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(RESEARCH ARTICLE)



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## The effects of wheat cultivar blend on the content of essential grain minerals

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#### Abstract

In western Europe, not so long ago, wheat was frequently grown in a blend (mixture) of two or more cultivars, which was not common in southeast European countries. However, in recent times again growing wheat cultivars in a blend become more acceptable. Why? There are several advantages to this practice. In a blend, we have the possibility to combine different characteristics of two or more cultivars. For instance tolerance to different diseases, lodging tolerance, to combine characters in negative relation (for instance high yield and good grain protein content), and finally nutritive value of wheat, especially when essential minerals are in question. The last one will be the subject of this communication. Seven high protein wheat lines and one registered cultivars are sown in sixteen wheat 1:1 blends, and the effect of blending was studied with an intention to get a higher concentration of essential minerals and better nutritive value of wheat flour.

Keywords: Wheat blend; Nutritive value; High protein; Essential minerals

#### 1. Introduction

Although recently the average crop yield raised up tremendously, this enhanced crop production proved to be unsustainable as it degrades soil, water, and environment, caused dramatic loss of biodiversity and associated traditional knowledge.

In 1936 the article "Modern miracle men" was published [1]. This article attracted much attention and was even submitted for discussion to the US Senate: "The alarming fact is that foods – fruits, vegetables, and grains - now being raised on millions of acres of land that no longer contains enough of certain needed minerals, are starving us - no matter how much of them we eat!"

It is well known saying of Nobel Prize-Winner Linus Pauling: "You can trace EVERY sickness... and EVERY ailment to a mineral deficiency." However, the diet of approximately three billion people worldwide is nutrient deficient. While grain yield has increased over time, the concentrations of all minerals except calcium have decreased. [17].

Agroecology is making agriculture, more productive and sustainable, resilient to natural stressors such as disease, pests, drought, wind, and salinity, and to human-constructed stressors such as chemical inputs, economic cycles, and trade barriers. If we intend to replace chemical inputs with ecological processes and regulations, the biodiversity of cropped plants should be a key leverage for agroecology [2].

Scientists from Western Canada [9], with intention of yield stabilizing across environments, control of air-born diseases, and manage pest populations, performed an experiment with five wheat cultivars and 20 their two-way and

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three-way blends, all this in four conventional and two organic environments. Cultivar mixtures may increase yield (3.3 to 14.1 %) across environments, control air-borne diseases, and manage pest populations in both conventional and organically managed systems, found them.

Half a way to the solution could be sowed by cultivar blend (mixture) again, what was, until recently, a common practice. There are at least three, even five, advantages that cultivar blend can provide [5]:

- Stabilization of yield due to larger genetic diversity, and better genotype by environment interaction.
- Compensation effects a stronger variety compensates for a weaker or injured variety.
- Better disease control due to more different resistance genes involved management of multiple disease tolerance [3], [4], [5], [6], [7], [8], [9], [10], [11], [15], [16].
- Better protection from insect attacks [14].
- Combine different characteristics of cultivars. For instance: extreme quality, tall, to lodging sensitive and low yielding plant, with supporting semidwarf, high yielding wheat, the characters which are in negative relation [12].

There was the report that the positive cultivar mixture effects on disease and yield remain despite large diversity in wheat height and earliness [22], [23].

Recently more attention is connected to nutritive value, more precisely to the grain mineral content of wheat grain. Until now there is no evidence of mineral content testing in such blends.

#### 2. Material and methods

A randomized complete block with three replicates per sample was conducted in Krizevci, Croatia (46°00′ N, 16°32′ E, at 140 m above sea level) under rainfed conditions (723 mm in vegetation period of wheat). For that period minimal monthly temperature was 1.1 °C and maximal monthly temperature 19.5 °C.

