component indices of Zn and Fe efficiency, including uptake of Zn and Fe by the roots, translocation, assimilation, and remobilization of them into the grains (Liu et al. 2019). According to Velu et al. (2014), efficient uptake of Zn and Fe from the soil can be achieved by modifying the morphological and structural traits of root.

Generation means analysis (GMA), a biometrical method developed by Mather and Jinks (1982), is a simple but useful tool for designing breeding strategies to take the advantage of gene interaction existing in succession breeding generations (Mather and Jinks 1982). In GMA, joint scaling test is commonly used to study the inheritance types of traits, in which the gene effects (additive and dominance) and digenic epistatic interactions (additive x additive; Additive x dominance; Dominance x dominance) are set up in a linear model and evaluated by the chi-square test (Mather and Jinks 1982). However, this method has several limitations that can restrict its utilization. This method cannot be used directly for models in which the number of effects is equal or greater than to the number of generation means. On the other hand it is possible that additive, dominance, and epistatic effects are over- or underestimated, since these effects are obtained without error via the least square analysis method (Balestre

et al. 2012). Additionally, the error terms usually computed based on within-plot variances (Mather and Jinks 1971). In this case, because between-plot variance for the segregating generations is ignored, results may not be very accurate (Piepho and Mohring 2010). Using Mixed linear model developed by Piepho and Mohering (2010), previous issues and problems have been resolved and estimates of genetic effects and generation means as well as all variance components are executed in a single step, while, the traditional method requires two analyses for means and variances, separately.

Genetic control of different traits under abiotic stress conditions has been studied in wheat. Dashti et al. (2010) reported significant additive gene effects and high broad-sense heritability for K/Na ratio,  $K^+$ , and  $Na^+$  concluding existence of possibilities to improve these traits under salinity stress condition. Moroni et al. (2013) stated that additive effects were larger than dominant effects for manganese tolerance. Abbasi Holasou et al. (2019) showed that additive, dominance and epistatic effects were involved in the inheritance of agronomic traits under water deficit stress condition. Amiri et al. (2020) reported that both additive and non-additive effects are important for Fe and Zn uptake efficiency at both crosses (Marvdasht  $\times$  Rassoul and Marvdasht  $\times$  Shahpasand) under normal and drought stress conditions.

To the best of our knowledge, there were no reported records about gene action of zinc and Fe absorption ability under salinity stress conditions in bread wheat. Thus, the main objectives of this study were understanding the types and value of gene effects controlling the inheritance of efficient Zn and Fe uptake and related characters *viz.* grain yield, 100-seed weight, shoot dry weight, root dry weight, proline, Malondialdehyde (MDA), Peroxidase (POX), Catalase (CAT) by means of six basic generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1$ , and  $BC_2$ ) of wheat using generation mean analysis according to mixed linear model analysis under salinity stress conditions. Ultimately, this information will be useful for selecting Zn and Fe-efficient wheat genotypes for cultivation in low- Zn and Fe soils under salinity stress, thereby providing health benefits to humans.

## **Materials and Methods**

### **Plant materials and experiment**

The experiment was conducted under hydroponic greenhouse condition (temperature between  $28:19\pm 2^\circ\text{C}$  day:night on a 16:8-h light:dark photoperiod; humidity between 35–45%; natural light conditions) using a factorial experiment (including two factors; generations and treatments) based on randomized complete block design with three

replications. The first factor was the six basic generations (P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>, BC<sub>2</sub>) derived from a cross of two Iranian facultative wheat variety; Navid (salt sensitive, and Fe and Zn-deficient) and Roshan (salt tolerant, and Fe and Zn-efficient). The second factor was the levels of NaCl salinity, including control (no NaCl), 100 and 200 mM NaCl. Parents were previously screened for salinity stress, Zn and Fe efficiency by Khoshgoftarmanesh et al. (2006a and b), Daneshbakhsh et al. (2013) and Sharifi-Soltani et al. (2016).

The seed of generations were planted in 126 plastic pots (25×25 cm) filled with perlite: vermiculite mixture (4:1 v/v). The 24 plants for each P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, BC<sub>1</sub>, and BC<sub>2</sub>, and 48 plants for F<sub>2</sub> were used in each replication. Prior to sowing, qualified seeds of each generation were surface sterilized with 5% (W/V) sodium hypochlorite (NaClO) for 8 min and rinsed well with distilled water several times. To provide uniform seed germination, the seeds were sown on filter paper moistened with deionized water for 7 days in the greenhouse conditions (28/20°C day/night max/min). Upon germination, the undamaged seedlings with uniform size and uniform root length were transplanted into pots.

The pots were irrigated using Hoagland solution (Hoagland and Arnon 1950) in 1/4, 1/2 and full ratios on two, four and six days after planting, respectively. The nutrient solutions were changed frequently after every 7 days during the salinity treatment the pH was adjusted to 6.5±0.5 (EC=1.3 dsm-1). Salt treatment was applied at 17 days after sowing (DAS), when the third leaf was appeared (Zodaks score (Z) 13; Zodaks et al. 1974). To avoid osmotic shock, salt treatment was imposed stepwise in aliquots of 50 mM in Hoagland's nutrient solution every alternate daily until the appropriate salt treatments were reached. Pots were variedly irrigated from 600 to 650 ml of tap water and saline solutions every alternate period of days to achieve 100% FC (field capacity) according to weather conditions and growth stage. Avoiding excess salinity due to adding Hoagland solution, perlite substrate within the pots was washed every 14 days, and non-saline and salinity treatments were reapplied. Recorded ECs were 2.1 + 0.18, 9.2 + 0.2 and 17.2+ 0.74 for 0, 100 and 200 mM NaCl, respectively, which were weekly measured.

### **Phenotypic evaluation**

Sixteen morpho-physiological traits including grain Fe and Zn concentration (GFeC and GZnC), root length (cm), root fresh weight, root dry weight, leaf Na<sup>+</sup> concentration (LeaNa<sup>+</sup>C), leaf K<sup>+</sup> concentration (LeaK<sup>+</sup>C), Na<sup>+</sup>/K<sup>+</sup> concentration ratio (LeaK<sup>+</sup>/Na<sup>+</sup>C), Relative water content (RWC), Electric leakage (EL), weight of 100 grains (100-SW), grain yield per plant (g/plant), proline content, Catalase (CAT), Peroxidase (POX) and Malondialdehyde (MDA) were measured after 10 weeks from sowing. The shoots were cut off at the perlite-vermiculite mixtures

surface, and the roots were separated gently from the perlite-vermiculite mixtures via soaking them in deionized water for 10 min. The remaining perlite-vermiculite mixture adhering to the roots was then washed away.

Electric leakage (EL) was determined by the method of Lutts et al. (1996) using the portable electrical conductivity meter on the third leaf. Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Proline concentration was assessed according to Bates et al. (1973). MDA, POX and CAT were assayed by Heath and Packer (1968), Gueta-Dahan et al. (1997) and Singh et al. (2010), respectively. Concentration of Na<sup>+</sup> and K<sup>+</sup> was measured on the second leaf after nine weeks using a flame photometer according to Poustini and Siosemardeh (2004) with minor modification, and Fe and Zn concentration was measured according to Chapman and Pratt (1961) using Atomic Absorption Spectrometer (SpectrAA-220, VARIAN, Australia).

