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Excellent Bread Making Wheat Germplasm Offered

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***Abstract* – The primary general goal, as well as ongoing and most important objective in wheat breeding was, and still is, improvement of grain yield. Since 1960 in this activity the worldwide great results were achieved: wheat production was improved and grain production has increased from about 220 million metric tons in 1996 to about -765 million metric tons in 2019 on more than 215 million ha. However, this great improvement in yield was accompanied with some undesirable side effects: lowering grain protein content as a result of negative correlation between grain yield and grain protein, as well as lowering nutritive value of grain (mineral content) as a result of dwarfing genes, and low mineral supply in soil, and finally, the constant increase of intolerance of people to wheat gluten, or more correctly, to glyphosate residua on grain (Celiac disease). This means that in the future more breeding effort should be directed to breeding wheat of improved nutritive value, as well as new growth technology. The results of 20 years of breeding effort, as well as the best wheat germplasm created and offered for sale is described.**

***Keywords* – Winter Wheat, Grain Yield, Grain Protein Content, Grain Mineral Content, Nutritive Value.**

I. INTRODUCTION

Modern day wheat (*Triticum aestivum* L.) is a hexaploid composed of AABBDD genome, and grows widely across different regions of the world, with altitude levels ranging from sea level to 4500 m a.s.l. (Tibet), and latitudinally, from 30° to 60° N, and 27° to 40° S. Wheat has one of the largest gene pools among the cereal crops and is notable for its diversity. According to sources [6, 42] wheat had spread from the north-western Caspian Sea region also to South China, and has been widely grown as a staple food source in China for about 4500 years. It is the leading global food crop providing 19% of the daily calories and 21% of protein requirements for humans [42]. With 134.3 million metric tonnes in 2017, China is the largest grain producer and consumer. It is interesting that these cereals were produced by millions of small household farms. Over 90 per cent of household farms work on an area that is not larger than 1-2 hectares, and produce per labourer on average about 1520 kg, and only half of that is sold to the market. China's grain production is still largely semi-subsistence [46].

The World Population Prospects:

The current world population of 7.6 billion is expected to reach 8.6 billion in 2030. According to United Nations report (2017) with about 83 million people being added to the world's population every year, wheat is, as stated earlier, the leading global food crop producer for humans.

Due to wheat genetic variability and its importance as human food, a lot of effort was involved in improvement of its characteristics. Wheat was among few crops for which genes are transferred efficiently from their wild relatives. The ultimate objective of any breeding program is to develop outstanding genotypes in terms of yield, adaptation, resistance to biotic and abiotic stresses, and end use quality. A way for increasing the

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genetic variability, as a basic premise of successful breeding, was found through the exploitation of primitive species (*T. monococum*, *Ae. speltoides*, *Ae. tauschii*, *T. urartu*, *T. turgidum*, *T. timopheevi*, *T. tauchi* etc.) [5], or other cereal species (rye or barley genome). For instance, it was found that 1RS/1BL and 1RS/1AL translocations of rye chromosome carry many desirable genes for increasing the stability and level of yield, water use efficiency, resistance to certain pests, diseases and tolerance to different stress conditions [17, 18, 21, 31, 47]. The chromosome arm 1RS of rye (*Secale cereal* L.) has been used worldwide as a source of genes for agronomic and resistant improvement.

About one century ago wheat plant was tall, prone to lodging. For example: two extremely tall cultivars (over 130 cm), one, Bankuti 1201, an old Hungarian wheat variety with superior quality-attributes for bread making, and the other U-1, also tall, but with not so good quality characteristics, were grown widely till about 1950 [29]. However, such wheat cultivars were not able to yield more than one to two tones per hectare.

Short wheat varieties originated in Korea and were introduced in Japan in the sixteenth century, during the Korean-Japanese War. As a result of introduction of genes Gai1 for gibberellin insensitivity, and Rht1 and Rht2 for short straw, first carried out in Japan by Gonjiro Inazuka, (1935) the famous cultivar Norin 10 was released.

Between the two World wars, Italian wheat breeder Nazareno Strampelli, by means of crossing the Italian wheat landraces with the Japanese variety Akakomugi, introduced the gene for photoperiod insensitivity (Ppd-D1) and reduced height (Rht8, both on the 2D chromosome) in Italian wheats [2].