Besides common NPK fertilizer (250 kg ha<sup>-1</sup> 7:20:30), the two wheat nutrition was done with KAN 27% N (on March 15, with 120 kg ha<sup>-1</sup>, and on April 20 with 100 kg ha<sup>-1</sup>). The addition of S fertilizer and humic acids with organic fertilizer Energen Akva (0,25 l ha<sup>-1</sup>) was applied on May 22. For plant protection, the lowest concentration (0.000 000 125 ppm) of the gastric pentadecapeptide BPC-157 was used for leaf treatment [18], and 2% stone powder Ekorast for seed and leaf treatments were applied several times [19]. Low dose of fungicide Elatus Era (0,8 l ha<sup>-1</sup>, on April 20) and Artea Plus 0,5 l ha<sup>-1</sup> + Condi Agro (7 l ha<sup>-1</sup>) on May 20 were applied. They were no attack of plant diseases and the effect on grain yield was as never before - wonderful.

#### 2.1. Metals determination method

- A sample of 0,5 g of flour on a 14 % moisture basis and after <0,48 mm sieve is weighed and placed in 10 ml sulfuric acid and 2 ml perchloric acid and keep it overnight.
- Next day the digestion tubes are heated on over 260 °C confirmed when the liquid becomes one color.
- After wet digestion the reading is made on an AAS (PinAAcle 900F instrument).
- Metal concentrations are given in mg kg<sup>-1</sup> of crop dry weight.
- After data analyzed with descriptive statistics (ANOVA) the results are displayed in tables and graphs.

Grain protein was determined by NIRS method measurement on DICKEY John on a dry weight basis.

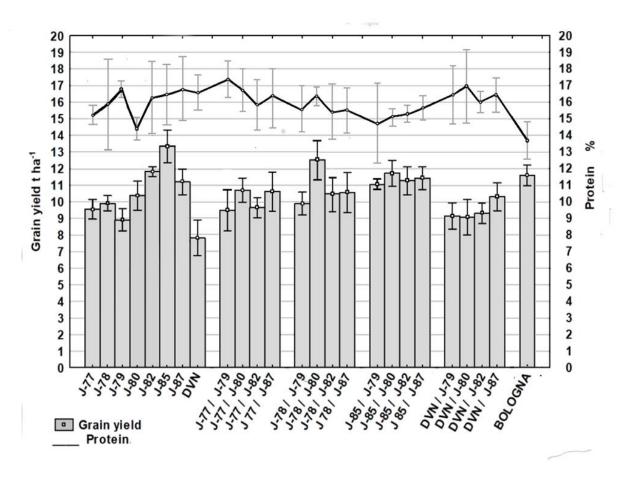
#### 3. Results

The climate in the 2020 growing season was optimal, and the grain yield (8 -13 t ha<sup>-1</sup>) and grain protein content (13 - 17 %) were as high as never before. Fig. 1.

We believe that it was the cumulative effect of optimal climate, no disease attack, low need for herbicide application, new technology applied, and of course superior new germplasm [13]. The average grain yield for eight germplasms (10 363 t ha<sup>-1</sup>) was similar to the grain yield of 16 of their blends (10. 445 t ha<sup>-1</sup>). The best pure line J-85 (with average grain yield 13 336 t ha<sup>-1</sup>) was better than the best blend J-78/J-80 (12 512 t ha<sup>-1</sup>). It means that these pure lines in blends 1:1 can not improve grain yield. However, for grain yield check cultivar Bologna (11 519 t ha<sup>-1</sup>) was significantly lower.

A similar case was with grain protein: nearly the same average values for 6 pure lines (16.03 %), and an average of 16 blends (16.12 %). The best pure line with 16.89 %, was not significantly better than the best blend J-77/J-79 (17.38 %). Check cultivar Bologna (13.87 %) was also significantly lower in grain protein.

For instance: In grain yield, as well as Fe and Cu mineral, the best was germplasm J-85. At the same time this germplasm has the lowest Zn content in grain. (Tab.2.)



**Figure 1** Grain yield and protein content of eight superior wheat cultivars and sixteen of their 1:1 blends, compared with check cultivar Bologna.

The wheat germplasm tested had rather good tolerance to prevalent diseases and good looking hard red type grain. In one replication, ever till now, the highest grain protein was recorded (20.75 %).



**Figure 2** Disease-free wheat plants (example line J-85) a few days before harvest, and the highest grain protein content (>20%), the example from the best replication.

As it was well known that cultivar with a specific accumulation of Zn and Fe exists, the nutritive value of wheat regarding minerals are also tested for six more important minerals: Mg, Ca, Mn, Fe, Zn and Cu. However, in this short preliminary review, the graphic presentation for only two of them Fe and Zn are given. (Figure 3 and 4).

