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EXAMINING THE GENETIC DIVERSITY IN WHEAT GERMPLASM FOR ACCUMULATION OF NUTRITIONAL AND ANTI-NUTRITIONAL MASSES IN GRAINS

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ABSTRACT

Micronutrient deficiency in soils coupled with poor utilization particularly in cereals has led to low crop productivity and malnutrition in over 3 billion human beings. Addressing this problem a field study was carried out to evaluate concentrations of minerals and anti-mineral components in grains of 23 wheat genotypes using randomized complete block design replicated thrice under similar field conditions during 2010-11 at Experimental farm of Nuclear Institute of Agriculture Tandojam. Nitrogen, P_2O_5 and K_2O were applied at the rate of 120, 90 and 60 kg ha^{-1}, respectively. The wheat grain samples were collected and kept in oven at 80 ^{0}C and ground in IKA FM-10 grinding mill. The analytical results revealed that wheat lines V-4403, MB-2, SD-333, MSH-5 and MSH-3 were the highest accumulator of protein. Genotypes V-4403, MNS-7, MB-3 and MNS-13 illustrated maximum ash content of 2.2, 2.1, 2.1 and 2%, respectively. Micronutrient (Fe, Zn and Cu) concentrations significantly varied in genotypes. Genotypes V-4403 and MB-3 illustrated maximum Fe densities (82.5 and 80.4 mg kg^{-1}). The genotype MB-3 accumulated the highest Zn (63.3 mg kg^{-1}), while V-0203 was the least Zn (18.1 mg kg^{-1}) accumulator. None of these genotypes contain ideal phytic acid: zinc ratio (<5:1), only 01 (SD-4085/3) was medium 5:1 -15:1, while rest of all were high (>15:1) in phytic acid. This research paper provides important information about the wheat grain and their nutritional values which will be helpfull for wheat breeders in improving nutritional quality of their upcoming lines through different agriculture and breeding strategies.

Keywords: Ash, micronutrients, phytic acid, proteins, wheat grain.

INTRODUCTION

Wheat (*Triticum aestivum* L.) ranks first among the cereal crops in Pakistan and occupies about 66% of the annual food crop area. It provides food for 36% of world population, about 40% (278.60 million tons) of the total world wheat production (681.4 million tons) is produced in Asia (Ali *et al*., 2013). It contributes 10.1% to the value added in the agriculture and 2.2% to Gross Domestic Production (GDP) (Pakistan Economic Survey, 2013-14). Wheat is cultivated on

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an area of 8.66 million hectares with annual production of 23.50 million tons (Ali *et al*., 2013).

Half of the world population (> 3 billion) is suffering from micronutrient (vitamins, Fe and Zn) deficiency. Globally nutrient deficiency related health problems are common and at high risk in rural population of developing countries (Welch, 2005). Insecure food cannot provide consistent supplement of micronutrients to all the human beings in appropriate and affordable quantity (Zuzana *et al.*, 2009), hence, retarded growth of body, brain, skin lesions, hypogonadism, night blindness, loss of hairs, poor healing of wound and destruction of immunity power are typical Fe and Zn deficiency symptoms in early preschool going children. Iron (Fe) alone hits greater than 47% of early stage children (Cakmak *et al*., 2004; Cakmak *et al.*, 2010). Cultivation of recently introduced high yielding wheat cultivars on vast area contain meager concentrations of Fe and Zn, which do not fulfill daily nutritional requirements of growing body (Cakmak *et al.*, 2010). In addition, wheat also contains sufficient amount of anti-nutritional compounds like phytic acid that decreases the absorption of micronutrients (Zn and Fe) in human intestine (Welch and Graham, 2004). Mostly, Zn^{2+} and Fe^{2+} concentrations in commercial wheat genotypes vary between 20 to 35 µg g^{-1} (Cakmak *et al*., 2004). These densities are insufficient for normal human diet.

