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Estimating genetic variation and genetic parameters for grain iron, zinc and protein concentrations in bread wheat genotypes grown in Iran

Reza Amiri, Sohbat Bahraminejad*, Kianoosh Cheghamirza

Department of Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, Razi University, 6715685438 Kermanshah, Iran

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The low grain micronutrient concentrations particularly iron and zinc are well documented problems in wheat. Eighty bread wheat genotypes were assessed for grain iron (GFeC), zinc (GZnC) and protein (GPC) concentrations along with agronomic traits for two consecutive years under normal and terminal drought stress conditions within each year. The results of combined ANOVA revealed high significant genotypic variations for GFeC, GZnC and GPC as well as agronomic traits under both conditions. The genotype × year interaction was significant for thousand kernel weight (TKW) and GPC under normal and for TKW under drought stress conditions. According to average of both years, drought stress caused reduction in kernel yield (KY) and its components, while raising trends in GFeC, GZnC and GPC were observed. In all environments, KY had negative phenotypic and genotypic correlations with GFeC, GZnC and GPC. Broad sense heritability of GZnC, GFeC and GPC were observed as moderate to high under normal conditions across two years. Cluster analysis showed that the older genotypes and landraces were located on a separate cluster with high GFeC, GZnC and GPC and low KY. Finally, the two extreme groups of genotypes were identified in order to be used as parent in crossing programs.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the main staple food grains worldwide providing significant amounts of daily calorie intake and the protein consumption and is a source of minerals such as iron (Fe) and zinc (Zn) for poor consumers throughout the world. Wheat is a staple foodstuff for more than one third of the world population, especially in the most of developing and low income countries such as Iran where, as much as 50–60% of the daily energy and around 50% of the daily protein intake is derived from it (Wang et al., 2011). Today, further yield increases are essential to meet the wheat production demands in the world's growing population. However, the nutritional quality (in particular micronutrients and protein) of wheat is equally important but often overlooked (Velu et al., 2014). Thus, both yield and the nutritional quality of the wheat grain have critical role in food security and human health of the world people. It is well documented that bread wheat has low micronutrients concentration (Cakmak et al., 2010). Currently, approximately 40% of the world's population face problem of micronutrients deficiency, which are often known as "hidden hunger", due to diets that are poor in essential micronutrients (FAOSTAT, 2013). This phenomenon is much more widespread in the populations of the developing countries where due to poverty a diversified and balanced diet is not affordable for the majority, and crops such as wheat eaten widely by the poor. Among the widespread micronutrient deficiencies, inadequate uptake of Fe and Zn represents a common micronutrients deficiency in human populations around the globe.

Regarding to the fact that the wheat is a staple food crop, even a small increase in its grain nutritional quality can help to decrease micronutrients deficiency. Therefore, emphasis should being given to improve wheat grain nutrition quality to meet the challenges cause due to hidden hunger and bridges the gap between

Abbreviations: ANOVA, analysis of variance; GFeC, grain iron concentrations; GFeY, grain Fe yield; GPC, grain protein concentration; GZnC, grain zinc concentrations; GZnY, grain Zn yield; HI, harvest index; KY, kernel yield; NKPS, number of kernel per spike; $NSPm^2$, number of spike per square meter; TKW, thousand kernel weight.

* Corresponding author. Tel.: +61 8 82727245.
E-mail addresses: rezaamiri20002007@yahoo.com (R. Amiri), sohbah72@hotmail.com, bahraminejad@razi.ac.ir, sohbat.bahraminejad@alumni.adelaide.edu.au (S. Bahraminejad), cheghamirza@yandex.ru (K. Cheghamirza).

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agriculture and health. Biofortification, which aims to enhance micronutrient concentrations in edible portions of crop foods as well as their bioavailability through the conventional breeding practices and modern biotechnology approaches, is one of the best evidenced solution and potentially a cost effective way to diminish malnutrition in developing countries (Khush et al., 2012).

The processes of micronutrient uptake, translocation and accumulation in the grains are under genetic control (Yang et al., 2007). Gomez-Becerra et al. (2010) demonstrated that variation in micronutrients and protein of wheat grain is highly attributed to genetic effects (Heidari et al., 2016). Moreover, more than twenty different QTL for increased grain iron (GFeC) and zinc (GZnC) concentrations have been reported on ten different chromosomes in *Triticum*, indicating the inheritance of this micronutrient is mostly quantitative in wheat (Xu et al., 2012; Velu et al., 2014), but seems that influenced by the environment and depend largely on genotype. Experiments at CIMMYT showed medium to high heritability for these traits across various environmental conditions (Velu et al., 2012). Therefore, identification of genotypes with higher ability in uptaking micronutrient from soil and selection those one with natural elevated GFeC and GZnC obtained through breeding in an adapted genetic background can facilitate biofortification strategy in developing countries (Bouis et al., 2011; Pandey et al., 2016).

