THE EFFECT OF COVER CROPS ON THE CONTENT OF PROTEIN IN GRAIN OF SWEET MAIZE

Željko Dolijanović[1], Vesna Dragičević[2], Milena Simić[2], Snežana Oljača[1], Dušan Kovačević[1], Biljana Janošević[1]

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
[2]Maize Research Institute „Zemun Polje“, Belgrade, Serbia

Corresponding author: dolijan@agrif.bg.ac.rs

Abstract
The study was conducted in the experimental field of the Maize Research Institute Zemun Polje, Serbia, during 2013/14-2014/15. The experiment was established as a block design with four replications. As winter cover crops-CC (factor A) the following plants were grown: CV–common vetch (*Vicia sativa* L.), FP-field pea (*Pisum sativum* L.), WO-winter oats, (*Avena sativa* L.), FK-fodder kale (*Brassica oleracea* (L.) convar. *acephala*), two mixture variants of legume crops with oats (CV+WO and FP+WO) and two control treatments: a variant in which the surface was covered with dead organic mulch (DOM) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (TV). Green biomass of the cover crops was incorporated in the soil, immediately after, half of the elementary plot was infested with bio-fertilizer (BF) - Uniker (mobilizer of nutrients) in an amount of 10 l ha^{-1} (factor B), which contains the strains of cellulolytic and proteolytic bacteria to support the mineralization of entered crop residues. The seeds of sweet maize ‘ZPSC 421*su* (FAO 400) were sown at the arrangement of 70 cm between rows and 22 cm between plants in the row (65,000 plants per ha). Preceding crop in both years was winter wheat. The kernel protein content was measured on infrared analyser. The data were processed by ANOVA. The investigated factors (CC and BF) showed significant effect on protein content in sweet maize kernel in both years. As it was expected, the greatest impact on protein content was exhibited in leguminous species grown alone, or in mixtures with oats, particularly in the dry, 2015. Small grains intercropped with legumes obtained higher values of protein content than small grain grown as monocrops.

Keywords: sweet maize, content of protein, microbiological fertilizer.