Protein content of grain influences the quality and nutritional status of bread and pasta. Proteins stored in the vegetative parts of wheat undergo hydrolysis and the resulting amino acids are largely translocated to the grains; these processes are accompanied by gradual plant senescence (Yang *et al*., 2004; Parrott *et al*., 2010). Grain protein content (GPC) is more economical as compared to meat, but it does not meet human and animal nutritional requirements, because of lack of essential amino acids (EAAs) like lysine, tryptophan and threonine, etc. Deficiency of these amino acids is degrading the quality of grains. The recommendation of World Health Organization (WHO) for these amino acids is lysine 1.5-4.5%, tryptophan 0.8-2.0% and threonine 2.7 to 3.9%, respectively (Bicar *et al*., 2008). There is an especial wild emmer wheat genotype (*T. turgidum* ssp. Dicoccoides) has capability to increase grain protein content (Olmos *et al*., 2003; Gonzalez-Hernandez *et al*., 2004; Brevis and Dubcovsky, 2010).

The growers select high yielding cereals to get maximum harvest and reduce the area of pulse crops which are important sources of protein and mineral nutrients. This farming trend leads to decline in cereal prices and enhancement in cost of legumes, fruits, vegetables, animals and fish proteins. This ultimately causes serious “hidden hunger” or micronutrient malnutrition in human beings who are utilizing cereals in daily life at affordable rates. The old land races of wheat which are imperative source of genetic diversity for micronutrients and other nutritional compounds are disappearing. Only 30 crop species out of 7000 cultivated by men are gratifying the food energy demand of about 95% of world people (Welch, 2002). Wheat grain quality is an important objective of bio-fortification program. Breeding of wheat for good yield and better quality nutritional elements has key position for food security of nation. Wheat grain contains large amount of chemical compounds and elements which are essential for human nutrition. It contains proteins, carbohydrates, fat, mineral elements (Fe, Zn and Cu) and vitamins which play an important role in bread and backing quality (Cakmak *et al.*, 2010). Breeding strategy of wheat has greater importance of quality traits due to imposition of high standards by end users, cookies manufacturers and bakers, etc. simultaneously high urbanization has also turned scenarios of quality protein (Peña, 2007). Therefore, breeding efforts are underway throughout the world, keeping in view the utilization of new breeding strategies to evolve quality wheat varieties capable to accumulate higher densities mineral nutrients with better grain yield and less anti-nutritional masses, this study was carried to determine quality, nutritional status and availability of anti-nutritional compounds in wheat grains of genotypes evolved at Nuclear Institute of Agriculture (NIA) Tandojam.

MATERIALS AND METHODS

A field study was carried out to evaluate concentrations of minerals and anti-mineral components in grains of 23 wheat genotypes using randomized complete block design replicated thrice under same field conditions during 2010-11 at Experimental farm of Nuclear Institute of Agriculture Tandojam. Nitrogen, P_2O_5 and K_2O in the form of urea, di-ammonium phosphate (DAP) and sulphate of potash (SOP) were applied at the rate of 120, 90 and 60 kg ha^{-1}, respectively. The recommended fertilizer levels were applied to examine the genotypic variation in wheat. After threshing, wheat grain samples (200 g) from each treatment were collected and kept at 80 ^{0}C in an oven and ground in IKA FM-10 grinding mill.

Ash and protein content

Five gram sample was taken in ash cup to determine ash content (%). The sample was burnt over night at 585 ^{0}C. The material cooled at room temperature and weighed. The ash content (%) was calculated by deducting the weight of burnt sample from dried sample. Ash content (%) = (weight of ash/weight of oven dry sample)*100. Protein content was analysed by using kjeldhal method.

