Assessment of Rapid Diagnostic Tools in the Nitrogen Status of Inoculated Pea Plants (*Pisum sativum* L.)

Adrijana FILIPOVIĆ[1,2] (✉)
Katarina BAGARIĆ[1]
Sanja SIKORA[3]
Ana MANDIĆ[1]
Višnja VASILJ[1]

Summary

The pea, (*Pisum sativum* L.) as other legume plants, has symbiotic relationship with bacteria from genus *Rhizobum* that are able to perform biological fixation of atmospheric nitrogen due to the fact that plants are able to meet their seasonal nitrogen requirements. Nitrogen content in pea plant after artificial inoculation with ten indigenous strains of *R. leguminosarum* previously isolated in Herzegovina soils, referent check *R. leguminosarum* 1001 and the control with no inoculant, was investigated. The field study was set up in RCBD with four replicates at Hodbina (Mostar, Bosnia and Herzegovina) in 2009. Nitrogen was analyzed based on ten whole plants per plot. The total nitrogen in aboveground plant parts and number of pods were measured at anthesis. The total nitrogen in grains was measured at technological maturity. Relative amount of chlorophyll (un-dimensioned units) content and nitrates (NO_3-N ppm) with the use of rapid diagnostic tools as Chlorophyll meter and Cardy ion meter was observed three times, just before flowering, at anthesis and at technological maturity. *R. leguminosarum* strains had affected total nitrogen content in the aboveground part of the peas comparing to the control (uninoculated seeds) and referent strain 1001. The treatment was not significant for number of pods per pea plant but significant differences appeared for applied strains and control, based on Cardy-ion meter readings with values in range from 246 to 387 mg NO_3-N kg^{-1}. Measured Chlorophyll content in the leaf showed no significant difference with respect to the applied indigenous strains *R. leguminosarum* compared to the control where values ranged from 46.87 to the 49.57 units. Measurements with both devices showed significant differences in obtained values due to the vegetation period.

Key words

pea, inoculation, indigenous strains, Chlorophyll meter, Cardy -ion meter

[1] University of Mostar, Faculty of Agriculture and Food-technology, Biskupa Čule 88000 Mostar, Bosnia and Herzegovina

[2] Federal Agro-Mediterranean Institute of Mostar, Biskupa Čule 10, 88000 Mostar, Bosnia and Herzegovina