Introduction

Over the last decade, substantial effort has been devoted to implementing alternative practices in agriculture (conservation and minimal tillage, permanent soil cover and diverse crop rotation) under sustainable and organic production because a combination of both strategies could have synergistic effects and further improve soil quality. From this point of view it is important to examine relations between applied cropping practices and nutritional value of produced crops. The overall strategies include growing of healthy plants with good defense capabilities, stressing pests and enhancing populations of beneficial organisms (Magdoff, 2007). From that point, sustainable agriculture includes combination of different crops at the same field and application of organic and microbiological fertilizers, enabling better utilization of space and nutrients, with lower inputs. On the other hand, organic farming means production of high quality food or raw material with maintaining and improving the soil quality. It is based on controlled input of allowed agrochemicals that are mainly natural products, like plant extracts, fertilizers originating from decomposed organic products or manure from organic cattle production (Dragičević et al. 2013). The application of reduced or no tillage practices in sustainability or organic production often increases problems related to weed control and crop nutrition (Armengot et al. 2015). One way to tackle these issues is the inclusion of cover crops in the crop rotation. Cover crops are implemented between two main crops and are known to provide various ecological services in agro-ecosystems, such as protection against soil erosion (Hartwig and Ammon 2002), reduction of nutrient losses (Janošević et al. 2017), improvement of soil and water quality, and to some extent (Dabney et al. 2001), the reduction of weeds and pests (Dorn et al. 2015). In adition, adding nitrogen (N) fixing legume species as a cover crop can improve N nutrition of the succeeding main crop and increase the soil N organic pool (Thorup-Kristensen et al. 2003). Despite these advantages, cover crops are generally not widely used by farmers, mainly due to additional costs and labour requirements. Cover crop effects on productivity, crop nutrition, soil erosion or weed control are variable and depend on cover crop species, soil type, and meteorological conditions. A very large number of plants have been used for cover-crops (Oljača and Dolijanović 2013). The selection of compatible crops depends on their growth habit, land, light, water and fertilizer utilization (Thayamini and Brintha, 2010). These may be divided into two groups, viz., the legumes, or nitrogen-gatherers, and the non-legumes, or those which are sometimes distinguished as nitrogen-consumers. The kind of crop to plant must be determined by the local conditions and the local needs; that is, whether a grass, cereal, legume, or cruciferous plant shall be used, will depend on whether the habits of growth and characteristics of the plant will accomplish the purpose desired. Weather conditions, in particular air temperature during the growing season, have the greatest influence on sweet maize growth, yield and yield quality (Stone et al. 1999). The chemical composition of the grain of the main crop depends to weediness. Cover crops contribute to the reduction of number and fresh weight of weeds in different ways. Incorporation of cover-crop plant material as a green manure is done at the expense of harvestable grain or seed yield, but maximizes the input to the soil of both slowly degraded organic N and secondary chemicals that may affect soil microbiological community structure. Many secondary metabolites of plants are inhibitory to a wide range of pathogenic fungi such as *Fusarium culmorum* and *Rhizoctonia solani* (Lovett, 1991). The concentrations of these metabolites are generally highest in plants at the flowering stage (Chen et al. 2012), and the incorporation of green manure at this stage offers the further advantage that it is unlikely to interfere with the sowing time of the next crop. Bio-fertilizers have an important role in keeping high soil fertility and crop yields increasing (Janošević et al. 2017). The positive impact of microbiological fertilizers is also observed in regards to quality of plant products, as for example increase in lycopene and vitamin C accumulation in sweet maize (Rosa, 2015) and higher glutathione content in maize grain (Dragicevic et al. 2013). Data obtained in this study from field experiments provides valuable knowledge regarding the (i) influences of different type of cover crops and (ii) the applied form of fertilizers (i.e. microbiological) on content of protein in grain of sweet maize.

Material and methods

A field experiment was carried out in 2013/14–2014/15 growing seasons, at the Experimental Field of Maize Research Institute in Zemun Polje near Belgrade (44°52'N; 20°20'E). The soil was slightly calcareous chernozem with 47% of clay and silt and 53% of sand. The soil at 0-30-cm layer were contained 3.22% of organic matter, 0.19% of total N, 1.9% of organic C, 16.2 and 22.4 mg per 100 g soil of available P and extractable K, respectively, 1.38% of total $CaCO_3$ and had pH 7.3. The experiment was established as a block design with four replications. As winter cover crops (factor A) the following plants were grown: CV–common vetch (*Vicia sativa* L.), FP-field pea (*Pisum sativum* L.), WO-winter oats, (*Avena sativa* L.), FK-fodder kale (*Brassica oleracea* (L.) *convar. acephala*), two mixture variants of legume crops with oats (CV+WO and FP+WO) and two control treatments: a variant in which the surface was covered with dead organic mulch (DOM) and traditional variant: after ploughing in the fall plot stayed uncovered during the winter (TV). The cover crops (CC) were sown in the amount: common vetch – 120 kg, field pea – 150 kg, oat – 160 kg, and fodder kale 15 kg per ha, and in mixture relation between legume and oats was 70:30. The plot size was 17.5 m^2. The seeds of the Institute for Forage Crops Institute of Field and Vegetable Crops in Novi Sad was used for planting in both years. The seeds of sweet maize 'ZPSC 421*su* (FAO 400) were sown at the arrangement of 70 cm between rows and 22 cm between plants in the row (65,000 plants per ha).