Iron, Zn and Cu analyses

Sample of ground material (0.50 g) was weighed and 10 ml of concentrated nitric acid (HNO_3) were added in digestion tubes. The tubes were left overnight. On day 2nd the samples were digested at 70 ^{0}C for 2 hours, and then gradually temperature was raised up to 110 ^{0}C for further 6 hours. The samples were cooled at room temperature, filtered (Whatman 40) and final volume was made to 100 ml by successive rinsing. The tubes were removed from the block, allowed to cool and then filtered in acid washed 100 ml volumetric flasks through a filter paper (Whatman 40) (Westerman, 1990). Successive rinsing of tubes was ensured with deionized water and thus the volume of the flask was made upto mark (100 ml). The micronutrient (Fe, Zn and Cu) densities were assessed through Atomic Absorption spectrophotometer (AAS), Novaa 400, Germany.

Phytic acid

The phytic acid is important chelating agent of metallic nutrients like Fe and Zn in wheat grains. Haug and Lantzsch (1983) reported a sensitive method for determination of phytic acid concentration in wheat flour. Sample was extracted in 0.2 N HCl on hot plate by adding known concentration of acidic Iron III. The sample was extracted (with 0.2N HCl) and heated with an acidic Iron III solution of known concentrations (0, 5, 10, 15, 20, 25 and 30 ppm). The decrease in Fe concentration was determined using color solution 2, 2-bipyridine at OD 510 nm (Wahab *et al*., 2004).

Statistical analysis

The statistical analysis of the data, CV and Tukey Honesty Significant Difference (HSD) assessed by using STATISTIX® VERSION 8.1, Analytical Software, Inc., Tallahassee, FL, USA. $HSD_{0.05.}$

RESULTS AND DISCUSSION

Protein and mineral nutrients (Fe, Zn and Cu) have very important role in biochemical processes involved in growth. They play a very crucial role in physiological processes and enzyme activation of digestive system. The nutrient densities of different wheat genotypes are given in Table 1- 2 .

Table 1. Analysis of variance of different quality parameters illustrated by various wheat genotypes.

Parameter	Sum of squares	Mean squares	F value	Significance
Protein	64.1	2.91	57.1	***
Ash content	2.9	0.1	58.9	***
Iron (Fe)	5120	232.8	17.2	***
Zinc (Zn)	11400	518	33.9	***
Copper (Cu)	576.7	26.2	141.7	***
Phytic acid	8041501	365523	212.1	***

Protein content

Significant genotypic variability was noted in protein content. Genotype V-4403 and MB-2 accumulated higher protein content of 15.4 and 15.2%, respectively. The wheat line MNS-13 contained the least densities (12.3%) of protein under normal nutrient management system. The rest of other genotypes categorized as medium in protein content according to standards of global trade association (GTA). Proteins are believed as crucial nutrient for development of human and animal body. Major portion (endosperm) of grain is consisted of carbohydrates; the remaining part contains 1.5% fats and 13% protein. The ash content (minerals) and fibres are in minute quantity 0.5% and 1.5% (Belderok *et al*., 2000). Quality protein is very essential trait to be considered in wheat breeding programs to fulfill the standards imposed by end users. In our study genotype V-4403 and MB-2 accumulated higher protein which may be due to high gluten trait of genotypes. These genotypes may be good for chewy texture and breads. The grain of line MNS-13 contained low protein and can be used for cookies, cakes and other tender products. The protein content of wheat grains may vary between 10% - 18% of the total dry matter (Zuzana *et al*., 2009). The findings of the study are in agreement with results of Brevis *et al.* (2010) who reported that the wheat line UC1041 and Anza contained grain protein of 13.08 and 13.77%.

Table 2. Protein, ash content, nutrients (Fe, Zn and Cu) and anti-mineral compound (Phytic acid) concentrations of different wheat genotypes.

