Hrvatski 55
Croatian

2020 15
International
Symposium on
Agriculture
Međunarodni
Simpozij
Agronoma

Proceedings
Zbornik radova

February 16 - 21, 2020
16.-21. veljače 2020.
Croatia / Hrvatska
Vodice, Olympia Sky Hotel

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Zbornik radova

Izdavač Published by	Sveučilište u Zagrebu, Agronomski fakultet, Zagreb, Hrvatska University of Zagreb, Faculty of Agriculture, Zagreb, Croatia
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Tehnički urednici – Technical Editors	Ivan Širić Darija Bendelja Ljoljić
Oblikovanje, prijelom Design, typeset	Martin Šok, www.martinsok.com
Tisak Print	Grafomark d.o.o., Zagreb
Naklada – Edition	40

ISSN 2459-5543

Web page **http://sa.agr.hr**

Službeni jezici Simpozija su hrvatski i engleski.
The official languages of the Symposium are Croatian and English.

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Effects of nitrogen deficiency on some physiological parameters and root traits of three Croatian common bean landraces

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Abstract

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes in the world and the lack of nitrogen in its cultivation can cause major negative changes leading to decrease in leaf protein content, such as RuBisCO, which causes a decrease in photosynthesis capacity and ultimately results in leaf damage and senescence. The aim of this study was to analyze the effect of nitrogen deficiency with 'N-' treatment, Hoagland solution with no added nitrogen, on the chlorophyll content index, 'greenness', digital biomass, quantum yield of photosystem II (Y (II)), maximum quantum yield of photosystem II (F_v/F_m) and the root system traits of Croatian common bean landraces 'Trešnjevac', 'Zelenčec' and 'Biser'. Nitrogen deficiency treatment caused a decrease in the chlorophyll content index and 'greenness', digital biomass and changes in root architecture for all three landraces. The landrace 'Zelenčec' developed the largest root volume, and the landrace 'Trešnjevac' the largest average root diameter, digital biomass and highest chlorophyll content index in nitrogen treatment (N-).

Keywords: Common bean, nitrogen deficiency, root architecture, photochemical reactions

