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THE IMPACT OF ZnO NANOPARTICLES APPLICATION ON YIELD COMPONENTS OF DIFFERENT WHEAT GENOTYPES

SUMMARY

The properties of zinc oxide nanoparticles (ZnO NPs) and their use have been shown as prominent for application in agriculture since it can bring certain benefits in agricultural production. The objective of this study was to estimate the impact of seed priming with ZnO NPs on yield components, plant height and spike length on wheat. In order to estimate the effects of ZnO nanoparticles on yield component, four winter wheat genotypes namely, NS Pobeda, NS Futura, NS 40S and NK Ingenio were selected. Seeds of each wheat genotypes were primed with different concentrations of ZnO NPs (0, 10, 100 and 1000 mg l^{-1}) for 48 h in dark box by continuous aeration. Primed seeds were after sown in soil pots with 60-70% moisture contents during the till maturity. Considerable improvement was observed in plant height and spike length which increased with rates of ZnO NPs compared to the control. At rates of 10 mg l^{-1} ZnO NPs, the greatest increases in plant height and spike length were observed for genotypes NS Pobeda and NS Futura. At 100 mg l^{-1} ZnO NPs, the greatest increase for both traits was observed for genotypes NS 40S and NK Ingenio. Maximum rates of ZnO nanoparticles reduced both observed traits of wheat. The result indicated that ZnO nanoparticles can significantly increase plant height and spike length of wheat, but also plant response to ZnO nanoparticles significantly depends on concentration of application, as well as from wheat genotype.

Keywords: *Triticum aestivum* L., yield components, zinc oxide, nanoparticles, correlation.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the World, grown on over 220 million hectares and representing 26% of the total harvested area (Popović, 2010; USDA, 2015). Wheat is a food source for over

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seven billion of people and is a major food item in many countries of the world (Pavićević, 1991; 1992; Popović, 2010; Dončić et al., 2019). According to FAO (2017), all types of wheat in the Republic of Serbia are cultivated in the about 588.820 ha. In addition to the main product, grain, significant quantities of by-products are remaining in the field, in warehouses and in industrial production and processing (Rakaščan et al., 2019). In 2016, Serbia had a very good wheat crop of over 2.89 million tones, which had harvested from 595,000 ha. The initial wheat stock in 2018 was 218,000 tonnes with 3.11 million tonnes of wheat, available for consumption. Wheat needs in grain, in Serbia were about 1.55 million tonnes. For domestic consumption it required 1,200,000 tones, for supplies 200,000 tonnes and for seed production 150,000 tonnes, while the rest was intended for export (about 1.34 million tonnes), (Gulan, 2017). Growing demands for wheat rising approximately 2% per year, which is twice of the current gain rate in genetic yields potential, hence plant breeders have to put many efforts to improve the grain yield of wheat (Reynolds et al., 2001; Ljubičić et al., 2015).

Grain yield in wheat is a complex polygenic trait influenced by many components that interact in a multiplicative manner (Slafer and Calderine, 1996; Popović, 2010). Since that increment in one yield component might have positive or negative effect on the other components, a large number of genetic studies have been made to investigate the genetic basis of these traits of wheat. Breeders frequently use yield components to improve the grain yield, despite the fact that these components compensate each other in practice and increase in one cause a decrease in the other (Foroozanfar and Zeynali, 2013; Ljubičić et al., 2015; Djuric et al., 2018; Biberdzic et al., 2020). A high and stable wheat yield can be achieved only when it is based on the cultivation of varieties of high genetic yield potential with the application of intensive agro-technology. Producers of wheat in our country have a wide range of domestic varieties that are highly yielding, not genetically modified (Popović, 2010; Popović et al., 2011; Glamočlija et al., 2015; Lakić et al., 2015; Maksimović et al., 2018; Milivojević et al., 2018; Rakaščan et al., 2019; Rakić et al., 2020) and adapted to our climate. Recent studies suggest that nanotechnology possess great potential to be successfully used in agriculture for different purposes and various conditions. Among different nanoparticles (NPs) in use, zinc oxide nanoparticles (ZnO NPs) are the most widely used, since they can bring certain benefits in agricultural production. It has been reported that zinc oxide nanoparticles (ZnO NPs) could promote seed germination, improve zinc deficiencies, root volume, increase plant growth and yield traits, as a biomass, stem height and spike length in wheat (Munir et al., 2018; Rizwan et al., 2019). On the other side, different methods have been developed for the application of ZnO NPs to crop, such as in the soil application, foliar application and by seed priming method. Seed priming method has been shown as a simple, cost effective and beneficial especially under adverse environmental conditions (Mahakham et al., 2017). Seed priming method can also improve the growth quality and production of crops (Munir et al., 2018).