A number of semi-dwarf and dwarf varieties Pitic62, Penjamo62, Sonora63 and 64, Mayo64, Lerma Rojo64, Inia66, Tobari66, Ciano67, Norteno67 and Siete Cerros, were released into the Mexican programme (CIMMYT, 1955), and grain yield was increased from 1.0 t ha^{-1} in 1955, to 2.8 t ha^{-1} in 1967. Beside dwarfism, as a result of introduction of Rht genes, leaf area decreased, but photosynthesis per unit area, as well as harvest index increased. The new semi-dwarf and dwarf varieties (dwarfing genes Rht1, Rht2 and Rht3), [11] have been the varieties responsible for a yield breakthrough in Pakistan, India, Turkey, Afghanistan and Tunisia [3].

According to our study, the effects of dwarfing Rht3 genes depend on their dosage: double (Rht3 Rht3) or single (Rht3 rht3). For instance, plant shortening for 54% or 28% respectively. In spite of 12% more heads per plant, other yield components, such as the number of grains per head, or grain weight per head were lower, and final yield results were significantly lower (-27% grain yield per plant). Based on these results we do not recommend applying Rht3 genes in our breeding work [23].

The new Strampely wheat cultivars Villa Gloria, Ardito, Mentana and Damiano, beside insensitivity to day length, were earlier, shorter and better yielding. Soon, in Italy, by means of further crossing, new and better cultivars were released: San Pastore, Abondanza, Mentana, Funo, Ardito, Villa Glori, and 30 others, and they were spreading in Czechoslovakia, Hungary, Bulgaria, Romania, the former Yugoslavia [2] and even in China (cv. Abondanza under the name Nanda 2419). Vavilov was among the first scientists who recognized the high potential value of plant genetic resources (PGR) for humankind [8]. China had improved yielding ability of cereals, (the highest being 123.3 million tonnes in 1997[46]) but not grain quality.

To our knowledge, as important characteristics of wheat, we understand:

1. The global grain yield for China it is estimated that the yield increase achieved by cultivar improvement during the 2001-2012 was 1.7% per annum, or 52% in total. Based on this improvement it was greatly over

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predicted that China will be self-sufficient in wheat production by 2020 [33]. The self-sufficiency in wheat production for China is possible, but it seems that more effort will be needed.

2. The bread making properties, the content of macronutrients and micronutrients as well as other grain quality traits are genetically determined [14] and are the results of many characteristics of wheat grain: grain size, grain hardness, milling ability, grain protein and gluten content, flour water absorption, dough extensibility, and finally taste, volume and colour of bread loaf. The flour from imported and selected Australian or USA wheat varieties had better gluten properties, dough mixing characteristics and starch properties compared to the commercial Chinese wheat flours evaluated. However, China is able to produce own quality cereals, instead of importing them.

   After World War II wheat gluten was accused of being the agent responsible for triggering the gluten intolerance in humans, named Celiac disease (CD). At the moment about 5 % of the population in the USA and Europe suffers from it. The disease description of the European Society for paediatric gastroenterology Hepatology and Nutrition, first published in 1970, has been revised twice. Namely, the composition of the gut microbiota also seems to influence the release of pro-inflammatory cytokines triggered by gluten peptides [37].

   Recently, the scientists [1] proposed that celiac disease is associated with imbalance in gut bacteria caused by glyphosate (the active component of Roundup total herbicide). Glyphosate residues in wheat are likely increasing recently due to the growing practice of weed desiccation in mature wheat with Roundup, just prior to harvest. US farmers have recently been practicing this technological step to remove green weeds just to make harvest more easy and comfortable, without green weeds. In 1990 as a result of glyphosate residua, Celac disease appeared, and each year both of them increased (R = 0.976 Source USDA-NASS).

3. Grain mineral content, especially Ca, Fe, Zn, Mn, Mg and Cu, have today more and more important nutritive value. Wheat provides about 40% intake of essential micronutrients by humans in the developing countries [41]. Today about 3 billion people may suffer from micronutrient deficiency with its tremendous impact on health. The consumption of 100 g whole wheat grain, produced under the most favourable conditions, might provide over 5 % of Ca, 26 % of Fe, 39 % of Mg and 32 % of Zn of the recommended daily intakes.[12] However, it was shown[41] that the Rht dwarfing genes have significantly reduced Zn, Fe, Mg and Mn concentrations in the grain. The magnitude depends on the genetic background. It is genotype dependent. The statement of the Nobel prize winner Linus Pauling is well known to everybody: “You can trace every sickness, every disease and every aliment to mineral deficiency.” In fact, more than 400,000 children die each year due to Zn deficiency globally [32].