Introduction

For normal physiological functions and development, plants need macro and micro nutrients. The lack of nutrients such as nitrogen, potassium and phosphorus is a big problem in agricultural production (Aleksandrov, 2019). Nitrogen is a major component of amino acids and is important in biochemistry of photosynthetic pigments and co-enzymes (Aleksandrov, 2019; Maathuis, 2009)magnesium, nitrogen, phosphorous, potassium and sulfur in relatively large amounts (>0.1% of dry mass. Common bean (*Phaseolus vulgaris* L.) forms a relationship with nitrogen-fixing rhizobia and through a process termed symbiotic nitrogen fixation provides the plant a source of nitrogen (George and Singleton, 1992; Wilker et al., 2019), but efficiency of process depends on soil fertility (Muñoz-Azcarate et al., 2017)and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. Therefore, optimization of nitrogen fixation of bean-rhizobia symbioses and selection of efficient rhizobial strains should be a priority, which begins with the study of the natural diversity of the symbioses and the rhizobial populations associated. Natural rhizobia biodiversity that nodulates common bean may be a source of adaptive alleles acting through phenotypic plasticity. Crosses between accessions differing for nitrogen fixation may combine alleles that never meet in nature. Another way to discover adaptive genes is to use association genetics to identify loci that common bean plants use for enhanced biological nitrogen fixation and, in consequence, for marker assisted selection for genetic improvement of symbiotic nitrogen fixation. In this review, rhizobial biodiversity resources will be discussed, together with what is known about the loci that underlie such genetic variation, and the potential candidate genes that may influence the symbiosis' fitness benefits, thus achieving an optimal nitrogen fixation capacity in order to help reduce reliance on nitrogen fertilizers in common bean.","author":[{"dropping-particle":"","family":"Muñoz-Azcarate","given":"Olaya","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"M González","given":"Ana","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Santalla","given":"Marta","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"AIMS Microbiology","id":"ITEM-1","issue":"3","issued":{"date-parts":[["2017"]]},"page":"435-466","publisher":"American Institute of Mathematical Sciences (AIMS. Because of the variability of nitrogen that can be ensured through symbiotic fixation it is necessary to add nitrogen as mineral fertilization for the initial growth of the beans (Muñoz-Azcarate et al., 2017)and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. 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Assuming that N persists in the root zone, N deficiency can act as external signal that affects root growth and development (Muñoz-Azcarate et al., 2017)and the most important legume for direct consumption by millions of people, especially in developing countries. It is a promiscuous host legume in terms of nodulation, able to associate with a broad and diverse range of rhizobia, although the competitiveness for nodulation and the nitrogen fixation capacity of most of these strains is generally low. As a result, common bean is very inefficient for symbiotic nitrogen fixation, and nitrogen has to be supplied with chemical fertilizers. In the last years, symbiotic nitrogen fixation has received increasing attention as a sustainable alternative to nitrogen fertilizers, and also as a more economic and available one in poor countries. 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Certain morphological traits such as length, volume, ramification and root diameter, can increase the efficiency of the root in nutrient acquisition from the soil (Lazarević, 2018; Lynch, 2013; Sinclair and Rufty, 2012)root systems with rapid exploitation of deep soil would optimize water and N capture in most maize production environments. The ideotype Specific phenes that may contribute to rooting depth in maize include (a. The need for nitrogen is also increased at the end of the growing season due to the synthesis of proteins in the seed (Sinclair and Rufty, 2012)improved plant genetics is viewed as the path to increased crop yields. However, in this manuscript, we argue that yield increases most often result from a combination of improved genetics and increased availability of nitrogen and water resources. At this time, it is likely that resource availability is the main impediment to yield increase in many cropping systems. In developing regions, it appears that nitrogen availability limits crop yield. In developed regions, rainfall and water availability commonly impose a substantial constraint on further crop yield increase. Strategies are examined to enhance resource accumulation and use in cropping systems of the future. © 2012 Elsevier B.V.","author":[{"dropping-particle":"","family":"Sinclair","given":"Thomas R.","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Rufty","given":"Thomas W.","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"Global Food Security","id":"ITEM-1","issue":"2","issued":{"date-parts":[["2012","12"]]},"page":"94-98","title":"Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics","type":"article","volume":"1"},"uris":["http://www.mendeley.com/documents/?uuid=58beb228-fa18-3d4d-9353-5a1b6dea9871"]}],"mendeley":{"formattedCitation":"(Sinclair & Rufty, 2012. Because of nitrogen deficiency in the soil, common bean plants become weaker and more susceptible to diseases, insects and adverse weather conditions (Wilker et al., 2019). Under nitrogen deficiency conditions breakdown of RuBisCO occurs, which leads to decrease in photosynthetic rate and reduction of plant growth (Aleksandrov, 2019).

Materials and methods

Plant material and experimental set up

The seeds of traditional Croatian common bean landraces; 'Trešnjevac', 'Zelenčec' and 'Biser' were obtained from the Department of Seed Science and Technology Collection at University of Zagreb Faculty of Agriculture. Experiment was conducted in a greenhouse. Seeds were surface-sterilized using 1.5% sodium hypochlorite for five minutes and 70% ethanol for 30 seconds and then washed with distilled water three times. The experiment was set in randomized design with three replications. Seeds were sown into 2L plastic pots filled with vermiculite. Three seeds were planted in each pot and 15 days after emergence two plants were removed. Half of the plants was irrigated with ½ Hoagland solution (N+) and the other half with ½ Hoagland solution without nitrogen (N-) (Hoagland and Arnon, 1950).

Measurements

Leaf chlorophyll content index (CCI), greenness (GR), digital biomass (DB) and chlorophyll fluorescence parameters maximum quantum yield of PSII (F_v/F_m) and quantum yield of PSII (Y(II)) were measured non-destructively once a week during four weeks of experiment (measurement time) on the same plants. Chlorophyll content index was measured on the first fully expanded leaf from the top of the plant (CCM-200, Opti-Sciences Inc, USA) and chlorophyll fluorescence measurements were done in dark and light adapted leaves using modulated fluorometers (Plant Stress Kit, Opti-Sciences Inc, USA). GR and DB were measured using PlantEye multispectral 3D scanner (Phenospex B.V., The Netherlands). At the end of the experiment roots were washed from the substrate and scanned with an Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan). Root images were used to analyze root morphological traits (length, volume, average diameter and number of tips) using WinRHIZO Pro software (Regent Instruments Inc., Quebec, QC, Canada).

Statistical analysis

The analysis of variance (ANOVA) was conducted in order to determine effect of nitrogen treatment, genotype, measurement time on measured physiological traits and effect of nitrogen treatment and genotype on root system traits. The general linear model in R software (R core team, 2017) was used and mean differences between the values of the variables were determined by the Tukey test ($P < 0.05$).

