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Orientation: genetics and plant breeding

Title

**Association analysis of some agronomic
and physiological traits of maize (*Zea
Mays*) under zinc deficiency stress**

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Abstract

The first step in maize breeding programs is to utilize the genetic diversity available among available populations, cultivars and genotypes. Maize (*Zea mays* L.) is the third most important cereal crop and one of the most important food crops in the world, and it has high genetic diversity, and is used as a model plant to study the genetics of various traits. Identifying gene loci controlling quantitative traits is one of the important topics in the field of genetics and breeding. Environmental stress, such as the stress of lack of nutrients, creates serious threats to the production of agricultural products. Zinc element is one of the essential elements with low consumption but with high nutritional value, which plays an important role in plant growth, crop yield, plant resistance to diseases, energy production, and increasing antioxidant enzymes and chlorophyll in plant tissues. About 50% of the soils used for grain production in the world do not have enough usable zinc. The effects of zinc deficiency in the soils of arid and semi-arid regions reduce grain production in most countries of the world, including Iran. One of the ways to deal with zinc deficiency is to improve cultivars. Therefore, it is necessary to carry out research to identify the genes that control it. In the current research, 95 corn lines were studied under optimal (normal) conditions and zinc deficiency stress. The aim was to investigate the diversity and identify genomic locations controlling morphological and physiological traits using the GWAS method based on SNP markers. In order to investigate the diversity, classification of lines and mapping at the genome level of morphological and physiological traits in corn lines, the number of 95 pure lines of corn during 2 consecutive crop years (1399 and 1400) in two normal conditions (use of zinc sulfate fertilizer) and zinc deficiency (Non-use of fertilizer) in the form of Alpha Lattice design was done in two repetitions at the Agricultural and Natural Resources Research Center of Sistan. The researched corn lines were obtained from Razi University of Kermanshah, Khorasan Razavi Agricultural and Natural Resources Research Center, and Karaj Seedling and Seed Breeding Research Institute in the form of research project No. 94/101/T.T approved by Bio-Technology Research School of Urmia University. 29 morphological traits and 21 physiological traits were measured and recorded. Population phenotypic evaluation was done using multivariate statistical methods. In order to evaluate the genotypic population of the genome-wide association study (GWAS) technique to investigate the association between markers and traits (MTA) for the investigated traits in each of the normal and deficiency conditions from the nested key model with Bayesian information and linkage disequilibrium. Replay (BLINK) was done. In GWAS, in order to identify SNPs related to traits, Manhattan analysis and QQplot diagram based on BLINK model were used. The results of phenotypic evaluation showed that there are many phenotypic changes among the corn lines used for all traits in both environmental conditions. Variance analysis confirmed the high phenotypic diversity, all traits were affected by the environment, in both environments, statistically significant differences were observed between lines for all traits, and interaction effects were significant for most of the traits. Descriptive statistics showed that there was significant variation among the lines. Thus, for the morphological traits in zinc deficiency and normal environments, the highest amount of variation related to yield traits per unit area, economic yield and the number of seeds per cob was observed. For the physiological traits in the deficiency environment, the most diversity was found for the traits of chlorophyll b, guaiacol peroxidase enzyme, and total chlorophyll, and for the normal environment, the most diversity was found for the traits of chlorophyll b, total chlorophyll, and malondialdehyde enzyme. The amount of heritability for morphological traits was high in deficiency conditions, so that the highest amount was obtained for the traits of peduncle length, height of the main ear from the ground, plant height, and in normal conditions for the traits of weight of five ears, number of seeds in an ear, and economic yield. For physiological traits in both environmental conditions, the heritability rate for all traits was high. In terms of correlation for morphological traits, traits of weight of five ears, number of seeds per ear and weight of 100 seeds in both normal and zinc deficiency environments showed positive and significant correlation with economic performance, while little correlation was observed for physiological traits. . Based on