### Statistical analysis

The analysis of variance was done using the PROC GLM procedure of SAS System ver. 9.2 (SAS Institute 2007). Generation mean analysis was performed based on genetic model of Mather and Jinks (1982) using mixed linear model (MLM) proposed by Piepho and Mohering (2010) as follows:

$$Y = m + \alpha[d] + \beta[h] + \alpha^2[i] + 2\alpha\beta[j] + \beta^2[l] \quad (1)$$

where Y, m, d, h, i, j and l are mean of one generation, mean of all generations, sum of additive effects, sum of dominance effects, sum of additive × additive interaction (complementary), sum of additive × dominant and sum of dominant × dominant interactions (duplicate), respectively. The  $\alpha$ ,  $\beta$ ,  $2\alpha\beta$ ,  $\alpha^2$ ,  $\beta^2$  are the coefficients for the additive, dominant effects and their interactions in the model, respectively. The data that was significant in the ANOVA, showing significant differences between the parents, P<sub>1</sub> and P<sub>2</sub>, was submitted to the lack of fit test for the additive-dominance model, using the SAS macros for PROC Mixed models, as indicated by Piepho and Mohring (2010). According to Piepho and Mohring (2010), the lack of fit test is equivalent to the Joint Scaling Test of Mather and Jinks (1971). The fitness of additive-dominance model was examined using Wald-type F-test (Piepho and Mohering 2010).

Broad-sense and narrow-sense heritabilities (Wright 1968) as well as degree of dominance (Halluer and Miranda 1988) were estimated using following formula:

$$h_B^2 = \frac{V_G}{V_G + V_E} \quad (2)$$

$$h_N^2 = \frac{V_A}{V_G + V_E} \quad (3)$$

$$\bar{a} = \sqrt{\frac{2V_D}{V_A}} \quad (4)$$

Where  $V_G$ ,  $V_A$ ,  $V_D$ , and  $V_E$  are genotypic variance, additive variance, dominant variance and environmental variance, respectively.

## Results

### ANOVA

Analysis of variance revealed significant ( $p \leq 0.01$ ) effects of salinity and generations for all the traits indicating the presence of sufficient genetic variability for carrying out generation mean analysis and estimating heritability. The salinity  $\times$  generations interaction was also significant ( $p \leq 0.05$ ) for all the traits except 100-SW, proline content, GFeC and GZnC (Table 1). The CV varied from 2.05% for RWC to 15.35% for  $\text{Na}^+/\text{K}^+$  ratio.

The means and standard errors of the six main generations at three salinity levels (0, 100 and 200 mM) are represented in Table 2. It is worth noting that the mean values for GFeC, GZnC, RWC,  $\text{LeaNa}^+\text{C}$ ,  $\text{LeaK}^+\text{C}$ ,  $\text{LeaK}^+/\text{Na}^+\text{C}$ , CAT, POX, MDA and proline in  $F_1$  was close to the average of two parents at all three levels of salinity stress, while the means for 100-SW, GY, RL, RFW and RDW in  $F_1$  was greater than that of the two parents (Table 2). These results indicated that heterosis relative to mean parents which may be used to help develop hybrid varieties. Heterosis (over best parent) for grain yield was 43.56%, 28.13% and 10.34% at control, 100 and 200 mM salinity levels, respectively. Salinity stress reduced GFeC, GZnC, 100-SW, GY, RL, RFW, RDW, RWC,  $\text{LeaK}^+\text{C}$  and  $\text{LeaK}^+/\text{Na}^+\text{C}$  although the decrease at 200 mM was more than 100 mM. The value of EL,  $\text{LeaNa}^+\text{C}$ , CAT, POX, MDA and proline were increased by imposing the salinity treatments in all generations (Table 2).

### Gene effects and genetic parameters

Wald-F test revealed significant digenic epistatic interactions for majority of the traits at different salinity conditions. However, the additive-dominance model explained the genetic basis of GFeC and RL at the two higher salinity regimes (100 and 200 mM), RFW, RDW, MDA and proline under non-saline condition, RWC, EL and CAT at 100 mM NaCl and GZnC and  $\text{LeaK}^+\text{C}$  at 200 mM NaCl.

Generally, the non-additive effects were greater than the additive effect. Additive effects were negative and significant for all traits at all salinity levels ( $P_1$  had smaller values than  $P_2$ ), except for 100-SW in control, and EL and  $\text{LeaNa}^+\text{C}$  in all salinity levels. The negative additive effect indicates the inheritance of favorable alleles from  $P_2$

(Roshan) to the progenies. The same trend was observed for the dominance effect for RWC and  $LeaK^+/Na^+C$  at all salinity levels, for 100-SW and  $LeaK^+C$  under non-saline condition and 100 mM level, for GY, MDA and RFW at 100 mM salinity level, for POX at 200 mM salinity level, and for RDW and proline at 100 and 200 mM salinity levels.

The additive  $\times$  additive effects were negative and significant for  $LeaK^+/Na^+C$  (at all salinity levels), 100-SW (at control and 100 mM NaCl), GY (at 100 mM NaCl), RFW (at 100 mM NaCl), RDW (at 100 mM salinity level), RWC (at non-saline condition),  $LeaK^+C$  (at non-saline and 100 mM salinity levels), POX (at 200 mM salinity level), MDA (at 200 mM salinity level) and proline (at 100 and 200 mM salinity levels), but positive and significant ( $p \leq 0.01$ ) for  $LeaNa^+C$  (at all salinity levels), EL (at 200 mM salinity level) and MDA (at 200 mM salinity level). Dominance  $\times$  dominance effects were positive and statistically significant for GY (at all salinity levels),  $LeaK^+/Na^+C$  (at all salinity levels), 100-SW (at non-saline and 100 mM salinity level), RFW (at 100 and 200 mM salinity levels), RDW (at 100 and 200 mM salinity levels), RWC (at non-saline and 200 mM salinity level),  $LeaK^+C$  (at non-saline and 100 mM salinity levels), POX (at 200 mM salinity level), MDA (at 200 mM salinity level), and proline (at 100 and 200 mM salinity levels), whereas for  $LeaNa^+C$  (at 100 and 200 mM salinity levels), EL (under 200 mM treatment) and POX (under non-saline condition), dominance  $\times$  dominance gene effects were negative and significant (Table 3).

Duplicate epistatic (dominance and dominance  $\times$  dominance components were in opposite directions) digenic effects were ascertained critical in the inheritance of  $LeaK^+/Na^+C$  (at all salinity levels), RDW,  $LeaNa^+C$ , proline (under 100 and 200 mM salinity levels), 100-SW,  $LeaK^+C$  (at non-saline and 100 mM salinity levels), RWC, POX (at non-saline and 200 mM salinity levels), GY, RFW, MDA (at 100 mM salinity level) and EL (at 200 mM salinity level). It was suggested that these traits were governed by complex genetic architecture. Delayed selection until the high level of gene fixation is suggested for improvement in these traits (Table 3).

The estimates of additive, dominance, additive  $\times$  dominance covariance, and environmental components of variance, broad-sense and narrow-sense heritabilities and degree of dominance for different traits in control and salinity treatments are presented in Table 4. The additive variance was greater than dominance under both control and salinity conditions for GFeC, RL, RFW, RDW,  $LeaK^+C$ ,  $LeaNa^+C$ , MDA and proline. Whereas, dominance effect was slightly higher than additive effect for 100-SW and CAT under control, 100 and 200 mM NaCl,  $LeaK^+/Na^+C$  under control and 200 mM salinity levels, GZnC, EL and POX under 100 mM salinity level, RWC

under 200 mM salinity level and GY under non-saline condition (Table 4). Most of the studied traits exhibited high broad-sense heritabilities (more than 80%) in all three salinity levels, indicating the environmental effects constitute a minor portion of the total phenotypic variation for these traits. The narrow sense heritability ranged from 0.03 for  $LeaK^+/Na^+C$  (at 200 mM salinity level) to 0.69 for MDA (at 100 mM salinity level). The average dominance ratio for all studied traits was greater than unity in the three salinity levels except GFeC, RFW,  $LeaNa^+C$ , POX and proline under 200 mM salinity level, GY, RWC and MDA under 100 mM salinity level and RDW under control condition, indicating the predominance of the dominant gene action for these traits.