[3] University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia

✉ Corresponding author: adrijana.filipovic@aptf.sum.ba

Received: May 20, 2020 | Accepted: August 17, 2020

INTRODUCTION

Pre-sowing inoculation treatments with *Rhizobium* strains have no soil contamination effect. They can provide a greater amount of fresh organic matter by stimulating effect on indigenous microbial populations, their enzymatic activity thus increasing soil biogenity, metabolism and soil organic matter levels, which positively affects the physical properties of the soil (Redžepović et al. 2006, 2007, Sikora et al. 2008). Seed inoculation of legumes with selected strains of *Rhizobium leguminosarum* enhances the nitrogen fixation and enables an alternative in the plant nitrogen supply (Blažinkov et al. 2007; Sikora et al. 2008; Mungai and Karubiu 2011). *Rhizobium* species provide nitrogen fixation with compatible species of legume plants, but only if they are present in the soil at the right time and sufficient quantity to create a symbiotic relationship (Sikora et al. 2008, Blažinkov et al. 2007, Bejandi et al. 2011). The plant leaves tissue analysis was rapidly used for determination of the nutrient status contrary to long standard laboratory analytical procedures. Chlorophyll and Cardy-ion meters tools were proposed as rapid diagnostic, non-destructive methods for indirect determination of nitrogen status in plant tissue (Vos and Bom, 1993). Sampling of leaves for this purpose was recommended in most physiologically active period during the daylight from 9 a.m. to 1 p.m. Fully developed leaves (4-5 leaves from the top of the main stem) (Vos and Bom, 1993; Minotti et al., 1994) from healthy plants should be taken for analysis. The Chlorophyll-meter readings (SPAD value) were performed from the central part of the leaf, taking 30 readings average. The petioles of some sampled leaves (about 3 cm of the petiole from the base to the leaf) could be used for measurements of NO_3-N by squeezing the juice sap on Cardy-ion meter and reading the nitrate concentration. However, the assessment of these tools in detecting plant nitrogen status should be tested for each cultivar or variety in particular, as well as agro-ecological growing conditions. The chlorophyll-meter is used to measure the content of chlorophyll (a molecule that absorbs sunlight and uses light energy to synthesize carbohydrates from water and CO_2) in the leaf blade. The content of identified chlorophyll reflects the nitrogen status in the plant, since chlorophyll contains about 70% of leaf N, and good correlations of chlorophyll with the concentration of the leaf nitrogen confirm the accuracy of method (Bullock and Anderson, 1998). Provided measurements are simple and without destruction of the leaf blade. Chlorophyll reading values are expressed in units of 0 to 99.9. The Chlorophyll-meter reads the total chlorophyll present in the crop leaf, with the help of light transmission, i.e. its wavelengths (red-650 nm and short-wave infrared-940 nm). During the measurement, the light is reflected and converted into electrical signals which amplify and convert into a digital signal. A different intensity of reflected light achieved gives SPAD values by further processing. The Cardy-ion meter has a flat surface design with ion-selective electrodes for nitrate detecting from the petiole sap. The meter has a scale in range 100 to 10000 NO_3-N ppm. Readings can be made directly in the field. According to Takebe (2000), in vegetable crops the petiole presents a tissue in which nitrates are stored and suitable for plant analyzes by Cardy-ion meter, because the concentration of nitrates is changing according to the N status in the plant. The same author points out that nitrate levels are higher in the petiole than in the whole plant. Increased nitrogen levels increase NO_3-N concentration, as confirmed by numerous studies (Wescott et al., 1991; Lewis and Love, 1994; Sharma, 1996). In some cases, the different nitrate content in the plant tissue depends on agroecological factors (tillage, fertilization, irrigation) and climatic conditions during the growing season (Cieślik, 1995). It has also been confirmed that different cultivars, soil, climate and weather, as the main factors influence the reading of SPAD values (Oliver et al., 1999). The main objective of the study is to evaluate the use of rapid diagnostic measurements by Chlorophyll and Cardy-ion meters in detecting of nitrogen status in plant tissue comparing to the nitrogen analysis by standard Kjldahl laboratory method. For plant tissue analyses the pea variety Petit Provencal was used, inoculated with ten indigenous strains of *R. leguminosarum* and one reference *R. leguminosarum* 1001 and obtained results were compared in relation to control. The obtained result should present the inoculation efficiency by different *Rhizobium* strains and selected diagnostic tools accessibility in detecting nitrogen status in pea plants.

MATERIALS AND METHODS

Experimental field was set in 2009 with peas (*Pisum sativum* L.) variety Petit Provencal at the Hodbina site (near Mostar, Bosnia and Herzegovina). Pea variety was inoculated with the ten indigenous *R. leguminosarum* strains V 1, V 5, V 6, V 7, V 10, V 13, V 14, V 18, V 19, V 20 (isolated from native soil of Herzegovina Table 1) and one reference strain *R. leguminosarum* 1001 (from the collection of the Institute of Microbiology). Randomized complete block design in four replicates was used with plot size 22m x 17m (374 m^2). Fields were sown manually on March 19th, 2009. The strains were multiplied and prepared for pre-sowing inoculation of peas at the Department of Microbiology, Faculty of Agriculture, University of Zagreb. Standard soil analyses were performed before seed sowing. pH reaction was done by potentiometric method (BAS ISO 10390:2009) w/v 1:2.5, the total nitrogen content in soil was detected by modified Kjeldahl method (BAS ISO 11261:2010), the concentration of available phosphorus (by spectrophotometer) and potassium (by flame photometer) in soil was determined due to ammonium lactate method (Egner et al., 1960) and the content of soil organic matter was analyzed by Kotzmann method (JDPZ, 1966). The number of pods and total nitrogen content in the aboveground part of the plant (%) were measured on 10 randomly sampled plants at anthesis. The plants in technological stage were sampled for the detection of total grain nitrogen content (%). The total nitrogen content of the aboveground plant part and grain was provided by the standard Kjeldahl method (AOAC, 1970). At the three stages, before flowering, anthesis and technological maturation, measurements on leaves by Chlorophyll meter (SPAD-502, Konica-Minolta) and petiole tissue by Cardy-ion meters (Horiba, Spectrum technology Inc.) were made and fully matured leaves were used for Chlorophyll meter readings. On the same leaves the sap from the petioles was taken to Cardy -ion meter reading of nitrates. Correlations between established measurements by rapid diagnostic tools and nitrogen content in the aboveground part of the plant and grain were determined and significant coefficients were shown (r). Meteorological data of average monthly temperatures (°C) and precipitation (mm) for the spring period of 2009 were used to interpret the results obtained from the field trial (Table 3). In this paper, some of the trials dates are selected and presented. The data have been statistically processed by analysis of variance (ANOVA). The LSD tests have been performed for differences between average values according to inoculated bacterial stains; less than or equal to P ≤ 0.05 being considered significant and their average values further tested by Student's t-test. The GENTSAT7 (GenStat for Windows 7th Ed) statistical program has been used for data analysis.