Results and discussion

N- caused significant decrease (*P<0.01*) of CCI through measurement time in all three examined common bean landraces (data not shown). Hence, the lowest CCI was observed in last week of measurement time for all three landraces (Figure 1). The decrease of GR through measurement time was also observed where N- significantly (*P<0.01*) decreased GR (data not shown). DB has been calculated as the product of height and 3D leaf area assuming that the plant is a regular body of which the volume can be computed by taking into account height and length. Decrease in DB in last week of measurement occurred as a result of epinasty of leaves which can be caused by stress conditions (Lazarević and Poljak, 2019), in this case nitrogen deficiency stress and changes in environmental conditions while measuring (transferring plants from greenhouse to phenotyping laboratory). Lowered DB was observed in all weeks of measurement time for plants grown in N- compared to N+ (Figure 2). The differences among landraces in DB have also been observed where landrace 'Trešnjevac' both in N+ and N- had the largest DB, and the landrace 'Biser' had the smallest. N- reduced (*P<0.05*) F_v/F_m through the measurement time on average for 14% (data not shown). No significant differences have been observed for the parameter Y(II).

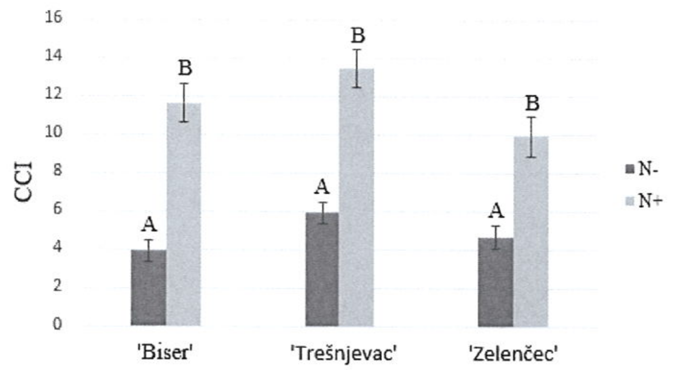

Figure 1. Chlorophyll content index (CCI) of three Croatian common bean landraces grown with (N+) or without nitrogen (N-). Vertical bars denote mean ± S.E. of means.

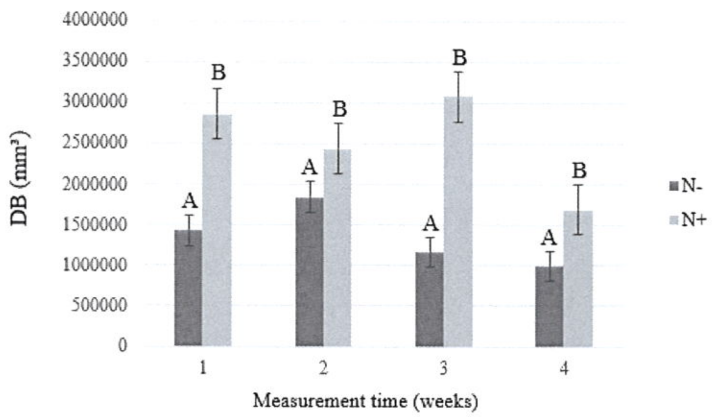

Figure 2. Differences in total digital biomass (DB) of all three genotypes between nitrogen treatment (N-) and control (N+) through measurement time. Vertical bars denote mean ± S.E. of means.

The differences among genotypes for root traits volume (V) and average root diameter (D) and between N+ and N- for traits length (L), D, number of tips (NT) have been observed (Table 1) (Figure 3). The interaction genotype x treatment showed significant negative effect on root trait V for all three landraces (Table 1). The landrace 'Zelenčec' has developed largest V (39.8 cm³) followed by landrace 'Trešnjevac' (38.75 cm³), while landrace 'Biser' developed the root with smallest V (18.68 cm³) in N-. The landrace 'Trešnjevac' developed root with larger D (0.48 mm) compared to landrace 'Zelenčec' (0.46 mm) and the landrace 'Biser' (0.39 mm) in N-.

	DF	L (cm)	V (cm^3)	D (mm)	NT
Genotype	2	ns	*	***	ns
Treatment	1	*	ns	*	***
Genotype x Treatment	2	ns	*	ns	ns

Table 1. Analysis of variance (ANOVA) results for measured root traits

L (cm) – length, V (cm^3) – root volume, D (mm) – average root diameter, NT – number of tips, DF – degrees of freedom; ns- not significant; *- significant at $P < 0.05$, **- significant at $P < 0.01$, ***-significant at $P < 0.001$.