   The basic premise for successful breeding is great genetic variability, and each breeding program tends to represent a pool of regional divergence. The government encouraged farmers to adjust their grain production in response to the WTO accession and to produce higher quality grains [46].

II. Material and Methods

In the last decade of the past century an extensive examination of 142 wheat cultivars from former Yugoslavia, released from 1967 to 1986 was conducted. The pedigrees [19, 20], their coefficients of parentage and cluster analysis [17], their identification by gliadin electrophoresis [15] and determination of their genetic

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variability of HMW glutenins [22, 39] were examined, and compared with the Chinese wheat improvement and pedigree analysis [16]. In spite of the fact that high-molecular-weight (HMW) glutenins make up only a small proportion of the total storage proteins in wheat, the combination of HMW glutenin subunits plays an important role in determining its grain quality [35], and low to medium-quality varieties were gathered in many countries, including China.

While the Chinese wheats exhibited the highest allelic diversity of the *Gli* loci, and showed relatively low genetic variability for the *Glu* loci, Chinese cultivars were close to Italian and Russian ones, sharing with them the common Gli alleles. Its moderate quality score of 7 is insufficiently high when compared to cultivars from the other countries. For instance, using SDS-PAGE analysis it was found that our first high protein wheat cv. DIVANA, with the HMW subunits composition 2*, 7-9 and 5-10, has the highest quality score of 10, and about 17% grain proteins, and 150 g/100g bread yield, or cv. Koleda has the subunits composition N, 7-9 and 5-10, and lower quality score of 7, near 15% of grain proteins, and about 142 g/100g bread yield [39]. Modern cultivars and the cultivars bred 10-15 years ago were essentially the same. However, the genetic diversity estimation suggests that Chinese cultivars have a potential for quality improvement [34].

Our first high protein wheat cv. Divana was released in 1995 [23] and used as a donor of the character to the progenies in further crossings [25]. Besides, a limited number of wheat germplasm from US (grain quality and disease resistance), Romania (grain quality and disease resistance) and New Zealand (grain yield and disease resistance) were studied, and some of them were selected for crossing with selected domestic germplasm.

The project of building heterozygotic effect for good bread-making characteristics, based on far suited regional breeding programs from the USA, Argentina, Romania, New Zealand was initiated many years ago. The germplasm of elevated grain protein NE7060 from the International wheat nursery [30], as well as resistance to the most virulent races of Hessian fly, and multiple resistance factors against a number of pathogens (Wheat Genetics Resource Center-WGRC) [5] has been used for crossing and further selection of locally adapted high protein lines from segregating progenies.

In wheat crop, some uncommon technological treatments were applied also: (1) seed treatment and plant protection with stone powder “Ekorast”, (2) repeated crop treatments with plant stimulant gastric pentadecapeptide BPC-751[27].

Zelemy sedimentation test (HRN ISO 5529:2010), Extensogram (*ISO* 5530-2:2012), voter absorption and rheological properties of flour by farinograph (ISO 5530-1:2013) were done.

Multielement analysis in wheat grain flours was performed by spectrometric method on ICP-MS, and following recommended provided by Agilent Technologies the results were expressed in mg kg^{-1}.

Statistical analyses were conducted using statistical analysis system 9.2 (SAS Institute, Cary, NC, USA).

III. Results

Since 2012 a large number (over one thousand) of F5 wheat lines were grown and preliminary investigation of grain protein and gluten content has been performed. Finally, in 2016, only fourteen best-selected wheat germplasms have been grown in replicated (five reps.) small plots (5 sqm) yield test in comparison with four check cultivars (Bologna - the principal one, and Falado and Ingenio the highest yielding cultivar in the region),

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and summary of the results has been presented in Fig. 1. Nine of them had over 16% grain protein: J77, J78, J79, J80, J85, J86, J87, J88, Divna.