Therefore, in the present study seed priming method was selected to evaluate the effect of ZnO NPs on yield traits, plant height and spike length in four winter wheat varieties. Assessing the impacts of NPs on these traits of wheat will provide new insights into the application of nanotechnology in improving yield traits of wheat.

MATERIAL AND METHODS

The present study was carried out at the experimental field in the greenhouse facility available in the University of Novi Sad, in Serbia, during the 2018-2019 growing season. The experimental material in this study was comprised of 4 winter wheat genotypes, namely, NS Pobeda, NS 40S, NK Ingenio and NS Futura. Seeds of each wheat genotypes were primed with different solutions containing appropriate concentrations of ZnO NPs (0, 10, 100 and 1000 mg L^{-1}) for 48 h in dark box by continuous aeration. Ten primed seeds of wheat were after sown in soil pots filled with 5.0 kg of soil, with 60-70% moisture contents during the experiment. The trial was set up according to the completely randomized design with three replications of each treatment on chernozem soil. To avoid the micronutrient deficiency in plants, the chernozem soil used for conducting trial was collected from the agricultural field, mainly used to grow wheat with usual agrotechnics measure was applied. At the stage of full maturity, ten plants from each replication of winter wheat genotypes were selected for recording data for plant height and spike length. Average values of three replication trait analysis were used. Components of phenotypic variance were calculated based on the following statistical parameters: the mean value ($\overline{X}$), the coefficient of variation (Cv) as an index of relative variability of the trait and analysis of variance. Significant differences between the mean values were estimated by LSD - test values. Pearson correlation coefficient (r) was used as a measure of correlation of NDVI with aboveground biomass and grain yield of wheat. All statistical analyses were carried out using software STATISTICA, version 13 (StatSoft Inc., USA). For the calculation of the yield components, we used a basic statistical method comprising of the following: for calculation of variation degree of yield coefficient of variation (Cv) was applied in equation: $Cv=b\bullet100/\overline{X}$.

RESULTS AND DISCUSSION

The yield per unit area is the result of the action of factors of variety in interaction with environmental factors. The yield is largely dependent on the genetic potential and considerably vary primarily as a result of agro-ecological conditions during the growing season (Popović et al., 2011; Vasileva, 2016; Đekić et al., 2017; 2018; Jaćimović et al., 2017; Milivojević et al., 2018; Terzić et al., 2018; Ugrenović et al., 2018; Rajičić et al., 2019; 2020; Vasileva and Vasilev, 2020). The studied yield components, plant height and spike length are complex variable traits which expression is largely depended on the environmental factors (Zečević et al., 2008). Within treatment the investigated wheat cultivars showed significant differences in the mean values of plant height and spike length and varied on overall basis.

Plant height. The results of plant height of four winter wheat varieties are presented in Table 1. Plant height increased with increasing ZnO NPs concentration applied. The greatest increase in plant height was found at 100 mg L^{-1} ZnO NPs for genotypes NS 40S (89.33 cm) and NK Ingenio (86 cm), while genotypes with greatest increase at 10 mg L^{-1} ZnO NPs applied were NS Futura (89 cm) and NS Pobeda (86 cm). Low values of plant height were observed at control plants, whereas the lowest values of these parameters were found in maximum concentration of ZnO NPs. It could be noted that ZnO NPs treatment had a twofold impact on wheat height. In general, wheat plants had advanced elongation under lower ZnO NPs concentration treatment (up to 100 mg l^{-1}), while the enriched concentration of nanomaterials diminished plant growth. Given results revealed that different treatments influenced the differences in plant height.

The plant height is considered a quantitative and variable trait which expression highly depends on the environmental factors. This is confirmed by values of the coefficient of variation which ranged from 0.70 % to 3.9 % The lowest variability was observed within treatments of 100 mg l^{-1} ZnO NPs (Cv=1.0%) and 10 mg l^{-1} ZnO NPs (Cv=1.2 %). The highest variation coefficient was observed 1000 mg l^{-1} ZnO NPs (Cv=3.1%), Table 1.

Table 1. Mean values and Cv for plant height of examined wheat varieties.