## **Discussion**

A good strategy to improve and sustain bread wheat production in Zn and Fe-deficient soils with minimum or no Zn and Fe application and knowledge of genetic components of tolerance have become a research priority. Understanding the inheritance and genetic basis of Zn and Fe efficient uptake-related traits may assisted to achieve high seed yield and salinity tolerance of bread wheat.

In this study, significant differences among generations for the studied traits indicted the presence of enough genetic variation for further analyses. The GMA for control and saline conditions showed the insufficiency of additive-dominance model for all the traits except GFeC and RL under 100 and 200 mM NaCl levels, RFW, RDW, MDA and proline under control condition, RWC, EL and CAT under 100 mM NaCl, and GZnC and  $LeaK^+C$  under 200 mM NaCl condition, indicating the presence of epistatic gene interactions in genetic control of the traits involve in Zn and Fe efficient uptake.

For all the studied traits, mean effects (m) were highly significant and had more value than additive gene effects (d), which implicated on existence of common genes between the two parents and the sufficient genetic variation in these traits. Data presented here indicate that dominance effects were generally greater than additives, explaining that the studied traits were generally controlled by major genes under all salinity levels. The negative or positive sign for dominance effect is a function of  $F_1$  generation mean value in relation to the mid parent value and indicates which parent is more contributing to the dominance effects. In the current study, the negative sign of dominance effect indicates that the  $F_1$  generation was more similar to the high Zn and Fe efficient parent “Roshan”, because dominance is originated from the parent containing alleles responsible for high value of the given trait. Although this study identified the important gene effects controlling Zn and Fe efficient uptake-related traits, it

should be noted that these traits are highly influenced by environmental conditions (high  $G \times E$  interaction), stage of plant development, and particularly water status in bread wheat (Gomez-Becerra et al. 2010; Amiri et al. 2018) as in other crops (Phuke et al. 2017; Gaddameedi et al. 2018).

For GZnC and GFeC, only additive gene effect was significant under both non-saline and saline conditions. On the other hand, the additive gene effects included the major ratio of genetic variation of GZnC and GFeC, suggesting necessity of effective selection in early generations. The sign of additive effects only reflects which parent is chosen as  $P_1$ . In other word, considering the low value parent as  $P_1$  resulted in negative sign of additive effects and vis versa (Reid et al. 2016). The occurrence of significant additive effects for GZnC, GFeC and Zn/Fe efficient uptake-related traits in all three salinity levels are different from the reports of Gaddameedi et al. (2018) that stated grain iron and zinc concentration and agronomic traits are largely inherited by both additive and dominant in sorghum. Mukamahirwa et al. (2015) found that additive and non-additive gene effects were important for Zn and Fe efficiency in common bean (*Phaseolus vulgaris* L.). On the basis of diallel analysis, Kumar et al. (2013) reported that additive and non-additive gene action were important for grain Zn and Fe concentration, respectively in sorghum and suggested that it is possible to improve grain Fe concentration through exploiting heterosis. Under field conditions, Velu et al. (2011) observed high ratio of combining ability variance components pointed out that the expression of grain Fe and Zn densities in pearl millet is governed predominantly by additive gene effects. Amiri et al. (2020) evidenced that dominant genetic effects mainly controlled GZnC and GFeC in bread wheat at the both crosses (Marvdasht  $\times$  Rassoul and Marvdasht  $\times$  Shahpasand). Chakraborti et al. (2010) reported non-additive gene effects for a large proportion of the variation in the kernel Zn and Fe concentrations in maize. Velu et al. (2017) studied the effects of *GPC-B1* gene on grain Zn and Fe concentrations in wheat and reported that incorporation of the *GPC-B1* allele into commercial elite wheat germplasm increased grain Zn and Fe concentrations and remobilization of Zn and Fe from leaves to the grains. This action of *GPC-B1* gene depended on the epistatic interaction with the background alleles and the environmental conditions.

Our results according with some other studies (e.g. Abu et al. 2017; Ravari et al. 2017) were shown that  $Na^+$ ,  $K^+$  and  $K^+/Na^+$  are important traits in relation to salinity tolerance in wheat. James et al. (2011) shown that by introgression of *Nax1* and *Nax2* genes from *T. monococcum* into hexaploid wheat, the  $Na^+$  concentration is reduced in leaves and increased in leaf sheaths, resulted in improving grain yield consequently. The results of the genome-wide association by Genc et al. (2019) revealed *Nax* genes associated with high  $Na^+$  accumulation, which may be

involved in osmotic stress/tissue tolerance. They stated that the most modern bread wheat, especially MW#293 genotype, are efficient in excluding  $\text{Na}^+$  and introduced as new paradigm in breeding for salinity tolerance. Utilizing of breeding programs in modern genotypes with high intrinsic Zn and Fe led to facilitation of uptake and transporting of these mineral nutrients from roots to the seeds under salinity stress.

The derived results showed the importance of the main and epistatic effects in  $\text{LeaN}^+\text{C}$ ,  $\text{LeaK}^+\text{C}$  and  $\text{LeaK}^+/\text{Na}^+\text{C}$  inheritance and demonstrated their concern in designing of breeding programs to improve salt tolerance in wheat cultivars and populations. Whereas additive gene effects did not contribute to  $\text{Na}^+$ ,  $\text{K}^+$  uptake and  $\text{K}^+/\text{Na}^+$  ratio under saline and non-saline conditions, it seems that progress in selecting for low  $\text{Na}^+$  uptake is very slow. These results are confirmed with Dashti et al. (2012) claimed complex epistatic effects for expressing  $\text{K}^+/\text{Na}^+$  ratio and leaf tissue  $\text{Na}^+$ . Therefore, it would be necessary to postpone the selection to later generation to improving this trait. Contrarily, the mentioned results are disagreed with those reported by Marzoghian et al. (2014) who found negligible amount of epistatic gene effects comparing with additive and dominance effects for traits plant height, biomass, electrolyte leakage and  $\text{K}^+/\text{Na}^+$  ratio at different salinity conditions. Ali et al. (2014) also reported both additive and dominance genetic effects involved in controlling root length, biomass, grains per spike, 100-grain weight,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{K}^+/\text{Na}^+$  ratio under both saline and non-saline conditions in bread wheat.

Halward and Wynne (1991) claimed that with increasing of difference between parents, dominance and epistatic effects may play more significant roles in the inheritance of quantitative traits. In this study the parent named Navid variety with pedigree (Kirkpinar 79) 63-112/66-2 $\times$ 7C improved from Iran, while Roshan is an Iranian local cultivar. The pattern of gene effects for quantitative traits (especially yield and 100-SW) were similar under the non-saline and saline conditions explaining fixable and non-fixable gene actions are the basis for yield components. In contrast, under field conditions, Ravari et al. (2017) showed that in both crosses (Kavir  $\times$  Arta and Kavir  $\times$  Moghan3) under acidic soil conditions, the dominance effects were more important than additive and epistatic effects in the genetic control of grain yield. The differences between those results and our study may be due to differences in the level of salt stress, different genetic backgrounds, and/or screening environment used. Concerning thousand seed weight and grain yield, Mwadingeni et al. (2018) observed significant effects for GCA under normal and drought-stress conditions indicated the influence of additive gene action.

Biochemical traits (e.g. CAT, POX, MDA and proline) perform important role in evaluating the salinity tolerance status of plants and could be used as an indirect selection index for screening Zn/Fe uptake efficient plants

(Tavallali et al. 2010; Saeidnejad et al. 2016; Babaei et al. 2017). Both fixable and non-fixable genetic effects under non-saline and saline conditions were important in inheritance of biochemical traits. Increased accumulation of antioxidants might improve abiotic stress in wheat by scavenging ROS (Ahmadi et al. 2018). Daneshbakhsh et al. (2013) found that under saline conditions, antioxidative defense mechanisms were activated to protect root membrane structure against oxidation. Plants potentially contain high Zn and Fe contents of seed could increase the activity of antioxidant enzymes, photosynthesis rate, and nutrient uptake under abiotic stress, ultimately could improve the yield performance (Faran et al. 2019).