Preceding crop in both years was winter wheat. The autumn soil preparation (ploughing and seedbed preparation) was performed immediately before sowing, when also soil samples were taken for available N analysis at depths of 0-20 cm and 20-40 cm. Further soil sampling from all CC and control treatments was done in the spring, after CC harvest, as well as after sweet maize harvesting. Before the sowing of CC (autumn) and sweet maize (spring) mineral fertilization was applied in order to obtain 120 kg ha^{-1} N, 90 kg ha^{-1} P and 60 kg ha^{-1} K. The total amount of P and K fertilizer was applied in autumn with mono-potassium phosphate fertilizer (a.m. 0:52:34) and the required N amount was incorporated together with sweet maize sowing (urea 46% a.m). Nitrogen fertilization followed: for non-legume crops and control treatments it was 120, for sole legume it was 80 and for mixture it was 90 kg ha^{-1} N. The remaining 40 or 30 kg ha^{-1} N was considered to be provided by nitrogen fixation. Green biomass of the cover crops was incorporated in the soil, immediately after, half of the elementary plot was infested with bio-fertilizer (BF) - Uniker (mobilizer of nutrients) in an amount of 10 l ha^{-1} (factor B), which contains the strains of cellulolytic and proteolytic bacteria to support the mineralization of entered crop residues. The ears were harvested at the stage of milk maturity of kernels. The schedule of the main works on the experiment is shown in Table 1.

Table 1. Chronology of field operations and length of vegetation period of sweet maize

Cover crops sowing	October, 30	November, 13
	2014	2015
Cover crops sampling	April, 23	May, 12
Cover crops and microbiological fertilizer incorporated	May, 12	May, 21
Sweet maize sowing	May, 20	May, 21
Hand weeding 1	June, 27	June, 22
Hand weeding 2	July, 17	July, 15
Sweet maize harvest	August, 14	August, 21
Length of vegetation period of sweet maize (in days)	86	92

The content of protein, starch and oil in grain was determined after drying in ventilation dryer at 80 °C, on infrared analyser (Infraneo, Chopin Technologies, France). The difference between fresh and dry mass (after drying at 60 °C, 105 °C and 130 °C) referred to contents of free, bulk and chemically bound water, calculated by free energy by sorption isotherm (Sun, 2002):

$$\Delta G = -RT\ln(a_w)$$

where a_w is the relative water content achieved after drying at T (60, 105 and 130 °C), R is the gas constant (8.3145 J mol^{-1} K^{-1}) and ΔG is differential free energy. The obtained data were processed using analysis of variance for two-factorial experiments (ANOVA). Statistical analysis was performed by SPSS 15.0 (IBM Corporation, Armonk, New York, USA) for Windows Evaluation version. For the individual comparisons, the least significant difference (LSD test) was used.

Meteorological conditions

The meteorological conditions during the investigation period are presented in Table 2. The first year investigation characterized by optimal air temperatures, enough of quantity and favourable distribution of precipitation. It has not had an effect on the investigation parameter of sweet maize, expect in traditional variant of growing. One of the many reasons for of introducing alternative systems of growing is the change weather conditions in recent years, such as the case in 2015. Air temperatures were higher and the quantity of precipitation were very lower, a particularly significant lack of precipitation for crop during the critical period for water. Precisely, in drought years, cover crops showed greater efficiency, while microbiological fertilizers affected the increase in quantity but not the yield quality of the main crop.

Table 2. Average air temperatures and precipitation sums from April to September at Zemun Polje

Months	Temperature (°C)		Precipitation (mm)	
	2014	2015	2014	2015
April	13.7	12.9	84.8	19.7
May	17.4	19.1	192.5	97.8
June	21.1	22.1	71.2	31.1
July	23.2	26.4	187.4	7.2
August	22.6	25.7	41.0	56.0
September	18.0	20.2	75.6	73.6
Average/Sum	19.3	21.1	652.5	285.4