Parameters	**Environments**							
Treatments	**K - 0 mg l^{-1}**		**10 mg l^{-1}**		**100 mg l^{-1}**		**1000 mg l^{-1}**	
Varieties	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv(%)
Pobeda	79.67	0.7	86.00	1.2	85.00	1.2	67.33	3.1
NS40S	75.00	1.3	83.67	1.8	89.33	0.7	63.67	3.6
Ingenio	74.67	3.9	82.67	0.7	86.00	1.2	65.33	3.9
Futura	75.00	2.7	89.00	1.1	88.00	1.1	64.33	1.8
$\overline{X}$	76.08	2.2	85.33	1.2	87.08	1.0	65.17	3.1

$\overline{X}$ - mean value (cm); Cv- coefficient of variation (%)

*Environmental labels represent control (K), and 10, 100 and 1000 mg l^{-1} primed concentrations of ZnO NPs applied.

Highest Cv of the plant height tells how consistent influence of the treatment was on the single plant. Due to CVs, high confidence in the positive impact of ZnO NPs to the wheat height could be underlined for 10-100 mg l^{-1} concentrations. Differences are caused by different plants response to environmental factors (treatment) with the experiment was performed. Overall, it is noticed that the greatest variability of stem height was obtained for the highest concentration of applied ZnO NPs of all varieties. This points out an increased interaction of genotype and the environment in terms of the more inconvenient conditions, compared to favorable conditions with lower levels of the applied concentration. According to Popović (2010) and Petrović et al. (2017) in the process of breeding wheat genotypes tolerant to abiotic stress, caused by unfavorable conditions, one of the selection criteria would be reducing genotype environment interaction for this trait, at higher mean values of trait.

According to ANOVA, the components of phenotypic variance were analyzed and significant differences in the average values for plant height was observed due to treatment (Table 2). The ANOVA showed that plant height was significantly affected by the treatment because of significant variance at 1% level, which explained 73.3 % of the total (G + E + GEI) variation. Variation was not significant when genotype was considered as the main effect, but was more obvious in GEI (genotype/environment interaction). Lower impact belongs to genetic/environment interaction (22.3 %) of the total sum of squares lower and non-significant impact belongs to genotypes (4.4 %), Table 2. These results are in agreement with previous study reported by Zečević et al. (2004) and Zečević et al. (2008).

Tab.2.ANOVA for plant height mean values for 4 wheat varieties in 4 treatments.

Effect	**SS**	**DF**	**MS**	**F**	**p**	**LSD 0.01**	**LSD 0.05**
Intercept	284284.1	1	284284.1	2509.773*	0.000000		
Genotype	254.1	3	84.7	0.748^{ns}	0.531698	11.848	8.825
Treatment	4280.4	3	1426.8	12.596*	0.000013	11.849*	8.826*
GEI	1302.8	9	144.8	1.278*	0.286478	23.695*	17.648*
Error	3624.7	32	113.3				

ns - non significant; *– Significant at P < 0.05 probability level, ** – Highly significant at P < 0.01 probability level; SS - Sum of squares; DF - Degree of freedom; MS - Mean square; F- F values

Spike length. The results of the spike length of four winter wheat varieties are presented in Table 3. The results revealed that spike length increases with increasing ZnO NPs concentrations in the priming solution, comparing than control. Depending on genotype, the highest increase in spike length was found with doses of 10 mg l^{-1} and 100 mg l^{-1}NPs applied, whereas the lower values of this parameter were found on control plants. The greatest increase in spike length within application dose of 10 mg l^{-1} ZnO NPs for genotypes NS Futura (11.30 cm) and NS Pobeda (9.87 cm), while genotypes with greatest increase at 100 mg l^{-1} ZnO NPs applied were NS 40S (9.80 cm) and NK Ingenio (11.07 cm). Low values of spike length were observed at control plants, and the lowest were found in highest concentration of ZnO NPs.

The present results indicated that different treatments influenced the differences in spike length. According to Zečević et al. (2008), spike length is genetically controlled, but it highly depends on environmental factors.

Table 3. Mean values and Cv for spike length of examined wheat varieties.

Parameters	**Environments**							
Treatments	**K - 0 mg l^{-1}**		**10 mg l^{-1}**		**100 mg l^{-1}**		**1000 mg l^{-1}**	
Varieties	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)
Pobeda	9.33	0.6	9.87	0.6	9.80	1.0	8.67	2.4
NS40S	10.20	1.7	11.10	0.9	11.20	0.9	9.73	0.6
Ingenio	9.97	0.6	10.73	0.5	11.07	0.5	9.33	3.3
Futura	10.93	0.5	11.30	0.0	11.27	0.5	9.87	0.6
$\overline{X}$	10.11	0.9	10.75	0.5	10.83	0.7	9.40	1.7

$\overline{X}$ - mean value (cm); Cv- coefficient of variation (%); *Environmental labels represents control (K), and 10, 100 and 1000 mg l^{-1} primed concentrations of ZnO NPs applied.