RESULTS AND DISCUSSION

At the field trial the soil was classified as deep, brown carbonate soil on gravels with neutral pH reaction (Vasilj at al, 2019). The detected level of soil humus was poor (< 3 %) with modest supply of nitrogen. The supplies of plant- available phosphorus and potassium were good to very good (Table 2). The average monthly temperature from period of germination, the first growing stage as well as the end of vegetation period were in acceptable range. A higher temperature average during May affected acceleration of pea pods filling (Table 3). Total annual precipitation was 1848.9 mm during 2009. The minimum precipitation in the vegetation period of 2009 occurred in May with average of 35.7 mm. The amounts of annual precipitation quite varied during the year.

Based on the analysis of variance (Table 4) it was found that inoculated plants with indigenous strain *R. leguminosarum* (V1) showed the highest nitrogen content in the aboveground part of the pea for 33,7 % as well as in nitrogen grain content for 46,2% compared to the control (uninoculated). These results are in line with the results of similar studies in the application of indigenous strains *R. leguminosarum* to pea plants (Argaw et al. 2017; Vasilj et al., 2016, 2019). In addition, the same indigenous strain V1 previously showed significantly higher aboveground nitrogen content for 22% at variety Petit Provancal that we also used in comparison to the variety the Miracle of America (Vasilj et al., 2016). The results from the Table 4 showed that total nitrogen contents in the aboveground parts of the pea were the lowest in control (2.49 N %) and inoculated variant by strain V13 (3.81% N), while all other inoculate plants showed nitrogen values significantly higher by 15-46 %. The results of this trial indicate that inoculated pea plants by strain V1, V14, V18, V19, V20 had a positive significantl effect on total grain nitrogen contents (3.94 % to 4.63 %N) compared to the control (2.49 N %). Clayton et al. (2004) found that inoculation of pea seeds with *Rhizobium* species increased the nitrogen content by approximately 19-42 mg plant^{-1}, thereby increasing the percentage of nitrogen recovered from the atmosphere from 10 to 61%. The number of pods per plant in the Petit Provencal variety show no significant differences regarding used indigenous *R. leguminosarum* strains, reference *R. leguminosarum* 1001 strain or control without inoculation. The number of pods per plant ranged from 3.40 to 4.46 pods. The reason of small number of pods per plant in this study could be explained with low precipitation occurrence during vegetation as well as sampling done before technology maturation stage. Our results of the number of pods per plant on inoculated variety Petit Provencal are lower than in other studies (Uher et al. 2006; Rapčan et al., 2017). Inoculated pea seeds led to the formation of more pods than the un-inoculated variant (Friščić et al., 2011; Uher et al., 2010). The measured chlorophyll content in the leaves showed no significant difference with respect to the applied indigenous strains *R. leguminosarum* compared to the control and values ranged from 46.87 to 49.57 units (Table 4). This could be explained by low chlorophyll meter sensitivity in SPAD value due to the similar effect of applied strains on the synthesis of chlorophyll content. Limited precipitation occurrence during vegetation can affect inoculation efficiency and nitrogen fixation process reducing the plant nitrogen content in leaves tissue used for SPAD analysis (Minguez and Sau, 1989; Djekoun and Planchon, 1991). Reduced nitrogen content in leaves could be the result of disturbed photosynthetic capacity affected by drought stress (Kao and Forseth, 1992; Atti et al., 2004). Bejandi et al. (2012) found that the chickpea inoculated by *Rhizobium* strains showed positive significant effect on chlorophyll content of 33.14 units comparing to an uninoculated variant (28.21 units). The chlorophyll meter has proved successful in use for monitoring of nitrogen status of several crops beans (Madeira et al., 2000) rice (Ladha et al., 1998), maize (Ma and Dwyer, 1997), wheat (Reeves et al., 1993) and potatoes (Minotti et al., 1994; Poljak et al., 2008; Majić et al., 2008), confirming the accuracy and reducing time consumption for analysis.