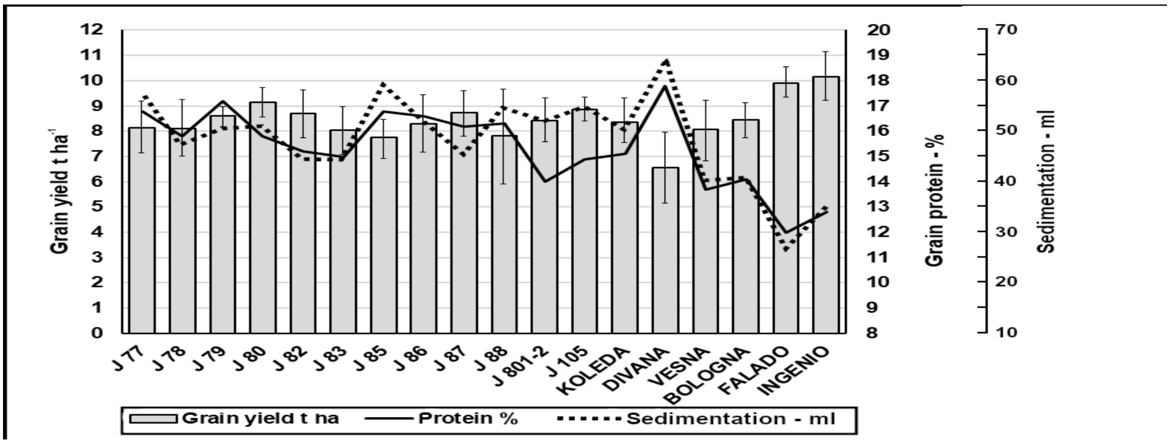

Fig. 1. Four years average grain yield of 12 advanced high-protein breeder lines, compared with six standard wheat cultivars at P> 0.05 (cv. BOLOGNA check).

Table 1. Plant characteristics of the tested 12 newly selected wheat lines and cultivars in comparison with high yielding check cv. Bologna (four years average 2015-2018).

No.	Characteristics	Four years averages		Bologna Check
		Average	Variation width - significant at P = 0.001	
1	Plant height - cm	102	Falado – Divana; 89 - 115*** cm	90 cm
2	Earlines – (heading May)	18	Falado – Vesna; May 4 - 25***	May 6
3	Population density - plants/sqm	831	Koleda – J-105; 654 - 1003***	782 pl/sqm
4	Powder mildew	tolerant	Resistant - tolerant	tolerant
5	Leaf rust	tolerant	Resistant - tolerant	tolerant
6	Stem rust	tolerant	Resistant - tolerant	tolerant
7	Yelow rust	tolerant	Tolerant - susceptible	tolerant
8	Fusarium	tolerant	Resistant - tolerant	tolerant
9	Head size - cm	7.7	J-79 - Pitoma; 7 - 9 cm **	7.5 cm
10	Number of kernels per spike	30	Divana - Falado; 24 - 43***	35
11	Grain yield – t ha^{-1}	7.829	Divana - Ingenio; 5.869 - 10.176 t ha^{-1} ***	8.542 t ha^{-1}
12	TKW - g	49.15	Bologna - J- 85; 37,81 - 55.85 g .*	37.81 g
13	Hectolitre mass – kg hl^{-1}	77.14	Falado - Koleda; 74.47 - 82.14 kg hl^{-1} **	75.92 kg hl^{-1}
14	Grain protein - % DM	16.43	Falado - Divana; 12.51 - 19.49 %***	13.34 % DM
15	Wet gluten - %	42.60	Falado - Divana; 28.70 - 45.96 %***	25.80 %
16	Zeleny sedimentation - ml	48,2	Falado – Divana; 27 - 63 ml ***	41 ml

Level of significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

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The values for the essential minerals in wheat grain determined in our wheat samples is a little higher in comparison with the data from other reports [44]. Beside genetic difference, one possible reason could be applied technology: instead of synthetic chemicals for seed protection, as well as synthetic fungicides and insecticides, we used “stone powder” (total about 100 kg ha^{-1}, several times during vegetation). It is milled flint-stone (in this case from Drava river), sold under commercial name “EKO-RAST”. It contains about 57% SiO_2, and other essential minerals (Mg, Fe, Zn, Mn, Cu and Ca) with favourable effects on plant growth [40].

Table 2. Essential minerals: average and variation in relations with grain protein content.