To meet this breeding aim there should be a wide variation in adapted genetic materials. On the other hand, the knowledge of nature and magnitude of such variation in the breeding materials are very important for selection of the breeding method and further improvement of the crop. Existence of a variation in GFeC, GZnC and grain protein concentration (GPC) in different wheat and its related species has been extensively documented (Ficco et al., 2009; Chatzav et al., 2010). However, persistent efforts are still needed to search genetic resources augmented with these micronutrients and GPC (Pandey et al., 2016). Therefore, it is imperative to raise greater awareness for breeders to study the nutritional status of diverse wheat genotypes to find genotypes with elevated grain micronutrients concentrations as the initial step for a breeding program.

It has been well emphasized that the quality of wheat grains is often related to cultivar's genetic background and different environmental factors particularly soil composition (Velu et al., 2012), temperature and water availability (Fernando et al., 2014). In Iran, soil is generally poor in available form. Soil experiments conducted in Iran showed that 37% of the irrigated wheat fields have problems in terms of availability to iron and 40% to zinc (Sharifi-soltani et al., 2016). In Iran with high temperatures and no rainfall during grain filling period, drought often causes severe reductions in wheat yield. But, it has been reported that wheat plants contain higher zinc concentrations when facing to heat and drought stress (Velu et al., 2016).

Generally, knowing genetic variation, heritability, genotype × environment interaction and association between the quantitative and qualitative traits is fundamental stage in developing appropriate breeding strategies and development of improved genotypes with high production potential and grain quality attributes. Thus, in the present study an effort has been made to (1) screen genetic diversity for GFeC, GZnC and GPC in 80 bread wheat genotypes including historical and modern varieties and advanced lines, released from 1942 to 2012 with respect to drought stress tolerance efficacy, (2) estimate of the heritability and the other genetic parameters for the different traits over two years, (3) identify the cultivars with a good production potential and/or grain quality attributes in order to enter the crossing programs and (4) understand the association between the quantitative and qualitative traits.

2. Materials and methods

2.1. Plant materials

Five elite lines and 75 historical and modern cultivars (released or introduced from 1942 to 2012) of bread-making wheat (listed in Table 1) were received from the Crop and Horticultural Science Research Department, Kermanshah Agricultural and Natural Resources Research and Education Center, AREEO, Kermanshah, Iran.

2.2. Field trials

Field experiments were conducted over two cropping years (2011–2013) at the research field of Razi University, Kermanshah located on the western part of Iran (latitude 34° 21′ N, longitude 47° 9′ E, altitude 1319 m) with 450–480 mm long-term average annual precipitation. At both years, the experiment was performed in a randomized complete block design with three replicates under normal and drought stress conditions. Each plot was 1.25 m wide by 1.2 m long, containing five rows as 400 seeds per square meter density. The rainfall at the two cropping seasons of the experiment was 308 and 393 mm, respectively. More information of monthly average T.max and T.min temperatures, absolute T.max and T.min temperatures and total rainfall are shown in Supplementary Fig. 1. Terminal drought stress was imposed in the last May; meanwhile, non-stressed plots were irrigated three times more. Chemical fertilizer, herbicide and pesticide were not used.

2.3. Determination of the soil properties

Each year, before sowing, a representative soil samples of 30 cm depth was taken from different part of the experimental sites, air dried, crushed to pass a 2 mm sieve, and saved for further analyses (Table 2). Soil different characteristics measurements have been explained in our previous study (Amiri et al., 2015). Soil Fe and Zn concentrations, were measured by Atomic Absorption Spectrophotometer which has been suggested for routine estimation of Fe and Zn in seeds.

2.4. Determination of agronomic traits and whole grain protein, Fe and Zn concentration

Harvesting plots took place at full physiological maturity to determine agronomic traits, GPC as well as GFeC and GZnC. Near Infrared Reflectance (NIR) spectrometer (Perten Instruments DA7200) was used directly at harvest to assess GPC in whole grains. According to the procedure suggested by Emami (1996) briefly, the hand threshed grains were washed thoroughly with distilled water to avoid any dust or contamination that could influence the analysis, put in a clean paper bags and oven dried at 40 °C for 1 day. Dried grains were ground by a laboratory non-rust steel miller (IKA® A11 B, Germany). Each powder sample was incinerated at 550 °C in a muffle furnace and subjected to hydrochloric acid-digestion and analyzed for Fe and Zn by using Atomic Absorption Spectrometer (SpectrAA-220, VARIAN, Australia). Both micronutrients were measured for two replicates of each trial. Further details about preparation of grain samples for Fe and Zn analysis is published in Amiri et al. (2015).

2.5. Estimation of genetic parameters

The phenotypic and genotypic variances and coefficients of variations were calculated based on the expected mean squares of the source of variations in combined ANOVA as suggested by Singh and Chaundhary (1977) and Allard (1960):