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The Thesis Submitted for Ph.D Degree in Plant Breeding

Association mapping of cadmium stress in *Zea mays* L.

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Abstract

Maize (*Zea mays* L.) is the third most important cereal crop in the world, after wheat and rice, it plays a role in food security and global economy, especially in tropical and subtropical regions. Maize is better than genetic diversity and is used as a model plant to study the genetics of various traits. Heavy metals, especially cadmium (Cd), which has potential toxicity and is harmful to plants and humans, are one of the most important abiotic stresses for grain products such as corn, which can be significantly reduced. These products are currently produced in Iran, among others. . One of the ways to deal with cadmium stress is to modify cultivars. Therefore, research is conducted to identify gene locations controlling quantitative traits. This study is conducted in order to evaluate the grain yield and phenological, morphological, agronomic, physiological and biochemical traits of corn, as well as the diversity and identification of genomic locations controlling these traits using GWAS method based on SNP markers, environmental conditions without stress and cadmium stress. In this study, 95 maize genotypes in the form of randomized complete block design with three replications, in two conditions without stress and cadmium stress, were grown in pots in the open environment at the agricultural research station, during two cropping seasons (1399-1400 and 1401-1400). were cultivated and evaluated. Phenotypic population evaluation has been done using univariate and multivariate statistical methods. The results of analysis of variance showed that there was a significant difference between the corn genotypes for most of the studied traits in two conditions without stress (normal) which indicated high phenotypic diversity among the studied genotypes. Most of the traits are strongly influenced by the genotype-environment interaction (GEI), which indicates the complex and independent genetic control of these traits under Cd stress. According to the description, in both non-stressed and stressed environments, the most variables were related to yield traits per unit area, cob wood weight, number of tassel branches and number of seeds in the row, and for physiological traits, the most variation was for the amount of cadmium in seeds and leaves. And the amount of copper in the leaves was observed. Examining the correlation coefficients between different traits in two environments without stress and stress showed that the traits of the number of seeds in the main cob, the weight of 1000 seeds, the number of seeds in a row and the weight of the cob wood have a positive and significant correlation with grain yield. Regarding physiological traits, a significant correlation is observed between photosynthetic pigments, so that in both environmental conditions, the amount of chlorophyll with the amount of total chlorophyll and cartons has a positive and very high correlation. Based on the results of step-by-step regression analysis and causality analysis of normal conditions and cadmium stress, the characteristics of the number of seeds in the cob and the weight of 1000 seeds are determined. These traits have a direct effect on performance and the maximum changes in performance were created by the provided traits. Since the grain yield in most studies is strongly affected

by the environment, therefore, it can be mentioned that this trait is used to improve the yield of corn lines. According to the results obtained from the cluster analysis in each of the non-stressed and cadmium stressed environments for agro-morphological traits, the corn genotypes have been placed in four groups. While for the physical and biochemical traits, the groups were divided into three categories and the results of the category detection determined which evaluated these groupings. By comparing between the groups resulting from cluster analysis, the genotypes that had a low average for most of the traits were identified as sensitive genotypes, some of the genotypes that had the highest level are tolerant genotypes. Therefore, by carefully selecting the parents from among the genotypes tolerant to cadmium stress and using targeted crossings between them, it is possible to find hybrids that are suitable in the environment with cadmium stress in order to take advantage of genetic phenomena such as segregation and heterosis. In the analysis of the main components in each of the non-stressed and cadmium-stressed environments for agro-morphological traits, respectively, the first nine and eight components had an eigenvalue or a root greater than one, which were 79.26 and 76.42% in total. They explained the diversity between the attributes of the case. While for physiological and biochemical traits in both environmental conditions, the first six components explain 79.76 and 71.94 percent of the total variation in stress-free environments, respectively. Factor analysis using the analysis and parallel analysis method divided 24 agro-morphological traits into six and seven main and independent factors in non-stress and cadmium stress conditions, and these factors accounted for 62 and 65% of the diversity of maize cultivars, respectively. they got. justified For physiological and biochemical traits, these traits were divided into 5 factors in both environmental conditions, which explained 66 and 52% of the variation in non-stressed and stressed environments, respectively. In order to evaluate the genotypic population, the genome-wide association study technique (GWAS) was used to investigate the relationship between markers and traits (MTA) to investigate the cases in each of the conditions without stress and cadmium stress from the nested key model using Bayesian information and methods. Repeated non-combination (BLINK) was used. In GWAS, in order to identify SNPs related to traits, Menthan analysis and QQplot design based on BLINK model were used. The results obtained from this research mean the identification of SNPs for all studied traits in both environmental conditions. In total, for 24 agro-morphological traits, 3783 and 4489 significant marker-trait relationships were identified in stressed and non-stressed conditions, respectively. In the environment of cadmium stress, the highest number of markers were respectively for the traits of cob weight (443), grain length (337), cob diameter (381), leaf width (299), grain width (294) and the lowest number of markers for grain yield traits (26), leaf length (23), number of seeds per row (57) were observed. In the non-stressed environment, the number of indicators for the traits of cob wood weight (469), cob wood diameter (455), number of leaves on the cob (411), row in cob (410), grain length (327), leaf length to width ratio (284) and the lowest number of