When grain minerals were compared some of germplasm (J-79) had significantly higher mean values for Ca, and Zn. While the other (J-85) was the best in Fe and Cu. (Table 2.)

It is important that in eight pure lines and 16 blends, the variation between low and high values for specific mineral content was significant, and the germplasm could be used as a source for breeding for increased mineral content.

It is important, there was no genotypic superiority in all mineral absorption, for instance: J-85 was superior in Fe absorption, and J-79 in Zn absorption (see Fig. 2 and 3). The effect of cultivar blending on mineral content till now was not examined, nor reported in other papers.

In 1:1 blends there is no special superiority in any characters recorded. More additional testing, perhaps in three, or four-component blend is necessary and will show the additional advantage of blending cultivars. Perhaps this optimal climate and record-high yield ever affects the final results, and the test should be repeated.

Or another example: the germplasm J-79 has the highest grain protein content, as well as Ca and Zn minerals in grain, but has the lowest Cu mineral. (Tab.2.).

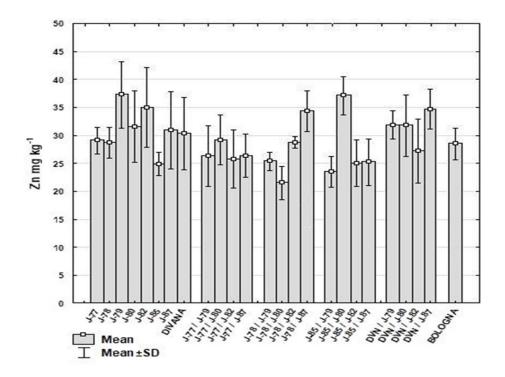


Figure 3 Grain Zn content in eight superior wheat cultivars and sixteen of their 1:1 blends, compared to the check cultivar Bologna

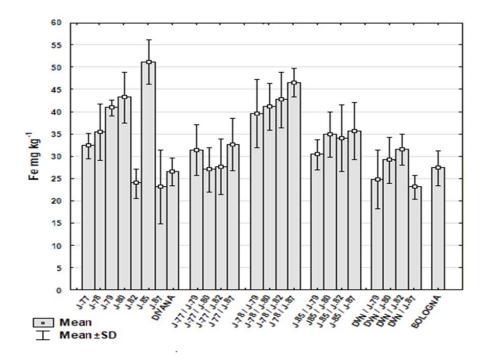


Figure 4 Grain Fe content in eight superior wheat cultivars and sixteen their 1:1 blends, compared to the check cultivar Bologna

From Table 2 as well as from Figures 3-4, you can see an absence of any regularity, on which the composing of wheat lines in blend could be done. It means more research in this direction will be needed.

Mineral	Correlation coefficient - r				
Millerai	Grain yield	Grain protein			
Fe	0.553***	0.783***			
Zn	0.617***	0.909***			
Mn	-	0.613***			
Са	-	0.528***			

**Table 1** Significant correlation coefficients of some grain mineral and grain yield, and grain protein content.

Level of significance: \*\*  $p \le 0.001$ 

Our data consent with the results of the Fe and Zn significant correlation with grain protein determined earlier [24].

From Table 2. as well as from Figures 3-4, you can see an absence of any regularity, on which the composing of wheat lines in blend could be done. It means more research in this direction will be needed.

**Table 2** The mean and range of grain yield, grain protein and essential minerals for eight germplasms and their sixteenblends in relations to check cultivar Bologna – Križevci, 2020.