Beside its its variable nature, the coefficient of variation ranged from 0.01% to 3.3 %. The lowest variability was observed within treatments of 10 mg l^{-1} ZnO NPs (Cv= 0.5 %), while the highest variation coefficient was observed 1000 mg l^{-1} ZnO NPs in genotype NK Ingenio (Cv=3.3%). Wheat genotype NS Futura expressed the highest homogeneity of this yield component across all treatments (Cv=0.01%), Table 3.

In general, the greatest variability of spike length was obtained for the highest concentration of applied ZnO NPs which fits within the lowest mean value for certain varieties. This indicated that in more inconvenient conditions an increase of genotype environment interaction is expressed. Analysis of variance identified the importance of sources of variation in the experiment. According to ANOVA, partitioning the total sum of squares for trial revealed that all effects (treatment, genotype and genotype/environment interaction) had been statistically highly significant and agronomically important.

Table 4. ANOVA for spike length mean values for 4 varieties in 4 environments.

Effect	**SS**	**DF**	**MS**	**F**	**p**	**LSD 0.01**	**LSD 0.05**
Intercept	5067.630	1	5067,6	352530.8*	0.000000		
Genotype	13.772	3	4.591	319.3*	0.000000	0.133*	0.098*
Treatment	16.065	3	5.355	372.5*	0.000000	0.134*	0.099*
GEI	0.833	9	0.093	6.4*	0.000035	0.267*	0.199*
Error	0.460	32	0.014				

ns - non significant; *– Significant at P < 0.05 probability level, ** – Highly significant at P < 0.01 probability level; SS - Sum of squares; DF - Degree of freedom; MS - Mean square; F- F values

The ANOVA showed that phenotypic variation of spike length was significantly affected by treatment which explained 52.3 % of the total variation, genotype explained 44.9 % of the total variation, while lower impact belongs to genetic/environment interaction (2.7 %) of the total sum of squares (Table 4).

Obtained results were expected since it is well known that many quantitative wheat components express different amounts of variability caused by variation, as well as due to different treatment or environmental factors, but also of the presence of genetic variability. These results are in agreement with previous reported by Petrović et al. (2007).

Analysis of correlations for the 2018/2019 season. It was observed the significant positive relationship between yield traits, plant height and spike length of wheat (r = 0.34*), Table 5.

Table 5. Pearson's correlation coefficients between examined traits

Parameter	**Genotype**	**Treatment**	**Plant height**	**Spike length**
Plant height	0.16^{ns}	-0.50*	1.00	0.34*
Spike length	0.56*	-0.54*	0.34*	1.00