stepwise regression analysis and causality analysis under normal and zinc deficiency conditions, the number of seeds in the cob and the weight of 100 seeds were determined as important traits affecting economic performance. In cluster analysis, the desired lines were classified into 5 and 4 clusters in terms of morphological traits under zinc deficiency and normal conditions, respectively, and based on physiological traits in both zinc deficiency and normal environmental conditions, they were classified into 5 groups. So that the results of analysis into main components confirmed the results of cluster analysis for morphological and physiological traits in both environmental conditions. In the factor analysis using the parallel analysis method, four hidden factors were determined for morphological traits in both normal and zinc deficiency conditions, which justified 63 and 57% of the total variation of genotypes, respectively, for physiological traits in deficiency conditions. and normal were determined as 3 and 7 hidden factors, which explained 33 and 54% of the total variation, respectively. Based on the results of correlation between tolerance indices, it showed that GMP, MP, STI and HM indices have a very high correlation with grain yield in both environmental conditions. Based on the results of principal component analysis and cluster analysis, high performance lines in both environmental conditions as highly tolerant lines (Ma015, Ma017, Ma022, Ma030, Ma035, Ma049, Ma054, Ma055, Ma064, Ma072, Ma096, Ma098, Ma104, Ma107, Ma111, Ma114, Ma123, G703, Simon), high yielding lines only under zinc deficiency stress conditions as tolerant lines (Ma004, Ma027, Ma028, Ma038, Ma083, Ma091, Ma105, Ma115) and yielding lines Above only in normal conditions as a very sensitive line (Ma021, Ma023, Ma025, Ma031, Ma033, Ma034, Ma037, Ma044, Ma045, Ma050, Ma073, Ma076, Ma077, Ma079, Ma080, Ma089, Ma118, Ma121) identification and separation became. In the genotypic evaluation, the obtained results showed that in the dendrogram obtained from the kinship matrix, corn lines were placed in 5 groups. In the genome-wide study (GWAS) based on the results of quantitative analysis and their validation with the QQ plot chart based on the BLINK model, significant SNPs were identified for all traits. In the relationship analysis of morphological traits based on the BLINK method, 3852 and 4664 marker-trait relationships were identified under zinc deficiency and normal conditions, respectively, and 3913 and 4711 marker-trait relationships were identified for physiological traits under zinc deficiency and normal conditions, respectively. SNPs with $-\log_{10}$ (P-value) higher than 5 as the most relevant (highly significant) markers for traits (morphology of normal environment including: number of days to pollen appearance, number of days to ear appearance, leaf width, tassel length, number of seed rows, ear diameter, wood weight of five cobs, yield per unit area - morphology of deficiency environment including: number of days to germination, leaf width, peduncle length, yield per unit area, biological function - physiology of normal environment including: leaf protein percentage, leaf nitrogen percentage, protein percentage seed, seed nitrogen percentage, seed carbohydrate, seed iron content, seed phosphorus content, physiology of deficiency environment including: carotenoid, proline, guaiacol peroxidase enzyme and oil percentage) were identified. In this research, 3 common markers were identified in normal environmental conditions. Common marker AX-91358146 between traits number of days until pollen emergence and number of days until earing on chromosome number 8, common marker AX-91527389 between traits percentage of protein and leaf nitrogen on chromosome number 2 and common marker AX-90552004 between traits percentage of protein and seed nitrogen were detected on chromosome number 7. The results obtained from the genotypic evaluation of this research provide valuable information in the field of selection with the help of markers and the genetic bases of the studied traits, which can be used in the selection of individuals during breeding programs and the production of new cultivars with high yield. Also, based on the results of the phenotypic evaluation, suitable parents can be selected and due to their crossbreeding, taking into account genetic phenomena such as heterosis and transgressive segregation, corn hybrids that are tolerant to zinc deficiency stress and have desirable agricultural characteristics can be obtained.

Key words: Association analysis, marker, alpha lattice, genetic variation, line