No.	Mineral	Average (mg kg^{-1})	Range (mg kg^{-1})	High content	Low content	Bologna Check (mg kg^{-1})
1	Mg	1 682.5	918.0 – 2 847.0 **	J-78, J-83, J-85, J-1301	Vesna, Boligna,	918.0
2	Ca	239.3	138.7 – 367.2 **	J-77, J-79, J-82, J-83	Falado, Ingenio,	224.9
3	Zn	57.9	32.6 – 75.4 **	J-77, J-78, J-87, Divana	Bol., Fld, Ing.	36.1
4	Cu	10.1	6.5 – 16.8 **	J-85, J-86	J-105, Bologna	8.0
5	Mn	44.5	28.3 – 54,5 **	J-85, J-86, Divana	Bologna, Falado	28.3
6	Fe	64.6	52,8 – 89.5 **	J-77, J-79, J-87, Divana	Vesna, Bologna	52.8
7	Protein - % DM	16.43 %	12.0 – 17.8 **	J-77, J-79, J-85, Divana	Falado, Ingenio	13.3 % DM

Level of significance: ** $P \leq 0.01$;

Tested germplasm exhibited rather wide and significant variation. As shown in table 2, some genotypes (J-77, J-78, J-79, J-85 and J-86) are repeatedly listed in different “high content” group, which gives a hope that by selection pressure even better wheats could be selected for multiple elements. It is interesting that all new high yielding cultivars (Vesna, Bologna, Falado and Ingenio) belong to minerals and protein low content group. Poor zinc (Zn) nutrition of wheat is one of the main causes of poor human health in developing countries, and foliar zinc application increased zinc in bread wheat [7].

Also high protein germplasm contains higher concentration of essential minerals, especially Zn and Fe. Correlation coefficients are shown in table 3.

Table 3. Correlation coefficients r between grain protein content and mineral concentration.

Protein : Zn = 0.910
Protein : Fe = 0.783
Protein : Mn = 0.613
Protein : Ca = 0.528
Protein : Mg = 0.286

Significant at $P<0.05$.

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3.1. *Baking Quality*

Complete analyses for some wheats (mixogram, farinogram, extensogram and alveogram, as well as experimental baking) were demonstrated in one earlier paper [28], and here baking quality is demonstrated by only one method - Zeleny sedimentation test [45]. Wheat strength and its bread volume are best predicted by this sedimentation test, and it provides a better measure of the overall bread-baking strength of wheat than any other known individual test, except for an actual bread-baking test. Its readings are shown in comparison with grain protein in Fig. 1. The sedimentation values of the new breeding lines are between 45 and 63 ml, while those of high yielding soft wheat are 27(Falado) to 35 ml (Ingenio), and at check cultivar Bologna 41 ml.

As wheat is mainly used for bread production, its baking quality is the most important property. There are several analytical methods for determination of the baking quality of certain wheat, but alone, none of them is complete accurate. The main quality tests are: grain protein content, wet gluten content, ash content, milling ability, flour water absorption, dough enzymatic characteristics, elasticity, mixing tolerance, but only evaluation of baked goods can give complete picture of wheat baking quality.

Fig. 2. The grain of high quality line J-85 in comparison with check for quality - cv. Divana [28].

1. Sedimentation values of 60 and over - wheat is always of high protein content and of superior gluten quality. It is suitable for mixing with weaker wheat for the production of bread flour. In normal years this type of wheat is in short supply and earns a considerable premium.
2. Sedimentation values of 40 to 59. This wheat is of the type most widely used for production of bread flour.
3. Sedimentation values of less than 20 to 39 have low protein content. Wheat in this range is best suited for use in mixing with stronger wheat for the production of bread flour.