According to Table 4, all the traits under non-saline and saline conditions showed high broad sense heritability and low to moderate narrow sense heritability, suggests that dominance gene action is more important than additives in controlling the majority of studied traits. Selection in the early segregation generations for such traits could be misleading, therefore, required further progeny testing. Such traits could be improved by crossing potential genotypes of segregating population by means of recombinant breeding approach (Samadia 2005). Similar results have been reported by Dashti et al. (2010) for  $K^+/Na^+$  ratio and  $LeaK^+C$ , Marzooghian et al. (2014) for  $K^+/Na^+$  ratio and EL and Amiri et al. (2020) for  $GFeC$  and  $GZnC$ . The degree of dominance ranged from partial dominance (0.47 in proline at 200 Mm salinity level) to over-dominance with the highest degree of dominance being 4.76 for  $K^+/Na^+$  ratio in the 200 Mm salinity treatment (Table 4). With two exceptions in the control treatment, all values ranged from partial to complete dominance.

## **Conclusions**

Based on our study, it is concluded that Zn/Fe efficient uptake-related traits are controlled by additive and non-additive genetic effects. When the contribution of additive effects is larger than non-additives, selection in the early segregation generations can be effective; otherwise, improvement of the characters needs intensive selection through later generations. Given that effective selection in early generations only occurs when genetic effects are additive and environmental effects have small impacts, this suggestion is appropriate. Finally, results of this study show that landraces would be excellent donors for combining beneficial salinity-tolerant and Zn/Fe efficient uptake traits into a modern variety to improve wheat for Zn/Fe deficient and salinity-prone environments.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interests.

**Author contribution statement** Hossein Abbasi Holasou is written the manuscript, designed the experiment, analyzed the data and developed the idea. Seyed Siamak Alavi Kia is developed the idea, designed the experiment and written the manuscript. Seyed Abolghasem Mohammadi is written the manuscript, designed and developed the experiment. Mohammad Moghaddam Vahed is developed some part of the methods.

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**Table 1** Analysis of variance for studied traits in bread wheat under Control (C), 100 and 200 mM NaCl

| S.O.V                 | df | Mean Square           |                      |                    |                    |                      |                    |
|-----------------------|----|-----------------------|----------------------|--------------------|--------------------|----------------------|--------------------|
|                       |    | GFeC (mg/kg)          | GZnC (mg/kg)         | 100-SW (g)         | GY (g)             | RL                   | RFW                |
| Rep                   | 2  | 82.03 <sup>ns</sup>   | 23.97 <sup>ns</sup>  | 0.01 <sup>ns</sup> | 0.02 <sup>ns</sup> | 0.78 <sup>ns</sup>   | 0.04 <sup>**</sup> |
| Salinity              | 2  | 950.19 <sup>**</sup>  | 484.09 <sup>**</sup> | 0.91 <sup>**</sup> | 2.17 <sup>**</sup> | 18.06 <sup>**</sup>  | 0.24 <sup>**</sup> |
| Generation            | 5  | 1161.85 <sup>**</sup> | 710.49 <sup>**</sup> | 5.27 <sup>**</sup> | 1.04 <sup>**</sup> | 461.93 <sup>**</sup> | 2.83 <sup>**</sup> |
| Salinity × Generation | 10 | 44.99 <sup>ns</sup>   | 29.32 <sup>ns</sup>  | 0.03 <sup>ns</sup> | 0.16 <sup>*</sup>  | 2.23 <sup>**</sup>   | 0.01 <sup>**</sup> |
| Error                 | 34 | 72.55                 | 15.47                | 0.02               | 0.06               | 0.54                 | 0.003              |
| C.V (%)               |    | 12.27                 | 6.12                 | 5.35               | 14.73              | 5.44                 | 4.76               |

ns, \*, \*\*: non-significant and significant at 0.05 and 0.01 probability levels, respectively.

Grain Fe Concentration (GFeC), Grain Zn Concentration (GZnC), weight of 100 grains (100-SW), Grain yield per plant (GY), Root length (RL), Root Fresh Weight (RFW).

**Table 1** (Continued)

| S.O.V                 | Df | Mean Square         |                     |                       |                     |                      |
|-----------------------|----|---------------------|---------------------|-----------------------|---------------------|----------------------|
|                       |    | RDW                 | RWC                 | EL                    | LeaNa+C             | LeaK+C               |
| Rep                   | 2  | 0.005 <sup>**</sup> | 2.41 <sup>ns</sup>  | 3.05 <sup>ns</sup>    | 0.59 <sup>ns</sup>  | 1.16 <sup>ns</sup>   |
| Salinity              | 2  | 0.034 <sup>**</sup> | 45.03 <sup>**</sup> | 213.80 <sup>**</sup>  | 70.39 <sup>**</sup> | 391.32 <sup>**</sup> |
| Generation            | 5  | 0.686 <sup>**</sup> | 75.74 <sup>**</sup> | 2440.15 <sup>**</sup> | 82.22 <sup>**</sup> | 494.93 <sup>**</sup> |
| Salinity × Generation | 10 | 0.003 <sup>**</sup> | 5.04 <sup>*</sup>   | 42.35 <sup>**</sup>   | 3.53 <sup>**</sup>  | 29.59 <sup>**</sup>  |
| Error                 | 34 | 0.0006              | 2.001               | 7.58                  | 0.29                | 5.03                 |
| C.V (%)               |    | 6.65                | 2.05                | 7.57                  | 6.77                | 6.87                 |

ns, \*, \*\*: non-significant and significant at 0.05 and 0.01 probability levels, respectively.

Root Dry Weight (RDW), Relative Water Content (RWC), Electric Leakage (EL), Leaf Na<sup>+</sup> Concentration (LeaNa<sup>+</sup>C), Leaf K<sup>+</sup> Concentration (LeaK<sup>+</sup>C)

**Table 1** (Continued)

| S.O.V                 | Df | Mean Square                          |                     |                      |                      |                    |
|-----------------------|----|--------------------------------------|---------------------|----------------------|----------------------|--------------------|
|                       |    | LeaK <sup>+</sup> /Na <sup>+</sup> C | CAT                 | POX                  | MDA                  | Proline            |
| Rep                   | 2  | 0.73 <sup>ns</sup>                   | 0.19 <sup>ns</sup>  | 3.98 <sup>ns</sup>   | 0.0002 <sup>ns</sup> | 0.05 <sup>ns</sup> |
| Salinity              | 2  | 47.14 <sup>**</sup>                  | 46.48 <sup>**</sup> | 121.15 <sup>**</sup> | 0.008 <sup>**</sup>  | 2.17 <sup>**</sup> |
| Generation            | 5  | 79.83 <sup>**</sup>                  | 78.65 <sup>**</sup> | 345.97 <sup>**</sup> | 0.017 <sup>**</sup>  | 3.15 <sup>**</sup> |
| Salinity × Generation | 10 | 3.63 <sup>**</sup>                   | 8.42 <sup>**</sup>  | 22.68 <sup>**</sup>  | 0.002 <sup>**</sup>  | 0.06 <sup>ns</sup> |
| Error                 | 34 | 0.62                                 | 0.87                | 2.55                 | 0.0003               | 0.05               |
| C.V (%)               |    | 15.35                                | 10.03               | 4.05                 | 2.98                 | 6.64               |

ns, \*, \*\*: non-significant and significant at 0.05 and 0.01 probability levels, respectively.