Table 1. Ten isolated *R. leguminosarum* strains from native soil of Herzegovina and their abbreviation

Location	Mostar – Vihovići	Ljuti Dolac	Čitluk – central part	Čitluk	Kruševo – field	Posušije – Osoj	Posušije	Mostar – Bijeli brijeg	Donja Jasenica
Abbrev.	V1	V5	V6	V7	V10	V13;V14	V18	V19	V20

Table 2. Soil chemical analysis for location of Hodbina in 2009

Soil depth	pH in H_2O	pH in 1 M KCl	Organic matter %	Total N %	P_2O_5 mg/ 100 g soil	K_2O mg /100g soil
0 – 30	8.02	6.51	2.76	0.13	21.8	27.2

Table 3. Average Monthly Air Temperature (°C) and Precipitation (mm) in Mostar Area for 2009

2009	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Monthly temperature average	6.3	5.9	9.5	15.6	21.3	22.0	26.3	26.8	22.3	14.8	10.4	7.5
Monthly precipitation average	336	117,4	173,6	68,1	35,7	190	15,8	57,8	127,8	246,6	164,2	315

(Source: FHMZFBIH, 2009)

Table 4. Average value of measured parameters affected by the selected strains

Bacterial strains	Total Plant nitrogen %	Total grain nitrogen %	Cardy ion meter mg NO_3-N kg^{-1}	Chlorophyll meter	Number of pods
control	2.93 c	2.49 d	284 b, c	46.87	3.45
V 1	4.42 a	4.63 a	341 a, b, c	48.53	4.08
V 5	3.85 a, b	3.56 b, c	232 c	47.80	4.08
V 6	4.16 a, b	3.06 c, d	306 a, b, c	46.96	3.40
V 7	3.86 a, b	3.43 b, c	267 b, c	48.71	3.44
V 10	4.20 a, b	3.81 b	387 a	48.99	3.67
V 13	3.81 b	3.01 c, d	246 c	47.26	3.44
V 14	3.87 a, b	4.13 a, b	276 b, c	48.82	4.46
V 18	4.16 a, b	4.2 a, b	372 a, b, c	48.79	3.79
V 19	4.04 a, b	3.99 a, b	372 a, b, c	49.57	3.51
V 20	4.01 a, b	3.94 a, b	382 a, b	48.21	3.59
V1001	4.18 a, b	3.33 b, c	286 b, c	47.51	4.17
$LSD_{5\%}$	0.58	0.78	101.9	No- significant F-test	
$LSD_{1\%}$	0.78	1.04	135.3		

The highest content of NO_3-N (387 mg kg^{-1}) was determined in the petiole sap for plants inoculated by indigenous *R. leguminosarum* strain V10 relative to control, and the lowest was determined in plants inoculated by V5 strain (232 mg kg^{-1} NO_3-N). Significantly higher contents of NO_3-N (mg kg^{-1}) were obtained in plants inoculated by strains V1, V6, V10, V 18, V19 and V20 comparing to control thus showing higher sensitivity of Cardy-ion meter and measured values of nitrates to inoculation effect of pea variety Petit Provencal. Nitrate content and chlorophyll values decline ontogenetically over the growing period (Table 5) indicating a significant difference in values. In order to interpret achieved results of nutrient status the researchers suggest that the crop's stage, its genetic characteristics and environmental factors should always be considered. The Cardy- ion meter is successful in use for monitoring nitrogen status in potatoes (Poljak et al., 2008; Majić et al., 2008; Westcott et al., 1991) and mint (Mitchell et al., 1995).