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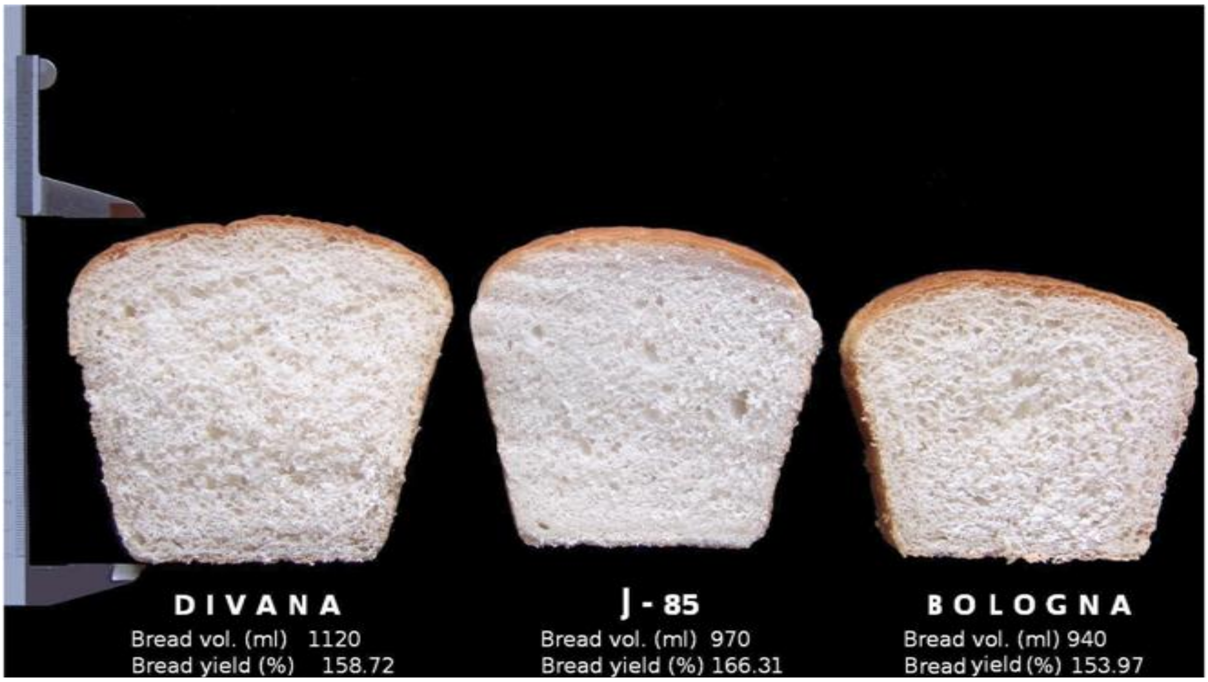

Fig. 3. Bread slices of cv. DIVANA (check for quality), line J-85, and cv. BOLOGNA (check for yield) [28].

3.2. *Technology*

Sustainable agriculture with at least four crop rotation system. In this way less herbicides and fungicides will be needed. As N fixing crop, before sowing wheat, white lupine is recommended.

Planting cultivars blend - is recommended. By choosing cultivars that complement each other for performance of important traits (disease tolerance), mixtures could be formulated to meet specific production requirements [4]. For instance complementary disease resistance traits [9, 10], or high yield and good baking quality [26]. In well chosen blend yield stability of blends exceeded that of pure cultivars.

Seed protection - just before planting use stone mill + 2% cuproblau. (Fig. 4.)

Plant protection - several times with water solution of stone mill. There are no dangerous concentrations

Plan stimulants - Gastric pentadecapeptide BPC-157 - several time during vegetation in extremely low dosage ($1.25x10^{-7}$ ppm), and Agrostemin - extract of (*Agrostema githago)* are recomended.

For plant treatment, to avoid soil damage, instead using heavy tractors, drones are recommended.

Fig. 4. Seed treatment: Check and Stone powder + CuSO4.

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IV. Conclusion

Agriculture can and should do much more than reduce hunger. It has an enormous potential to significantly improve the nutrition and health of people around the world, including China.

The area under wheat in China, over the last four years, was about 23.5 million ha, and it is not likely to expand [38]. At the same time, self-sufficiency in grains is a policy that was in China officially introduced in 1996, and has been implemented thereafter. In China it is a political imperative which is still not fulfilled [36].

The dough rheological properties of wheat genetic resources in China have greatly improved from 1986, although the rate of improvement is slow. However, flour quality in the form of protein content has not markedly improved. Therefore, demand for improved wheat quality in China is growing, and the Chinese grains industry is paying increased attention to produce grains of higher quality. China has the potential to increase its cereal output and it should produce more higher quality wheat to meet special demand [34, 41].

It was shown that wheat flour with high grain protein concentration and high mineral nutritional value can be produced by using specific genotypes, grown in specific environment. Their interaction (GEI) plays an important role in the final expression of grain yield and quality. In this paper, such genotypes are described, and placed at disposal.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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