Genotype	Protein	Ash	Iron	Zinc	Copper	Phytic acid
	------ % ------		------ mg kg^{-1} ------		------mg 100 g^{-1} ------	
SD-4085/3	13.63±0.72	1.66±0.02	53.7±4.50	40.6±0.61	7.28±0.58	570±10.2
SD-4047	13.01±0.45	1.72±0.02	53.4±5.00	26.7±0.85	6.34±0.64	821±11.3
SD-777	13.96±0.85	1.85±0.03	56.0±0.20	26.6±0.35	6.93±0.73	867±10.1
SD-222	14.63±1.77	1.77±0.02	54.2±0.80	23.7±3.60	5.93±0.73	846±13.2
SD-333	15.14±1.49	1.49±0.11	45.8±0.50	30.3±1.90	7.84±0.59	1145±12.4
NIA-6-12	14.63±0.79	1.79±0.01	62.5±3.65	25.6±0.50	16.86±0.96	642±12.3
CIM-04-10	14.01±0.62	1.62±0.18	59.6±6.60	26.4±1.65	6.88±0.66	849±11.2
MSH-3	15.04±0.59	1.59±0.03	49.3±0.57	35.0±1.95	6.97±0.15	883±13.4
MSH-5	15.03±0.52	1.52±0.01	57.9±2.75	29.3±2.00	15.7±1.11	885±12.1
ESAW-9525	14.41±1.46	1.46±0.02	63.8±0.55	33.8±1.60	7.08±0.53	808±12.4
Sarsabz	13.73±1.46	1.46±0.07	59.4±6.20	30.9±2.50	7.00±0.55	911±13.2
V-0203	14.47±0.64	1.64±0.01	78.3±4.32	18.1±1.01	8.01±0.40	1075±13.5
V-4403	15.44±0.23	2.23±0.01	62.5±1.40	33.0±1.90	5.97±0.15	1348±15.4
MN-01	14.23±0.75	1.75±0.02	67.0±0.80	46.4±0.45	6.80±0.36	2042±14.6
MN-08	12.99±0.73	1.73±0.01	60.4±0.80	46.2±1.95	7.29±1.17	1017±13.8
MN-12	12.69±0.88	1.88±0.01	61.1±0.45	50.0±0.45	9.04±0.83	1069±12.4
MB-1	12.47±0.75	1.75±0.02	55.6±2.15	28.5±2.12	8.90±0.50	1354±11.2
MB-2	15.24±1.04	2.04±0.03	79.1±4.50	62.4±2.70	11.3±1.10	1350±12.4
MB-3	14.26±1.07	2.07±0.03	80.4±3.95	63.3±4.85	5.47±0.35	1317±11.1
MNS-3	12.66±0.86	1.86±0.01	58.0±3.25	54.7±1.25	4.97±0.64	1850±15.2
MNS-6	13.21±1.75	1.75±0.02	59.7±4.25	54.7±0.90	10.65±0.55	1054±11.2
MNS-7	12.80±1.05	2.05±0.04	58.8±2.40	46.8±2.15	8.90±0.60	1355±12.5
MNS-13	12.25±1.04	2.04±0.01	55.3±1.10	51.2±0.95	7.85±0.45	1357±12.6
SED	0.184	0.038	3.00	3.19	0.35	33.89
Tukey HSD	0.707	0.148	11.55	12.27	1.35	130.31

Ash content

Ash content is an important trait of genotypes that resembles the mineral nutrient masses. Wheat genotypes significantly varied in ash content, ranging from 1.5 to 2.2% (Table 1 - 2). The genotypes V-4403, MB-3 and MNS- 7 contained highest ash 2.2, 2.1 and 2.1%, while the SD-333 (1.5%) was the least performer in this study. Ash content has key importance in the interest of flour millers, because the ash content of flour shows the total mineral contents. The millers need to increase mineral content of the flour to get ideal or specified ash levels. Since ash is primarily concentrated in the bran, ash content in flour is an indication of the yield that can be expected during milling. Ash can affect flour colour, viz., white colour indicates lower ash content while red colour of other products, such as whole wheat flour, have high ash content (USDA, 2007).