K<sup>+</sup>/Na<sup>+</sup> Concentration ratio (LeaK<sup>+</sup>/Na<sup>+</sup>C), Catalase (CAT), Peroxidase (POX), Malondialdehyde (MDA), Proline content.

**Table 2** Mean and its standard error of six generations for different traits of bread wheat

|     | GFeC        |            |            | GZnC       |            |            |
|-----|-------------|------------|------------|------------|------------|------------|
|     | C           | 100        | 200        | C          | 100        | 200        |
| P1  | 66.42±3.38  | 59.08±1.21 | 59.36±3.24 | 50.24±4.52 | 51.22±1.59 | 38.63±0.69 |
| P2  | 103.22±8.14 | 79.63±3.38 | 79.20±2.85 | 67.12±2.31 | 61.88±1.89 | 61.26±1.95 |
| F1  | 82.24±4.19  | 67.29±1.50 | 61.02±2.45 | 60.73±1.52 | 53.34±3.80 | 42.63±1.91 |
| F2  | 72.90±1.61  | 60.17±0.92 | 61.47±2.21 | 52.38±1.42 | 41.20±1.29 | 40.40±1.43 |
| BC1 | 67.40±3.04  | 57.51±1.59 | 52.25±1.16 | 51.26±2.67 | 41.95±1.45 | 36.97±1.40 |
| BC2 | 79.70±3.42  | 70.44±6.97 | 70.36±7.86 | 58.14±3.79 | 50.31±1.92 | 44.64±1.27 |

**Table 2** (Continued)

|     | 100-SW    |           |           | GY        |           |           |
|-----|-----------|-----------|-----------|-----------|-----------|-----------|
|     | C         | 100       | 200       | C         | 100       | 200       |
| P1  | 2.97±0.12 | 2.31±0.04 | 1.61±0.10 | 1.31±0.04 | 1.61±0.08 | 0.79±0.09 |
| P2  | 3.25±0.09 | 2.98±0.08 | 2.44±0.05 | 2.02±0.12 | 2.24±0.17 | 1.74±0.06 |
| F1  | 3.75±0.10 | 3.29±0.12 | 2.64±0.07 | 2.90±0.28 | 2.87±0.20 | 1.92±0.10 |
| F2  | 3.32±0.04 | 2.78±0.04 | 2.20±0.03 | 1.77±0.06 | 1.79±0.06 | 1.55±0.05 |
| BC1 | 3.10±0.06 | 2.42±0.07 | 2.07±0.03 | 1.52±0.12 | 1.18±0.08 | 1.32±0.04 |
| BC2 | 3.22±0.05 | 2.72±0.10 | 2.16±0.05 | 1.62±0.12 | 1.55±0.12 | 1.36±0.05 |

**Table 2** (Continued)

|     | RL         |            |            | RFW       |           |           |
|-----|------------|------------|------------|-----------|-----------|-----------|
|     | C          | 100        | 200        | C         | 100       | 200       |
| P1  | 16.28±0.34 | 12.05±0.25 | 5.77±0.29  | 1.39±0.02 | 0.87±0.03 | 0.49±0.04 |
| P2  | 18.97±0.58 | 15.43±0.28 | 9.36±0.29  | 1.67±0.03 | 1.26±0.04 | 1.04±0.04 |
| F1  | 21.75±0.65 | 14.09±0.35 | 10.03±0.23 | 1.73±0.04 | 1.23±0.03 | 1.07±0.03 |
| F2  | 17.72±0.25 | 13.73±0.22 | 8.86±0.23  | 1.65±0.02 | 1.16±0.03 | 0.82±0.04 |
| BC1 | 16.95±0.43 | 13.19±0.34 | 7.68±0.26  | 1.53±0.04 | 0.89±0.03 | 0.75±0.02 |
| BC2 | 18.98±0.52 | 14.95±0.37 | 8.28±0.28  | 1.67±0.04 | 1.04±0.04 | 0.80±0.03 |

**Table 2** (Continued)

|     | RDW       |           |           | RWC        |            |            |
|-----|-----------|-----------|-----------|------------|------------|------------|
|     | C         | 100       | 200       | C          | 100        | 200        |
| P1  | 0.38±0.01 | 0.42±0.01 | 0.09±0.01 | 67.63±0.43 | 65.51±0.56 | 65.95±0.38 |
| P2  | 0.50±0.02 | 0.57±0.02 | 0.20±0.03 | 73.00±0.65 | 69.95±0.62 | 71.92±0.63 |
| F1  | 0.51±0.02 | 0.56±0.02 | 0.30±0.02 | 72.40±0.65 | 69.65±0.37 | 69.15±2.11 |
| F2  | 0.45±0.01 | 0.51±0.01 | 0.10±0.00 | 73.31±0.41 | 69.61±0.47 | 66.91±0.40 |
| BC1 | 0.43±0.02 | 0.45±0.02 | 0.08±0.00 | 69.13±0.54 | 66.45±0.71 | 62.74±0.58 |
| BC2 | 0.47±0.01 | 0.49±0.01 | 0.09±0.00 | 71.88±0.65 | 67.78±0.77 | 67.32±0.42 |

**Table 2** (Continued)

|     | EL         |            |            | LeaNa <sup>+</sup> C |            |            |
|-----|------------|------------|------------|----------------------|------------|------------|
|     | C          | 100        | 200        | C                    | 100        | 200        |
| P1  | 28.57±0.74 | 43.11±1.26 | 60.13±0.99 | 9.80±0.43            | 12.80±0.60 | 16.59±0.57 |
| P2  | 21.77±0.68 | 33.42±1.24 | 42.20±1.07 | 4.48±0.32            | 5.74±0.24  | 6.19±0.23  |
| F1  | 22.62±0.73 | 27.45±1.50 | 40.69±1.14 | 5.18±0.31            | 5.68±0.28  | 7.42±0.31  |
| F2  | 30.28±1.00 | 29.77±0.72 | 45.04±0.84 | 4.89±0.14            | 6.02±0.17  | 9.11±0.28  |
| BC1 | 26.17±0.95 | 34.63±1.40 | 55.14±1.97 | 6.14±0.32            | 8.47±0.43  | 12.56±0.55 |
| BC2 | 27.00±1.08 | 35.50±1.89 | 50.75±0.98 | 5.21±0.24            | 7.25±0.38  | 9.31±0.46  |

**Table 2** (Continued)

|     | LeaK <sup>+</sup> C |            |            | LeaK <sup>+</sup> /Na <sup>+</sup> C |           |           |
|-----|---------------------|------------|------------|--------------------------------------|-----------|-----------|
|     | C                   | 100        | 200        | C                                    | 100       | 200       |
| P1  | 28.97±1.52          | 26.36±1.46 | 22.66±0.94 | 3.01±0.19                            | 2.12±0.17 | 1.39±0.08 |
| P2  | 55.87±2.06          | 42.40±1.88 | 34.84±1.27 | 13.13±0.93                           | 7.52±0.42 | 5.72±0.30 |
| F1  | 38.84±2.37          | 31.07±1.35 | 27.97±1.04 | 7.84±0.75                            | 5.67±0.44 | 3.81±0.15 |
| F2  | 40.86±0.83          | 36.37±0.83 | 28.41±0.88 | 8.61±0.31                            | 6.20±0.21 | 3.22±0.15 |
| BC1 | 30.66±1.10          | 29.11±1.82 | 25.16±1.15 | 5.13±0.29                            | 3.51±0.26 | 2.05±0.14 |
| BC2 | 33.16±1.12          | 28.64±0.23 | 26.50±1.54 | 6.50±0.36                            | 4.05±0.23 | 2.93±0.25 |