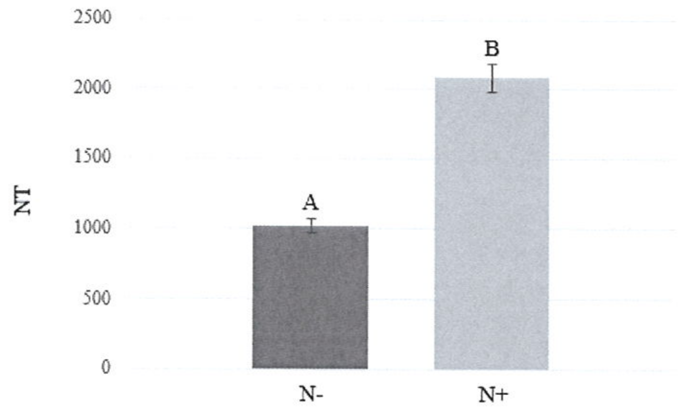

Figure 3. Total number of tips at the end of the experiment for control (N+) and nitrogen treatment (N-). Vertical bars denote mean ± S.E. of means.

Taken together, results of this study indicate that nitrogen deficiency reduced DB of all three genotypes. The decrease of CCI was followed by lowered GR in N-. Lima et al. (1999)isolated or in combination, on leaf gas exchange and fast chlorophyll (Chl reported similar reduction of CCI in 28 days old Negrito bean variety irrigated with low-dose nitrogen, compared to plants irrigated with control ('N+' Hoagland's solution). NT was significantly lowered in nitrogen deficiency treatment. The similar results occurred in nitrogen deficiency experiment with soybean conducted by Castell (2018) where N-deficient soybean had smaller root system; diameter of roots, evidence of root hairs, depth of taproot. While some studies, in a term of photosystem II (PSII) photochemistry, have demonstrated that N-deficiency has no effect on the maximum quantum yield of PSII (Khamis et al., 1990), others have shown that the lack of nitrogen reduces the maximum quantum yield of PSII photochemistry (F_v/F_m), indicating that N-deficiency causes damage to PSII (Huang et al., 2004; Verhoeven et al., 1997) which also occurred in this research.

Conclusions

Nitrogen deficiency decreased the CCI, GR and DB of common bean plants of all three landraces. Through the experiment the small changes of F_v/F_m occurred which can indicate that nitrogen deficiency caused stress that affected photosystem II in a dark adapted state. The changes in root architecture were observed through the reduction in L, V, D and NT when exposed to nitrogen deficiency stress. The landrace 'Zelenčec' developed the largest V, and the landrace 'Trešnjevac' the largest D, DB and highest CCI in N-.

Acknowledgement

This research has been funded/supported by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

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Utjecaj nedostatka dušika na neke fiziološke parametre i svojstva korijenovog sustava triju hrvatskih tradicijskih kultivara graha

Sažetak

Grah (*Phaseolus vulgaris* L.) predstavlja važnu mahunarku u svijetu, a nedostatak dušika u njegovom uzgoj može uzrokovati velike negativne promjene vodeći do smanjenja sadržaja proteina u listovima, poput RuBisCO-a, što uzrokuje smanjenje kapaciteta fotosinteze i na kraju rezultira oštećenjem listova i starenjem. Cilj ovog istraživanja bio je analizirati utjecaj nedostatka dušika, tretmanom Hoaglandovom otopinom bez dodanog dušika, na indeks sadržaja klorofila, 'greenness', digitalnu biomasu, kvantni prinos fotosustava II (Y (II)), maksimalni kvantni prinos fotosustava II (F_v/F_m) i svojstva korijenovog sustava triju hrvatskih tradicijskih kultivara graha 'Trešnjevac›, ‹Zelenčec› i ‹Biser›. Tretman nedostatkom dušika uzrokovao je pad indeksa sadržaja klorofila. Manjak dušika također je uzrokovao pad u 'greenness'-u, digitalnoj biomasi te promijene u arhitekturi korijena za sva tri kultivara. Kultivar 'Zelenčec' razvio je najveći volumen korijena, a kultivar 'Trešnjevac" najveći prosječni promjer korijena, digitalnu biomasu i najviši indeks sadržaja klorofila u N- tretmanu.

Ključne riječi: Grah, nedostatak dušika, arhitektura korijena, fotokemijske reakcije