markers for plant height traits (43), number of seeds in the main ear (44), seed yield (51) and number of seeds in a row (60) were obtained. Also, for each of the environmental conditions of cadmium stress and no stress, respectively, 53 and 75 SNP positions with negative logarithm greater than 5 were identified, which were very significant. In stress conditions, these SNPs are only for the traits number of days to yield, number of days to end of pollination, number of days to payment, number of rows in ear, leaf width, diameter of ear wood, length and width of grain and ratio of length to width of grain (respectively : 1, 9, 1, 7, 2, 30, 1, 1, 1) were significant. In non-stressed conditions, these SNPs were only significant for the traits number of days to yield, number of rows per ear, 1000 seed weight, ear wood diameter, grain length to width ratio, grain length and width (respectively 1, 2, 2, 64, 1, 4, 1) were significant. In the following, 5102 and 4826 significant marker-trait relationships were identified for 16 physiological and biochemical traits under cadmium stress and non-stress conditions, respectively. In the cadmium stress environment, the highest number of markers for the traits of seed cadmium content (1464), chlorophyll a/b ratio (784), leaf cadmium content (746), total chlorophyll (278) and the lowest number of markers for carbohydrate traits (67), ratio The carotenoid was converted to chlorophyll (76) and the enzyme malondialdehyde (77). In the non-stressed environment, the highest number of markers for leaf cadmium traits (2505), seed cadmium content (851), catalase enzyme (223), carbohydrate content (200) and the lowest number of markers for chlorophyll b (14) and total chlorophyll traits. (57) and the amount on the leaf (57) was obtained. Also, 78 and 8 SNP positions were identified for these physiological and biochemical traits in both stress and stress environmental conditions, respectively, which were without negative logarithm of 5. In stress conditions, these SNPs were significant only for chlorophyll a, chlorophyll a/b ratio, total chlorophyll, seed cadmium content, seed content and leaf cadmium content (1, 67, 3, 2, 3, 2 respectively). While in non-stress conditions, these SNPs were significant only for chlorophyll b traits, chlorophyll a/b ratio, catalase enzyme and seed cadmium content (1, 3, 3, 1 respectively). Therefore, due to the large number of indicators according to the study cases, there is a need for additional investigation of saturation maps and types of indicators in racial programs, as well as carrying out communication mapping in several years and places. If these markers are confirmed in additional tests, it will be possible to use them in corn breeding programs and indirect selection in order to improve and modify traits, especially under cadmium stress conditions. In this study, a large number of common markers were identified in non-stressed environmental conditions with cadmium stress. Some shared markers include AX-91748390 for days to tillering, two markers AX-91146137 and AX91146134 for number of rows in ear, AX-91742418 for grain length, AX-91161258 for grain width, AX-90594004 for grain length to width ratio. and the number of 30 common markers (AX-90729619, AX-90729611, AX-91509046, AX-90729647, AX-91509045, AX-90729654, AX-91208803, AX-91509069, AX-90729645, AX-90729648, AX-90729648 729686, AX -91675279, AX-90903214, AX-91630484, AX-91676113,

AX-90969278, AX-90994052, AX-90902252, AX-91214923, AX-90562152, AX-90615396, AX-90622634, AX-91 509023, AX-90970294 , AX-91509037, AX-90617430, AX-90902565, AX-90903322, AX-90580538 and AX-91629284) were for the trait of cob wood diameter. Further, a common marker AX-91350830 for chlorophyll a was identified with total chlorophyll under cadmium stress conditions. Common markers show that these markers have very high stability. Also, the common locations for the desired traits probably exist due to the pleiotropy effect or the connection between the gene locations that control the traits. These observations strengthen the hypothesis of co-localization or multiple effects of QTLs. Based on the genotypic evaluation results, it can be concluded that finding markers with high correlation as well as common markers is promising for saturating linkage maps. Linkage mapping can be used as an alternative method for linkage mapping of diverging populations. In general, correlation analysis is introduced as an efficient, low-cost and fast method in identifying markers related to crop traits.

Key words: Maize, Cd stress, Association mapping, Linkage Disequilibrium, SNP markers