**Table 2** (Continued)

|     | CAT       |            |            | POX        |            |            |
|-----|-----------|------------|------------|------------|------------|------------|
|     | C         | 100        | 200        | C          | 100        | 200        |
| P1  | 5.73±0.26 | 6.08±0.29  | 7.93±0.39  | 30.67±0.76 | 34.42±0.76 | 38.17±1.09 |
| P2  | 8.09±0.42 | 13.31±0.59 | 17.34±0.39 | 40.00±0.56 | 44.50±0.79 | 51.33±0.78 |
| F1  | 7.86±0.27 | 11.00±0.57 | 13.55±0.68 | 34.33±0.67 | 40.67±1.18 | 49.58±1.00 |
| F2  | 7.59±0.20 | 9.90±0.28  | 9.21±0.27  | 35.14±0.46 | 36.72±0.52 | 45.56±0.73 |
| BC1 | 6.77±0.51 | 7.39±0.39  | 8.87±0.65  | 38.17±0.67 | 38.08±1.16 | 38.33±0.97 |
| BC2 | 6.24±0.28 | 11.42±0.56 | 10.10±0.47 | 36.08±0.67 | 35.33±0.99 | 42.67±0.83 |

**Table 2** (Continued)

|     | MDA       |           |           | Proline   |           |           |
|-----|-----------|-----------|-----------|-----------|-----------|-----------|
|     | C         | 100       | 200       | C         | 100       | 200       |
| P1  | 0.52±0.01 | 0.61±0.01 | 0.63±0.01 | 1.98±0.13 | 2.38±0.14 | 2.94±0.09 |
| P2  | 0.64±0.01 | 0.64±0.01 | 0.68±0.01 | 3.31±0.11 | 3.87±0.09 | 4.06±0.11 |
| F1  | 0.58±0.01 | 0.56±0.01 | 0.64±0.01 | 3.03±0.13 | 3.74±0.12 | 3.92±0.10 |
| F2  | 0.58±0.01 | 0.58±0.01 | 0.59±0.01 | 2.81±0.10 | 3.72±0.06 | 3.90±0.07 |
| BC1 | 0.55±0.01 | 0.52±0.01 | 0.61±0.01 | 2.68±0.17 | 2.99±0.13 | 3.07±0.11 |
| BC2 | 0.57±0.01 | 0.57±0.01 | 0.63±0.01 | 2.95±0.16 | 3.55±0.09 | 3.76±0.12 |

**Table 3** Mean, additive, dominance and epistatic effects, and type of non-allelic interaction regarding Zn and Fe efficiency indices and shoot and root traits a for the cross Navid × Roshan, under salinity stress

| Traits                              | m             | [d]                       | [h]                         | [i]                        | [j]                       | [l]                        | Wald-F             |
|-------------------------------------|---------------|---------------------------|-----------------------------|----------------------------|---------------------------|----------------------------|--------------------|
| <b>GFeC</b>                         |               |                           |                             |                            |                           |                            |                    |
| C                                   | 81.18*±13.35  | -10.85*±3.17              | -44.26 <sup>ns</sup> ±34.91 | -4.26 <sup>ns</sup> ±13.01 | -2.59 <sup>ns</sup> ±1.08 | 46.85 <sup>ns</sup> ±23.77 | 3.46*              |
| S1                                  | 80.22*±15.09  | -21.34**±2.51             | -38.02 <sup>ns</sup> ±17.22 | -                          | -                         | -                          | 1.02 <sup>ns</sup> |
| S2                                  | 78.39*±27.31  | -10.22*±3.73              | -52.68 <sup>ns</sup> ±36.56 | -                          | -                         | -                          | 0.38 <sup>ns</sup> |
| <b>GZnC</b>                         |               |                           |                             |                            |                           |                            |                    |
| C                                   | 58.47**±10.79 | -10.90*±1.29              | 16.44 <sup>ns</sup> ±10.72  | 11.80 <sup>ns</sup> ±10.68 | 6.99 <sup>ns</sup> ±5.01  | -0.41 <sup>ns</sup> ±0.38  | 3.36*              |
| S1                                  | 53.66**±11.61 | -5.24*±2.11               | -1.93 <sup>ns</sup> ±1.52   | 18.35 <sup>ns</sup> ±11.39 | -6.74 <sup>ns</sup> ±5.22 | 19.90 <sup>ns</sup> ±18.21 | 13.43**            |
| S2                                  | 38.07**±3.24  | -7.13*±3.07               | -3.33 <sup>ns</sup> ±1.41   | -                          | -                         | -                          | 1.08 <sup>ns</sup> |
| <b>100-SW</b>                       |               |                           |                             |                            |                           |                            |                    |
| C                                   | 3.91**±0.26   | -0.13 <sup>ns</sup> ±0.08 | -2.13**±0.69                | -0.76**±0.25               | 0.04 <sup>ns</sup> ±0.32  | 2.01**±0.48                | 4.65**             |
| S1                                  | 3.55**±0.36   | -0.34**±0.06              | -2.55*±1.05                 | -0.83*±0.36                | -0.01 <sup>ns</sup> ±0.04 | 2.37**±0.70                | 6.79**             |
| S2                                  | 2.37**±0.32   | -0.39**±0.05              | -0.96 <sup>ns</sup> ±0.77   | -0.36 <sup>ns</sup> ±0.31  | 0.57*±0.22                | 1.17 <sup>ns</sup> ±0.49   | 10.00**            |
| <b>GY</b>                           |               |                           |                             |                            |                           |                            |                    |
| C                                   | 3.62**±0.57   | -0.36*±0.16               | -3.69 <sup>ns</sup> ±2.31   | -0.94 <sup>ns</sup> ±0.92  | 0.51 <sup>ns</sup> ±0.65  | 3.98*±1.46                 | 7.20**             |
| S1                                  | 2.63**±0.94   | -0.31*±0.13               | -6.28**±1.49                | -1.59*±0.55                | -0.05 <sup>ns</sup> ±0.49 | 5.63**±1.01                | 16.54**            |
| S2                                  | 2.13**±0.41   | -0.42**±0.06              | -2.07 <sup>ns</sup> ±0.97   | -0.87 <sup>ns</sup> ±0.40  | 0.77*±0.26                | 1.80*±0.60                 | 6.22*              |
| <b>RL</b>                           |               |                           |                             |                            |                           |                            |                    |
| C                                   | 14.35**±2.08  | -1.11*±0.38               | 5.92 <sup>ns</sup> ±5.67    | 3.41 <sup>ns</sup> ±2.03   | -3.43 <sup>ns</sup> ±1.85 | 1.77 <sup>ns</sup> ±3.78   | 5.87*              |
| S1                                  | 12.47**±1.91  | -1.67**±0.26              | 4.43 <sup>ns</sup> ±4.90    | -                          | -                         | -                          | 0.23 <sup>ns</sup> |
| S2                                  | 11.46**±1.77  | -1.69**±0.23              | -8.58 <sup>ns</sup> ±4.37   | -                          | -                         | -                          | 3.15 <sup>ns</sup> |
| <b>RFW</b>                          |               |                           |                             |                            |                           |                            |                    |
| C                                   | 1.91**±0.18   | -0.14**±0.02              | -0.63 <sup>ns</sup> ±0.44   | -                          | -                         | -                          | 0.83 <sup>ns</sup> |
| S1                                  | 1.86**±0.16   | -0.18**±0.02              | -2.32**±0.45                | -0.85**±0.17               | 0.009 <sup>ns</sup> ±0.02 | 1.66**±0.28                | 14.75**            |
| S2                                  | 0.96**±0.20   | -0.28**±0.03              | -0.77 <sup>ns</sup> ±0.46   | -0.23 <sup>ns</sup> ±0.20  | 0.51**±0.10               | 0.84*±0.27                 | 13.79**            |
| <b>RDW</b>                          |               |                           |                             |                            |                           |                            |                    |
| C                                   | 0.63**±0.05   | -0.05**±0.01              | -0.18 <sup>ns</sup> ±0.15   | -                          | -                         | -                          | 1.64 <sup>ns</sup> |
| S1                                  | 0.49**±0.02   | -0.07**±0.009             | -0.44**±0.14                | -0.14*±0.05                | 0.06 <sup>ns</sup> ±0.04  | 0.37**±0.09                | 6.65**             |
| S2                                  | 0.17**±0.02   | -0.04**±0.01              | -0.45**±0.05                | -0.04 <sup>ns</sup> ±0.02  | 0.08**±0.02               | 0.59**±0.04                | 37.50**            |
| <b>RWC</b>                          |               |                           |                             |                            |                           |                            |                    |
| C                                   | 82.47**±2.76  | -2.69**±0.46              | -26.82**±7.12               | -12.34**±2.66              | -0.32 <sup>ns</sup> ±0.25 | 16.59**±4.65               | 7.74**             |
| S1                                  | 79.17**±2.99  | -2.21**±0.42              | -27.62**±7.75               | -                          | -                         | -                          | 2.91 <sup>ns</sup> |
| S2                                  | 76.2**±3.74   | -3.1**±0.61               | -32.16*±9.28                | -7.65 <sup>ns</sup> ±3.64  | 3.04 <sup>ns</sup> ±2.54  | 26.85**±5.97               | 3.45*              |
| <b>EL</b>                           |               |                           |                             |                            |                           |                            |                    |
| C                                   | 41.04**±7.61  | 3.42**±1.03               | -20.24 <sup>ns</sup> ±18.83 | -14.53 <sup>ns</sup> ±7.54 | -9.25 <sup>ns</sup> ±5.2  | 3.21 <sup>ns</sup> ±2.77   | 6.46**             |
| S1                                  | 32.83**±1.96  | 4.69**±1.06               | 56.05*±18.09                | -                          | -                         | -                          | 3.98 <sup>ns</sup> |
| S2                                  | 24.29**±5.55  | 8.93**±0.84               | 65.87**±14.67               | 26.59**±5.50               | -14.97**±4.36             | -49.74**±9.5               | 12.72**            |
| <b>LeaNa<sup>+</sup></b>            |               |                           |                             |                            |                           |                            |                    |
| <b>C</b>                            |               |                           |                             |                            |                           |                            |                    |
| C                                   | 4.08**±1.18   | 2.84**±0.23               | 2.62 <sup>ns</sup> ±2.25    | 3.14**±1.14                | -3.90**±1.08              | -1.50 <sup>ns</sup> ±1.19  | 12.99**            |
| S1                                  | 3.56**±0.23   | 3.47**±0.32               | 12.76*±4.96                 | 7.51**±1.73                | -4.26*±1.62               | -8.73*±3.33                | 12.21**            |
| S2                                  | 1.76**±0.77   | 5.16**±0.30               | 15.98*±6.07                 | 7.11**±2.17                | -4.01 <sup>ns</sup> ±1.95 | -12.84**±3.99              | 9.52**             |
| <b>LeaK<sup>+</sup>C</b>            |               |                           |                             |                            |                           |                            |                    |
| <b>C</b>                            |               |                           |                             |                            |                           |                            |                    |
| C                                   | 77.48**±5.40  | -13.27**±1.52             | -                           | -35.66**±5.17              | 22.88**±4.90              | 71.07**±9.78               | 19.16**            |
|                                     |               |                           | 109.89**±14.19              |                            |                           |                            |                    |
| S1                                  | 70.6**±5.41   | -7.63**±1.12              | -95.87**±14.62              | -35.69**±5.38              | 14.05**±4.98              | 56.77**±9.73               | 14.93**            |
| S2                                  | 41.75**±6.56  | -6.44**±0.97              | -39.5 <sup>ns</sup> ±15.94  | -                          | -                         | -                          | 2.20 <sup>ns</sup> |
| <b>K<sup>+</sup>/Na<sup>+</sup></b> |               |                           |                             |                            |                           |                            |                    |
| <b>C</b>                            |               |                           |                             |                            |                           |                            |                    |
| C                                   | 18.52**±1.8   | -5.13**±0.55              | -29.97**±4.53               | -10.73**±1.67              | 7.91**±1.57               | 18.85**±3.17               | 17.43**            |
| S1                                  | 15.11**±1.08  | -2.69**±0.28              | -25.49**±2.67               | -10.18**±1.07              | 4.34**±0.89               | 16.12**±1.75               | 25.85**            |
| S2                                  | 6.37**±1.06   | -2.15**±0.18              | -10.49**±2.54               | -3.01*±1.03                | 2.85**±0.70               | 7.79**±1.61                | 11.33**            |