Results and discussion

Results about the effects of different cover crops and microbiological fertilizer on the content of protein in grain of sweet maize are presented in Table 3. The investigated factors (CC and BF) showed significant effect on protein content in sweet maize kernel in both years (table 3). As it was expected, the greatest impact on protein content was exhibited in leguminous species grown alone, or in mixtures with oats, particularly in the dry, 2015. Advantages of cover crops are more pronounced in drought years, which is a very important fact in today's weather, because we are witnessing the existence of climate change. Small grains intercropped with legumes obtained higher values of protein content than small grain grown as monocrops (Kadžiulienė et al. 2011). Microbiological fertilizer application in the first and second year of investigation did not increase the protein content in the sweet maize grain, except in the variants with a mixture of leguminous crops and winter oats. In previous studies on the same location (Dolijanović et al. 2012, Janošević et al. 2017) treatment with a microbiological fertilizer had a positive impact on the yield, the chlorophyll content and content of vitamin C in the grain. In investigation with cover crops in Poland, the average protein content of sweet corn kernels was 3.62% FM (Rosa, 2015). The crop following hairy vetch, white clover and Italian ryegrass as well as farmyard manure contained more protein than the remaining catch crops. It was probably due to the fact that nitrogen was more available for corn as it was more rapidly released from farmyard manure, leguminous catch crops (hairy vetch and white clover) and Italian ryegrass. Numerous investigations have demonstrated that incorporation of leguminous catch crops (crimson clover, hairy vetch, common vetch, fodder kale, subclover, red clover) preceeding popcorn and sweet maize resulted in increased N content in kernels compared with growing without cover crops (Salmerón et al. 2011, Kramberger et al. 2014, Rosa, 2015, Janošević et al. 2017). Tejada et al. (2008) cited that protein contents in kernels of maize following oilseed rape and red clover cover crops were similar when the same mineral fertilisation of cover crops had been applied. Since the highest seed yield (Yeganehpoor et al. 2015) and oil, starch and protein contents of corn seeds (Yeganehpoor et al. 2013) were obtained from synchronic cultivation of companion crops with corn, especially red clover, corn plants cultivated simultaneously with clover had also higher oil, starch and protein yields. Kramberger et al. (2014) in their investigation found a decline in N content in maize kernels harvested from an Italian ryegrass-manured treatment compared with the non-manured control and crimson clover manured plots. Błażewicz-Woźniak and Mitura (2004), Błażewicz-Woźniak et al. (2008) observed a positive effect of leguminous biomass on nitrogen accumulation of other vegetable species (onion, parsley, white cabbage).

Table 3. The protein content (%) in sweet maize grain

Treatments	2014			2015		
	BFØ	BF	Average	BFØ	BF	Average
CV	10.92	10.87	10.58	11.68	11.16	11.42
FP	11.23	10.68	10.96	11.54	11.36	11.45
WO	10.68	10.09	10.39	11.56	10.04	10.80
FK	11.08	10.79	10.94	11.18	10.87	11.03
CV+WO	11.14	11.82	11.48	11.32	11.33	11.33
FP+WO	10.22	10.14	10.18	10.58	11.64	11.11
DOM	10.80	10.29	10.55	10.42	10.27	10.35
TV	11.39	10.81	11.10	11.01	11.36	11.19
Average	10.93	10.68	10.81	11.16	11.00	11.08

LSD 0.05	CC^{**}	BF^{**}	$CC \times BF^{**}$
Protein content 2014	0.063	0.032	0.090
LSD 0.05	CC^{**}	BF^{**}	$CC \times BF^{**}$
Protein content 2015	0.021	0.010	0.029

$p<0.01$ very significant (**); $p<0.05$ significant (*); $p>0.05$ no significant (^{ns})

Salmerón et al. (2011) noticed that N content in kernels of corn grown after oilseed rape and winter rape cover crops was significantly lower compared with common vetch although it did not differ significantly from the control without cover crop. Caporali et al. (2004) reported a decline in N content after ryegrass compared with cultivation after subclover and hairy vetch cover crops, the N content being similar to the control. According to investigation of Dragičević et al. (2016), the highest protein content was in field pea + winter oats treatment, followed by common vetch and field pea, what could be explained by the positive response of sweet maize to nitrogen enrichment caused by leguminous plants present in those treatments (Idikut et al. 2009). Leguminous cover crops were important for protein accumulation, together with decrease in available sorption sites and decrease in endergonic reactions, what could in result contribute to the increased nutritional quality of produced kernel (Dragičević et al. 2016).