The correlation between the read values of the Chlorophyll meter and the Cardy-ion meter during the pre-flowering, anthesis and technological maturity phases of measurements is positive and weak (r = 0.21, 0.23, 0.24). Chlorophyll meter and Cardy-ion meter values show moderate positive correlations compared to the nitrogen content in the aboveground mass (0.26-0.32) during pre-flowering and anthesis phase, and significantly weaker coefficient in the technological phase. An increase in the correlation coefficient of SPAD and NO_3-N values in relation to the nitrogen content in the grain has been found as the plant enters the technological phase of maturity, although these correlations are quite weak and insignificant. What these correlations confirm is that the measured values of SPAD and NO_3-N show the status and dynamics of nitrogen in plant tissue, which is expected to be significantly higher in vegetative mass at the beginning of vegetation. In maturation phase when redistribution of assimilates occurs from vegetative parts to grain, they will start to show a higher correlation coefficient between measured total nitrogen in grain and SPAD and NO_3-N values. Weak correlation can be interpreted by low oscillations or too similar oscillations between the measured SPAD and NO_3-N values, comparing to the total N values. However, there is an obvious tendency of changing the nitrogen status in plant tissue that can be followed by proposed rapid diagnostic methods. Probably these differences in measured values should be more pre-announced by applying some other agrotechnical measures that could result in higher correlation coefficient. By applying different N fertilization to crops, strong and positive correlations can be achieved between nitrogen content and established SPAD and NO_3-N values, which has been confirmed by numerous researchers (Wescot et al., 1991; Vos and Boom, 1993; Minotti et al, 1994; Gianquinto et al., 2004).

Table 5. Cardy-ion and chlorophyll meters reading in different vegetation stages

Readings	Before flowering	Anthesis	Technol. maturation	$LSD_{5\%}$	$LSD_{1\%}$
Cardy-ion meter (NO_3-N mg/kg)	378 a	279 b	280 b	51	68
Chlorophyll meter	49.78 a	50.01 a	44.72 b	1.49	1.97

CONCLUSION

The presented study shows that the Cardy-ion meter readings significantly differed with respect to the applied indigenous strains *R. leguminosarum* V1, V6, V10, V18, V19 and V20 compared to the control reference strain 1001 and V5, V7, V13 and V14 indigenous strains. The Chlorophyll meter showed opposite results probably due to the small differences in strains nitrogen fixation amount. In this trail Cardy-ion meter showed higher sensitivity for determination of nitrogen status in pea variety Petit Provencal due to the inoculation effect. Measurements with both devices showed significant differences in obtained values with respect to the pea vegetation sampling stage. The established correlations between the measured values by rapid diagnostic tools and nitrogen content in plant and grain confirm the possibility of using leaves plant tissue for the monitoring of nitrogen status in pea variety Petit Provancal. However, it can be assumed that the short vegetation period of peas, as well as inoculation effect or some abiotic effects did not show highly significant correlation reflecting the nitrogen status change compared to the measured values. One of the important reasons of slow adoption of plant tissue analysis is difficulties in interpreting results against reliable referent values or standards, and measurements often fluctuate under the different external factors and cultivar or variety.

REFERENCES

Argaw A., Mnalku A. (2017). Symbiotic Effectiveness of *Rhizobium leguminosarum bv. vicieae* Isolated from Major Highland Pulses on Field Pea (*Pisum sativum* L.) in Soil with Abundant Rhizobial Population. Annals of Agrarian Science. 15 (3) 410-419.

Association of Official Analytical Chemists (1970). Official Method of Analysis XI Edn. Association of Official Analytical Chemists, Washington D. C.

Atti S., Bonnell R., Smith D., Prasher S. (2004). Response of an Indeterminate Soybean {Glycine Max (L.) Merr} to Chronic Water Deficit during Reproductive Development under Greenhouse Conditions. Canadian Water Resources Journal/Revue canadienne des ressources hydriques. 29 (4):209–222. doi: 10.4296/cwrj209.

BAS ISO 10390:2009. Soil Quality – Determination of pH

BAS ISO 11261:2010 Soil Quality–Determination of Total Nitrogen-Modified Kjeldahl Method

Bejandi T.K, Raouf S.S., Mohammad S., Ali N. (2011). Effects of Plant Density, *Rhizobium* Inoculation and Microelements on Nodulation, Chlorophyll Content and Yield of Chickpea (*Cicer arietinum* L.). Journal of Biology and Aric Res. 23: 1067-1178.