**Table 3** (Continued)

| Traits  | m                       | [d]                        | [h]                        | [i]                       | [j]                       | [l]                        | Wald-F             |
|---------|-------------------------|----------------------------|----------------------------|---------------------------|---------------------------|----------------------------|--------------------|
| CAT     |                         |                            |                            |                           |                           |                            |                    |
| C       | 11.06**±2.05            | -1.37**±0.22               | -10.25 <sup>ns</sup> ±5.89 | -4.07 <sup>ns</sup> ±2.04 | 4.31 <sup>+</sup> ±1.91   | 7.13 <sup>ns</sup> ±3.91   | 3.98 <sup>*</sup>  |
| S1      | 8.25**±1.96             | -3.59**±0.48               | 0.90 <sup>ns</sup> ±0.49   | -                         | -                         | -                          | 1.71 <sup>ns</sup> |
| S2      | 10.67**±2.41            | -4.68**±0.36               | -10.07 <sup>ns</sup> ±6.58 | 1.53 <sup>ns</sup> ±1.32  | 7.19**±2.14               | 13.06**±4.37               | 34.30**            |
| POX     |                         |                            |                            |                           |                           |                            |                    |
| C       | 26.27**±3.38            | -4.85**±0.54               | 25.25 <sup>*</sup> ±8.59   | 8.27 <sup>ns</sup> ±3.32  | 15.06**±2.59              | -18.01 <sup>+</sup> ±15.51 | 16.35**            |
| S1      | 39.21**±4.30            | -5.29**±0.62               | -11.69 <sup>ns</sup> ±10.1 | 0.12 <sup>ns</sup> ±0.27  | 17.99**±3.85              | 13.69 <sup>ns</sup> ±8.05  | 13.36**            |
| S2      | 63.58**±5.50            | -6.51**±0.86               | -61.97**±13.21             | -20.19**±5.37             | 3.69 <sup>ns</sup> ±3.63  | 46.69**±8.26               | 15.55**            |
| MDA     |                         |                            |                            |                           |                           |                            |                    |
| C       | 0.66**±0.04             | -0.06**±0.009              | -0.23 <sup>ns</sup> ±0.12  | -                         | -                         | -                          | 2.75 <sup>ns</sup> |
| S1      | 0.79**±0.05             | -0.01 <sup>ns</sup> ±0.007 | -0.58**±0.12               | -0.15**±0.05              | -0.11**±0.03              | 0.36**±0.07                | 12.05**            |
| S2      | 0.51 <sup>+</sup> ±0.05 | -0.02**±0.007              | 0.22 <sup>ns</sup> ±0.13   | 0.15**±0.05               | 0.01 <sup>ns</sup> ±0.04  | -0.09 <sup>ns</sup> ±0.08  | 6.27 <sup>*</sup>  |
| Proline |                         |                            |                            |                           |                           |                            |                    |
| C       | 2.32 <sup>+</sup> ±0.58 | -0.67**±0.10               | 1.21 <sup>ns</sup> ±1.05   | -                         | -                         | -                          | 0.77 <sup>ns</sup> |
| S1      | 5.11**±0.58             | -0.77**±0.09               | -4.18 <sup>+</sup> ±1.52   | -1.99 <sup>+</sup> ±0.57  | 0.61 <sup>ns</sup> ±0.46  | 2.80 <sup>+</sup> ±0.99    | 4.22 <sup>*</sup>  |
| S2      | 5.63**±0.62             | -0.57**±0.1                | -4.93 <sup>+</sup> ±1.56   | -2.05 <sup>+</sup> ±0.62  | -0.15 <sup>ns</sup> ±0.14 | 3.29**±0.99                | 3.99 <sup>*</sup>  |