Mineral (Fe, Zn and Cu) nutrient concentrations

Iron is one of the important constituents of haemoglobin required for normal health of human body. Iron concentration within the grains showed variable densities. The genotype V-4403 showed highest concentration (82.5 mg kg^{-1}) whereas lowest concentration (45.8 µg g^{-1}) was recorded in SD-333 (Table 1 and 2). The genotype MB -3 (80.4 mg kg^{-1}) and MB-2 (79.1 mg kg^{-1}) were the highest soil Fe extractor, followed by V-0203 (78.8 mg kg^{-1}).

Zinc accumulation of wheat grain in different genotypes varied from 18.1 to 63.3 mg kg^{-1} (Table 1 and 2). The wheat genotype MB-3 and MB-2 extracted highest Zn concentration (63.3 and 62.4 mg kg^{-1}) from soil, whereas minimum was observed in V-0203 (12.50 mg kg^{-1}). It is illustrated that out of 23 genotypes two had high concentrations of Zn (> 60 mg kg^{-1}), four between 50 to 60 mg kg^{-1}, rest of all 17 genotypes had minimum concentrations of Zn (< 50 mg kg^{-1}). Copper concentrations also varied significantly among genotypes. Compared to Fe and Zn, the Cu densities were quite low and varied from 5.0 to 16.9 mg kg^{-1} (Table 1 and 2). The wheat genotype NIA-6-12 and MSH -5 showed higher Cu concentrations (16.9 and 15.7 mg kg^{-1}), which were statistically differed from rest genotypes. The genotype MNS -3 was found to be the poorest in Cu content (5 mg kg^{-1}).

Hidden hunger (malnutrition) of mineral nutrient distresses more than 47% of global population and consistently increasing due to introduction of high yielding varieties with poor mineral contents. The highest Fe concentration was observed in genotype V-4403, followed genotypes MB -3 and MNS-7, while the highest Zn concentration was observed in the seeds of MB-3 and MB-2 genotypes (Fig. 4). These genotypes proved to be more micronutrients accumulator and can increase Zn^{2+} and Fe^{2+} concentration in food. These genotypes can also be better performer for high yield and health improvement (Zuzana *et al.*, 2009).

Phytic acid concentration

Phytic acid is an organic compound which reduces the bioavailability of mineral elements (Fe, Zn and cu) by binding tightly, the metallic nutrients then pass away from human intestine without absorption. A huge genotypic variability in phytic acid content of wheat grains was noted (Table 2). The wheat genotype SD-4085 accumulated least concentration of 570 mg 100 g^{-1}, among all tested genotypes. Genotype MN-01 and MB-03 contained maximum concentrations of phytic acid i.e. 2042 and 1842 mg 100g^{-1}, respectively. The genotypes extracted more mineral nutrients from soil showed the lesser amount of phytic acid as compared to those genotypes, which absorbed the least quantity of mineral elements. Phytic acid is a strong chelating agent of divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+} and

Zn^{2+}) reduces the bioavailability of minerals from plant derived food stuffs (Oberlease *et al*., 1961). Variable response of different genotypes was observed in phytic acid content. The wheat genotype SD-4085 accumulated least concentration of phytic acid among all tested genotypes. The wheat varieties containing the lowest phytic acid can prove better to provide Fe^{2+} in sufficient quantities in the areas where the inhabitants are suffering from anemia (Wahab *et al*., 2004). Such wheat varieties will be best suited for bread production. The varieties possessing lower phytic acid are also recommended for inclusion in the wheat breeding programme to produce progenies with lower phytate contents.

CONCLUSION

It can be concluded that the wheat genotypes V-4403, MB-3 and MB-2 are best accumulator for protein content and mineral nutrient densities. Genotype SD-4085 proved as low phytic acid bearer and may better to provide Fe and Zn concentration for human health, if this genotype manages with application of micronutrient fertilization. The Zn content of most genotypes depicted the lower concentrations as compared to the standards of “Harvest Plus” (50-60 mg kg^{-1}); therefore, application of Zn fertilizer at the rate of 5 kg Zn ha^{-1} is essential for maintaining the amount of bio-available Zn in grains of these genotypes.

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