ns - non significant; * Significant at P < 0.05 probability level

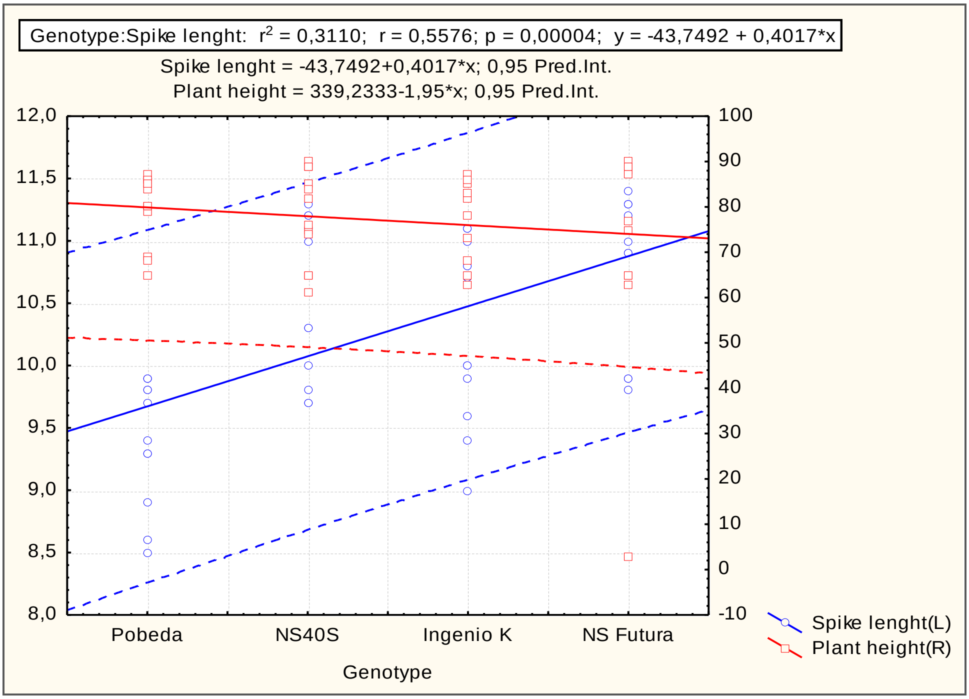

Figure 1. Effect of genotype of wheat plant height and spike length

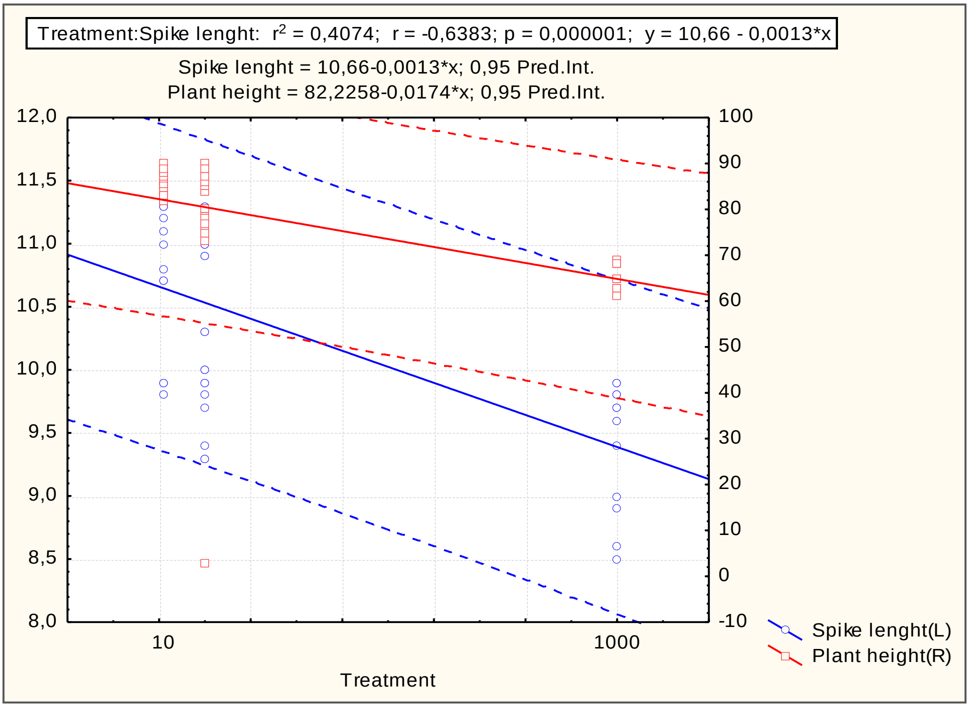

Figure 2. Effect of treatment of wheat plant height and spike length

The values of the correlation coefficients can be explained by the plant response to the applied treatments. This means that favorable conditions in the experiment caused increased values of plant height and spike length. Our findings in this study support the research of Banjec et al. (2000). The authors state highly significant positive correlations were observed between the length of the spikes and the number of grains per spike. Positive but very weak correlation manifested between plant height and grain weight per class (0.104), as well as between plant height and class mass (0.123).

CONCLUSIONS

Based on the obtained research, it can be concluded that the seed priming with different concentrations of ZnO NPs might be a suitable method to improve, plant height and spike length of wheat. Plant height and spike length varied widely within different treatment and different wheat genotypes. Seed priming with 100 mg/L ZnO NPs provided, the highest plant height for the Pobeda and Futura varieties, while under 100 mg/L treatment the largest values was noticed for Ingenio and NS40S varieties. Similar results, was obtained in case of spike length. The levels of the mean values of the analyzed yield components were the lowest in condition of maximum amount of ZnO NPs applied. In order to achieve a stable wheat yield component, appropriate measures of applying ZnO NPs should be applied. Overall this results showed that seed priming might be an effective method for improve important yield components of wheat and could provide valuable information for fertilizer industries in planning production of nanofertilizers based on ZnO NPs for plant nutrition.

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