**Table 4** Estimates of variance components using restricted maximum likelihood for mixed linear model

| Trait                | Salinity rate | $\sigma_A^2$ | $\sigma_D^2$ | $\sigma_{AD}$ | $\sigma_E^2$ | $h^2_{bs}$ | $h^2_{ns}$ | $\bar{a}$ |
|----------------------|---------------|--------------|--------------|---------------|--------------|------------|------------|-----------|
| GF <sub>Fe</sub> C   | C             | 72.74        | 66.04        | 31.41         | 15.84        | 0.96       | 0.50       | 1.34      |
|                      | S1            | 41.24        | 26.25        | 17.11         | 9.45         | 0.96       | 0.47       | 1.12      |
|                      | S2            | 39.66        | 15.73        | 19.16         | 27.35        | 0.89       | 0.47       | 0.89      |
| GZ <sub>n</sub> C    | C             | 173.99       | 149.83       | 94.51         | 97.16        | 0.92       | 0.38       | 1.31      |
|                      | S1            | 6.71         | 37.83        | 7.82          | 76.12        | 0.67       | 0.08       | 3.35      |
|                      | S2            | 64.40        | 32.94        | 21.16         | 21.74        | 0.94       | 0.51       | 1.01      |
| 100-SW               | C             | 0.13         | 0.14         | 0.03          | 0.06         | 0.93       | 0.40       | 1.47      |
|                      | S1            | 0.01         | 0.07         | 0.02          | 0.11         | 0.73       | 0.07       | 3.74      |
|                      | S2            | 0.05         | 0.06         | 0.03          | 0.03         | 0.93       | 0.33       | 1.54      |
| GY                   | C             | 0.15         | 0.43         | 0.07          | 0.41         | 0.83       | 0.19       | 2.39      |
|                      | S1            | 0.21         | 0.04         | 0.11          | 0.26         | 0.82       | 0.47       | 0.61      |
|                      | S2            | 0.08         | 0.05         | 0.01          | 0.05         | 0.90       | 0.51       | 1.11      |
| RL                   | C             | 0.90         | 0.63         | 0.99          | 3.52         | 0.68       | 0.24       | 1.18      |
|                      | S1            | 1.95         | 1.57         | 0.09          | 1.34         | 0.89       | 0.48       | 1.26      |
|                      | S2            | 3.18         | 2.03         | 0.01          | 0.83         | 0.95       | 0.58       | 1.12      |
| RFW                  | C             | 0.026        | 0.025        | 0.0007        | 0.018        | 0.90       | 0.40       | 1.38      |
|                      | S1            | 0.041        | 0.024        | 0.003         | 0.012        | 0.94       | 0.56       | 1.08      |
|                      | S2            | 0.065        | 0.024        | 0.007         | 0.007        | 0.98       | 0.66       | 0.85      |
| RDW                  | C             | 0.002        | 0.0005       | 0.0004        | 0.003        | 0.74       | 0.51       | 0.70      |
|                      | S1            | 0.003        | 0.002        | 0.001         | 0.002        | 0.90       | 0.45       | 1.15      |
|                      | S2            | 0.006        | 0.006        | 0.003         | 0.0007       | 0.98       | 0.39       | 1.41      |
| RWC                  | C             | 5.13         | 3.76         | 1.63          | 4.73         | 0.87       | 0.42       | 1.21      |
|                      | S1            | 7.27         | 3.09         | 0.54          | 3.83         | 0.93       | 0.37       | 0.92      |
|                      | S2            | 8.42         | 21.11        | 0.19          | 18.68        | 0.82       | 0.23       | 2.24      |
| EL                   | C             | 43.14        | 29.02        | 3.54          | 5.76         | 0.97       | 0.55       | 1.16      |
|                      | S1            | 1.19         | 8.42         | 0.97          | 26.75        | 0.54       | 0.06       | 3.76      |
|                      | S2            | 23.03        | 20.51        | 0.96          | 22.71        | 0.85       | 0.44       | 1.33      |
| LeaNa <sup>+</sup> C | C             | 1.96         | 1.64         | 0.66          | 1.01         | 0.93       | 0.42       | 1.29      |
|                      | S1            | 3.09         | 2.44         | 1.82          | 1.63         | 0.93       | 0.39       | 1.25      |
|                      | S2            | 1.11         | 0.49         | 1.60          | 2.29         | 0.80       | 0.28       | 0.93      |
| LeaK <sup>+</sup> C  | C             | 28.75        | 18.91        | 11.40         | 34.18        | 0.84       | 0.40       | 1.14      |
|                      | S1            | 22.34        | 20.71        | 9.91          | 25.99        | 0.86       | 0.36       | 1.36      |
|                      | S2            | 21.03        | 12.34        | 6.80          | 15.93        | 0.88       | 0.46       | 1.08      |

**Table 4** (Continued)

| Trait                                | Salinity rate | $\sigma_A^2$ | $\sigma_D^2$ | $\sigma_{AD}$ | $\sigma_E^2$ | $h_{bs}^2$ | $h_{ns}^2$ | $\bar{a}$ |
|--------------------------------------|---------------|--------------|--------------|---------------|--------------|------------|------------|-----------|
| LeaK <sup>+</sup> /Na <sup>+</sup> C | C             | 3.22         | 3.39         | 4.46          | 3.19         | 0.91       | 0.26       | 1.45      |
|                                      | S1            | 0.90         | 0.47         | 0.84          | 1.23         | 0.84       | 0.34       | 1.02      |
|                                      | S2            | 0.03         | 0.34         | 0.56          | 0.28         | 0.91       | 0.03       | 4.76      |
| CAT                                  | C             | 0.30         | 0.35         | 0.71          | 1.53         | 0.73       | 0.16       | 1.53      |
|                                      | S1            | 0.64         | 0.83         | 1.25          | 2.82         | 0.74       | 0.17       | 1.61      |
|                                      | S2            | 1.57         | 3.11         | 0.01          | 4.29         | 0.76       | 0.25       | 1.99      |
| POX                                  | C             | 5.34         | 3.07         | 0.27          | 5.13         | 0.83       | 0.51       | 1.07      |
|                                      | S1            | 5.08         | 10.46        | 0.41          | 15.21        | 0.76       | 0.24       | 2.03      |
|                                      | S2            | 21.16        | 9.86         | 0.74          | 8.48         | 0.92       | 0.61       | 0.96      |
| MDA                                  | C             | 0.0001       | 0.0005       | 0.0001        | 0.0014       | 0.63       | 0.09       | 3.16      |
|                                      | S1            | 0.003        | 0.0008       | 0.0003        | 0.0008       | 0.95       | 0.69       | 0.73      |
|                                      | S2            | 0.004        | 0.002        | 0.0003        | 0.0014       | 0.94       | 0.60       | 1.00      |
| Proline                              | C             | 0.22         | 0.21         | 0.04          | 0.28         | 0.84       | 0.39       | 1.38      |
|                                      | S1            | 0.06         | 0.05         | 0.06          | 0.13         | 0.81       | 0.28       | 1.29      |
|                                      | S2            | 0.09         | 0.01         | 0.03          | 0.11         | 0.62       | 0.56       | 0.47      |