Conclusions

Based on results, obtained effects of the cover crops, with and without microbiological fertilizer, on content of protein in grain of sweet maize grown on chernozem under rain fed conditions, the following can be concluded: Meteorological conditions during the investigation had an important impact on content of protein in all the cropping systems. In both years, microbiological fertilizer not have an effect on the content of protein, except in mixtures treatments. The greatest impact on protein content was exhibited in variant of mixture legumes and winter oats but the lowest in variant with winter oats and dead organic mulch.

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ASSIMILATION OF NUTRIENTS FROM COMMON WHEAT (*TRITICUM AESTIVUM* L.) DEPENDING ON SOME AGRONOMY FACTORS

Albena Ivanova[1], Margarita Nankova[2], Plamen Chamurliyski[1], Nikolay Tsenov[3], Magdalena Koleva[1]

[1]Shumen University "Bishop Konstantin Preslavsky", College Dobrich, Bulgaria
[2]Dobrudzha Agricultural Institute, General Toshevo
[3]Agronom I Holding, Dobrich, Bulgaria

Corresponding author: plam_s@yahoo.com

Abstract
The genotype specificity in the uptake of the main nutrients according to the fertilization rate was investigated in *Triticum aestivum* L. varieties under conditions of a vegetation experiment. Four fertilization rates were tested: $N_0P_0K_0$, $N_{200}P_{200}K_{200}$, $N_{400}P_{200}K_{200}$ and $N_{600}P_{200}K_{200}$. The response of the varieties to the nutrition conditions provided was studied during several stages of growth and development. The different nutrition regimes used lead to manifestation of the specific abilities of the varieties to take up nutrients. Genotype and mineral fertilization had a higher effect on nutrients uptake than stage of development. During the initial stage of wheat development, the differences established in the uptake of macro elements were greater between the varieties than between the individual fertilization rates. The differences were most evident at maturation. This specificity was well expressed in nitrogen and phosphorus uptake in total biomass and to a lesser extends - in potassium uptake. Varieties Slaveya and Milena had highest amounts of nitrogen uptake in grain.

Keywords: Wheat, mineral fertilization, uptake, nutrients.

Introduction
The variation of grain yield when growing winter wheat cultivars is always different as a result from the strong and constant effect of the environment. (Hagos and Abay, 2013, Tsenov and Atanasova, 2013a). This is the main reason for the various degrees to which the productive potential of each cultivar is being realized. Grain yield depends directly on the expression of several traits which are considered essential for productivity (Yagdi, 2009, Anderson et al, 2011). Varieties and hybrids of different cultures differ not only in yield potential, but also in requirements for nutrients and behaviour fertilization (Clark, 1990, Klimashevskiy, 1991). These differences are referred to the growth or genetic specificity of mineral nutrition. Wheat genotypes adaptive differently to various levels of fertilization and their productivity are connected mainly to the absorption of major macro elements - nitrogen, phosphorus and potassium (Ivanov et al, 1993; Le Gouis et al, 2000). The amount of absorbed nutrients depends on the plant genotype, the energy balance within it, the internal nutritional status, and the needs of the plants and the presence of nutrients in the soil (Misas et al, 2003). Energetically rational varieties absorb high levels of nutrients from fertilizers and soil (Klimashevskiy, 1990). One way to increase the wheat yielding is to create new varieties which grow fastly and have high potential and effective productivity (Dimova et al, 2002). The aim of the study was to characterize genotypic specificity in absorbing the main macronutrients (nitrogen, phosphorus and potassium) depending on the variety and level of fertilization.

Material and methods
In terms of vegetation experiment, 12 varieties of common wheat (*Triticum aestivum* L.) were tested: Sadovo 1 (st), Aglika, Iveta, Bolyarka, Milena, Slaveya, Enola, Kristi, Pryaspa, Todora, Karat and Pobeda (st).