Bejandi T.K., Sharifii R.S., Sedghi M., Namvar A. (2012). Effects of Plant Density, *Rhizobium* Inoculation and Microelements on Nodulation, Chlorophyll Content and Yield of Chickpea (*Cicer arietinum* L.). Annals of Biological Research. 3 (2): 951-958.

Blažinkov M., Sikora S., Uher D., Maćešić D., Redžepović S. (2007). Genotypic Characterisation of Indigenous *Rhizobium leguminosarum* bv. *viciae* Field Population in Croatia. Agriculturae Conspectus Scientificus. 72 (2):153–158.

Bullock D.G., Anderson D.S., (1998). Evaluation of the Minolta SPAD-502 Chlorophyll Meter for Nitrogen Management in Corn. Journal of Plant Nutrition. 21 (4): 741–755.

Cieślik E. (1995). Czynniki kształtujące zawartość azotanów i azotynów w ziemniakach [Factors affecting the contents of nitrates and nitrites in potato tubers]. Postępów Nauk Rolniczych. 6: 67-71.

Clayton G.W., Rice W.A., Lupwayi N.Z., Johnston A.M., Lafond G.P., Grant C.A., Walley F. (2004). Inoculant Formulation and Fertilizer Nitrogen Effects on Field Pea: Nodulation, N2 Fixation and Nitrogen Partitioning. Canadian Journal of Plant Science. 84 (1): 79-88.

Djekoun A., Planchon C. (1991). Water Status Effect on Dinitrogen Fixation and Photosynthesis in Soybean. Agronomy Journal. 83 (2): 316–322.

Đurđević B., (2015). Praktikum iz ishrane bilja. Poljoprivredni fakultet Osijek Sveučilište J J Strossamyera Osijek.

Egner H., Riehm H., Domingo W.R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nahrstoffzustandes der Boden, II: Chemische Extractionsmetoden zu Phosphorund Kaliumbestimmung. Kungliga Lantbrukshügskolans Annaler, 26: 199-215.

Friščić O., Uher D., Abramović-Friščić K. (2011). Utjecaj bakterizacije na gospodarska svojstva novih kultivara ozimog graška. Sjemenarstvo. 28 (1-2): 25-42.

FHMZFBIH, (2009). Klimatološka analiza sezone, Sarajevo.

Gianquinto, G., Goffart, J.P., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., Delle Vedove, G., Vos, J., MacKerron, D.K.L. (2004). The Use of Hand-Held Chlorophyll Meters as a Tool to Assess the Nitrogen Status and to Guide Nitrogen Fertilization of Potato Crop. Potato Research. 47:35–80.

Grupa autora, (1966). Fizikalne metode istraživanja zemljišta. Jugoslovensko društvo za proučavanje zemljišta (JDPZ), Beograd.

Kao W.Y., Forseth I. N. (1992). Chlorophyll; a Fluorescence of NO_3-FED vs N2-fixing Soybean Grown under Two Water Treatments. Environmental and Experimental Botany. 32 (4): 343–349. doi: 10.1016/0098-8472(92)90046-5.

Ladha J.K., Kirk G.J., Bennett D., Peng J., Reddy S., Reddy C.K., Singh P.M. (1998). Opportunities for Increased Nitrogen Use Efficiency from Improved Lowland Rice Germplasm. Field Crops Research. 56 (1):41–71.

Lewis, R.J., Love, S.L. (1994). Potato Genotype Differs in Petiole Nitrate-Nitrogen Concentrations over Time. Horticultural Science. 29:175-179.

Ma B.L., Dwyer, L.M. (1997). Determination of Nitrogen Status in Maize Senescent Leaves. Journal of Plant Nutrition. 20 (1):1-8.

Madeira A.C., Mendonca A., Ferreira M.E., Taborda M.L. (2000). Relationship between Spectroradiometric and Chlorophyll Measurements in Green Beans. Communications in Soil and Plant Analysis. 31 (5-6): 631–643.

Majić A., Poljak M., Sabljo A., Knezović Z., Horvat T. (2008). Efficiency of Use of Chlorophyll Meter and Cardy-Ion Meter in Potato Nitrogen Nutrition Supply VII. Alps-Adria Scientific Workshop, Stara Lesna. 36 (suppl.):1431-1434.

Minguez M. I., Sau F. (1989). Responses of Nitrate-Fed and Nitrogen-Fixing Soybeans to Progressive Water Stress. Journal of Experimental Botany. 40 (4): 497–502. doi: 10.1093/jxb/40.4.497.