No	Component -	Cultivar		Cultivar blend			Bologna -	
		Mean	Range	Low -high	Mean	Range	Low - high	Check
1	Grain yield - t ha <sup>-1</sup>	10 363	7 831 - 13 336**	Divana - J-85	10 445	9 071 - 12 512**	DVN/J-80 - J- 78/J-80	11 591
2	Grain protein - %	16.03	14.48 - 16.89 **	J-80 - J-79	16.12	14.85 - 17.38 **	J-85/J-79 - J- 77/J-79	13.87
3	Mg - mg kg <sup>-1</sup>	1 791	1480 - 2 133 **	J-80 - Divana	1 993	1 200 - 3 080 **	J-78/J-79 - J-85/J-80	1 686
4	Ca - mg kg-1	196.5	169.7 - 221.9 **	J-82 - J-79	206.9	171 - 235 **	J-78/J-79 - J- 78/J-80	218.4
5	Mn - mg kg-1	39.1	33.1 - 49.1 **	J-82 - J-78	38.48	30.1 - 48.4 **	DVN/J-79 - J- 78/J-87	38.6
6	Fe - mg kg <sup>-1</sup>	33.1	19.6 - 57.6**	J-87 - J-85	33.2	23.1 - 46.5 **	DVN/J-87- J-78/J87	27.3
7	Zn - mg kg <sup>-1</sup>	30.2	24,9 - 37.3**	J-85 - J 79	34.7	21.5 - 37.1 **	J-78/J-80 - J-85/J-80	31.8
8	Cu - mg kg-1	5.79	4.43 - 6.77**	J-79 - J-85	5.98	4.77 - 6.87 **	J-77/J-82 - DVN/J-79	6.71

#### 4. Post Scriptum

The least expensive and most effective means of increasing protein production and upgrading its quality is through the improvement of wheat grain protein. Wheat, therefore, has the major contribution of any single crop to world protein production. At the same time, wheat is the number-one source of calories in the American Diet.

After more than 30 years of high protein wheat breeding (based on high protein germplasm developed in Nebraska University by V. A. Johnson and J. W. Schmidt), the grain yield was rather low. Now, this is also a report of success in breeding for high grain protein and breaking the negative relation between grain yield and grain protein in wheat.

But it is a pity since about 1990 an allergy to wheat gluten called Celiakia disease starts to spread rapidly and consuming non-gluten bread is recommended. And suddenly, bread, in all its various forms, the most widely consumed food since old Egypt time (10 000 years before Christ), old Greek and old Roman time as energy source food are not recommended anymore. Just to remember: To supply energy for the fight, the old roman soldier each day were supplied with about 1 kg of bread. It is a well-known phrase of the Roman poet Juvenal: "Panem et circenses".

In recent times, scientists [6], [20], proposed that glyphosate, the active ingredient in the herbicide, Roundup(®), is the causal factor in this epidemic. The glyphosate cause an imbalance in gut bacteria and inhibit cytochrome P450 enzymes, important for detoxifying environmental toxins, activating vitamin D3, catabolizing vitamin A, and maintaining bile acid production and sulfate supplies to the gut. It is interesting, and well documented by the graph in the mentioned paper: Celiakia disease appears at the same time as Roundup, and frequency grows at the same speed as herbicide Roundup spread in agricultural production. Glyphosate residues in wheat and other crops are likely increasing recently due to the growing practice of wheat desiccation just prior to the harvest, points the scientists. However, the medical profession is rather stubborn, not willing to accept these findings.

### 5. Conclusion

- Significant variation in grain yield, grain protein content, and mineral content for six elements (Cu, Ca, Fe, Mg, Mn and Zn.) in eight wheat germplasm studied were determined. It means that wheat blends with a higher content of analyzed traits, in fact the crop of higher nutritive value, could be selected.
- In this way, the more valuable nutritive wheat cultivars could be breed.
- In spite of evidence of opposite relation between grain yield and grain protein content in wheat, high grain yield, and high grain protein germplasm were developed by conventional plant breeding.
- It is proven by many other authors, that gluten, the most important component for high-quality bread shape, elasticity, and taste, is not the cause of Celiac disease, and the real fact, the glyphosate, a component of Roundup herbicide should be forbidden all over the World. like in California. (See Post Scriptum.)
- Good adsorption of all minerals is not a general characteristic. Certain cultivars prefer certain but different minerals.
- Finally, nutritive more valuable wheat blend with increased mineral content could be produced in near future.
- By growing cultivar blend (at least three, or better four components), it would be possible to improve the nutritive value (especially for minerals Fe and Zn).

More work on combining more nutritive valuable blend, as well as low content of phytates is needed.

#### **Compliance with ethical standards**

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#### Disclosure of conflict of interest

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

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