Minotti P.L., Halseth D.E., Sieczka J.B. (1994). Field Chlorophyll Measurements to Assess the Nitrogen Status of Potato Varieties. HortScience. 29 (12): 1497–1500.

Mitchell A.R., Farris N.A., Light, J.E. (1995). "New Tools for Plant-Response Nitrogen Testing of Peppermint.". In Central Oregon Agricultural Research Center Annual Report 19–32. Madras, OR: Agric. Exp. Stn., Oregon State University: Spec. Rep. 941.

Mungai N.W., Karubiu N.M. (2011). Effectiveness of Rhizobia Isolates from Njoro Soils (Kenya) and Commercial Inoculants in Nodulation of Common Beans (*Phaseolus* vulgaris). Journal of Agriculture, Science and Technology. 12, 47–59.

Oliver, M., Goffart J. P., Sinnave G., (1999). Evaluation of Invasive Method to Assess the Nitrogen Status of Potato in Course of the Season, 14th triennal Conference of the EAPR, Sorrento, Italy. 134-135.

Poljak M., Majić A., Horvat T., Pospišil A., Ćosić T. (2008). Efficiency of Use of Chlorophyll Meter and Cardy- Ion Meter in Potato Nitrogen Nutrition. Stara Lesna, Slovakia: VII. Alps-Adria Scientific Workshop. 36 (suppl.): 1795-1798.

Rapčan I., Milaković Z., Kanižaji Šarić G., Šeput J., Galić Subašić D. (2017). Reaction of Garden Pea (*Pisum sativum* L.) to Inoculation and Nitrogen Fertilization in Eastern Croatia. Journal of Central European Agriculture, 18 (4): 889-901.

Redžepović S., Čolo J., Blažinkov M., Poljak M., Pecina M., Sikora S., Šeput M. (2006) Effect of Inoculation and Growth Regulator on Soyabean Yield and Photosynthetic Pigment Content. Agriculturea Conspectus Scientificus. 71 (3): 75–80.

Redžepović S., Sikora S., Čolo J., Blažinkov M., Pecina M. (2007). Influence of Plant Growth Regulator and Rhizobia Inoculation on Nodulation and Soybean Nitrogen Content. Cereal Research Communication. 35 (2): 993-996.

Reeves D.W., Mask P.L., Wood C.W., Delaney, D.P., (1993). Determination of Wheat Nitrogen Status with a Hand-Held Chlorophyll Meter: Influence of Management Practices. Journal of Plant Nutrition. 16 (5): 781–796.

Sharma, U.C. (1996). Use of Half-Life Technique to Diagnose Nitrate-Nitrogen Status in Petioles of Potato (*Solanum tuberosum*) during Growing Season. Indian Journal Agroicultural Sciences 66:1-6.

Sikora S., Blažinkov M., Babić K., Sudarić A., Redžepović S. (2008). Symbiotic Nitrogen Fixation and Sustainable Soybean Production. Cereal Research Communication 36 (1-4): 1483-1486.

Takebe, M., (2000). The Latest Research of Diagnosis- Plant Nutrition and the Problem of Determination of Diagnostic Critical Value. Hokkaido Dojouhiryo Kennkyu-Tsushin 46: 57-71.

Uher D., Štafa Z., Blažinkov M., Pisačić A., Kmet M., Ščavničar M. (2010). Importance of Winter Pea cv. Maksimirski rani in Milk Production on Family Farms. Mljekarstvo. 60 (1): 37-49.

Vasilj V., Šego M., Filipović A., Sikora S., Mandić A. (2016). Effect of Inoculation on Some Important Characteristics of Peas Grown in Herzegovina Region (B&H). VII Int. Sci. Agric. Symp., "Agrosym 2016", Jahorina, B&H. Proceedings ref.13. 965-971

Vasilj V., Blažnikov M., Filipović A., Knezović Z., Sikora S. (2019). Genetska identifikacija i simbiozna učinkovitost sojeva Rhizobium leguminosarum izoliranih iz različitih tipova tala Hercegovine, Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu. 64 (69/1): 20-38.

Westcott, M.P., Stewart V.R., Lund R. E. (1991). Critical Petiole Nitrate Levels in Potato. Agronomy Journal. 83:844-850.

aCS86_3