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ASSESSMENT OF THE NUTRITION OF POTATO PLANTS WITH NITROGEN ACCORDING TO THE NNI TEST AND SPAD INDICATOR*

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ABSTRACT

The aim of three-year-long field trials was to determine an optimal nutritional status of potato plants with nitrogen in a growing season using a Minolta optical measuring Camera SPAD 502, called the N-Tester. The research was carried out on three potato cultivars (Gwiazda - early, Etiuda - medium early, and Gustaw - medium late) grown under conditions of different levels of mineral fertilization with nitrogen (0, 50, 100, 150 and 200 kg ha⁻¹ N). Nitrogen fertilization on plots under 100 kg ha⁻¹ N was applied directly before planting tubers and on plots above 100 kg ha⁻¹ N in two doses before planting and before the emergence of potato plants. During the growing season, three dates (28, 42 and 56 DAE - BBCH 30-70 phase) were selected for determination of the total yield of dry matter of potato plants (leaves, stems, roots, tubers) and the nitrogen content. Three plants were harvested from each plot to make 30 measurements of SPAD in total on their leaves. It was found that as the potato plants grew, the yield of the plants' dry matter increased and the nitrogen content decreased. Under the influence of nitrogen fertilization, a significant increase in the yield of dry matter of plants in response to a dose of 100 kg N ha⁻¹ was obtained and the nitrogen content in the yield increased under a dose of 200 kg N ha⁻¹. With the increased nitrogen fertilization dose, the NNI index and SPAD indicators increased as well. In order to calibrate the SPAD test, its dependence with the values of the NNI test was determined. A linear regression model was implemented to describe the relationship between SPAD measurements and NNI indices. It was found that the critical values of leaf greenness indices evaluated using an N-Tester on a scale of 0-80 from the phase from crop cover in inter rows to fruit setting on plants amounted to 43.9 for the early cultivar Gwiazda and the medium early cv. Etiuda, and to 43.7 SPAD units for the medium late cultivar Gustaw. With these SPAD unit values, the potato plants were optimally nourished with nitrogen.

Keywords: leaf greenness index, SPAD, nitrogen nutritional status, NNI, potato.

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INTRODUCTION

Optimization of nitrogen fertilization of potato can be achieved by foliar application of supplementary doses of this compound during the plant growing season, based on a diagnosis of their nutritional status (VOS, BOM 1993). For this purpose, the results of analyses of the plants' total dry matter yield and nitrogen content, which must be calibrated to constitute a plant test, can be used. Calibration is best done based on the NNI test (Nitrogen Nutrition Index). The NNI test allows us to determine precisely an optimal nutritional status of plants with nitrogen through a mathematical description of the relationship between the nitrogen content and the total amount of accumulated dry matter of plants cultivated under conditions of an optimal nitrogen supply. The method which employs the NNI test is based on the ratio (N_a) of the actual nitrogen content to (N_c) critical nitrogen content, that is the minimum nitrogen content needed to achieve the maximum dry matter yield of plants. The ratio of the actual nitrogen content to critical content of nitrogen in the total yield of dry matter of plants determines the nutritional status of plants with this element (LI et al. 2012). The relationship between the nitrogen content in plants optimally nourished with this element, called the critical content, and their dry matter yield (W) is described by the power function equation $N_c = aW^{-b}$ by GREENWOOD et al. (1990). The concept of nutritional status evaluation of potato plants with nitrogen based on its content in whole plants tested by GREENWOOD et al. (1990) found confirmation in later studies (DUCHEUNE et al. 1997, LEMAIRE, GASTAL 1997, BÉLANGER et al. 2001). However, this method of assessing the nutritional status of plants with nitrogen proves to be time-consuming and expensive, and the information about the state of plant nutrition with nitrogen on a plantation is obtained in a relatively long time. Plant tests performed with optical instruments can be a significant simplification of the methods applied to attain precise determination of the nutritional status of plants during a growing season (GIANQUITO et al. 2004). An example of this type of plant tests is the leaf greenness index, obtained with an N-Tester and expressed in SPAD units (Soil Plant Analysis Development), which translates into a specific leaf chlorophyll content (GIANQUITO et al. 2003). SPAD units are an abbreviation adopted by Minolta, which constructed the instrument (MARKWELL et al. 1995). The instrument measures the difference between the absorption of light passing through a leaf at 650 and 940 nm. The quotient of these differences is the greenness index of the leaf, or the chlorophyll content, which can be an indicator of plant nitrogen nutrition (NITCH, VARIS 1991, OLIVIER et al. 2006). There is a significant relationship between the value of SPAD indicators and nitrogen content (GITELSON et al. 2003). Both Polish and foreign studies report that the SPAD test performed with an N-Tester is the most prospective and useful technique in agricultural practice owing to the ease of performing non-invasive measurements of the chlorophyll content

in leaves. A test performed with an N-Tester is relatively simple and rapid, as measurements are carried out in a field directly on leaves of plants, while preserving their integrity. Repeated multiple times during a growing season, these measurements can be used for precise nitrogen dosing, thus preventing yield reduction or accumulation of nitrogen in the environment (FOTYMA 2000, GOFFART et al. 2008).

The aim of this study has been to determine an optimal nutritional status of potato plants with nitrogen in a growing season, using to this end an optical instrument and establishing a relationship between values of an NNI test and a leaf greenness index SPAD measurement.

MATERIAL AND METHODS

Controlled field experiments carried out in 2008-2010 at the Plant Breeding and Acclimatization Institute, the Jadwisin branch (52°45' N, 21°63' E), enabled us to determine the nutritional status of potato plants with nitrogen during a growing season, where cultivars of different earliness were cultivated. A three-factor experiment was established using the random block method in triplicate. The first factor was mineral fertilization with nitrogen (5): 0, 50, 100, 150, 200 kg ha⁻¹ N; the second factor consisted of cultivars different in earliness (3): early – cv. Gwiazda, medium early – cv. Etiuda, medium late – cv. Gustaw; the third factor was composed of the dates (3) on which measurements were taken, i.e.: 28, 42, 56 days after the emergence of potato plants. The research was carried out on arable light soil classified as belonging to soils with clay translocation, type Luvisols, subtype Stagnic Luvisols (MARCINEK et al. 2011). The soil was characterized by acid reaction (pH in KCl 5.3 - 5.4), high content of phosphorus (75 - 86 mg kg⁻¹ P), average potassium content (108 - 112 mg kg⁻¹ K) and average magnesium content (32 - 33 mg kg⁻¹ Mg). The content of mineral nitrogen, determined in the soil profile of 0-60 cm and assessed every year in early spring before the application of nitrogen fertilizer, was 60 kg ha⁻¹ N. The years of the research were wet ones. The Selyaninov's Hydrothermal (HT) coefficient for the period of April to the end of September was on average: 1.32 in 2008, 1.12 in 2009, and 1.99 in 2010 (BAC et al. 1998). Winter triticale preceded the cultivation of potatoes. Organic fertilizer consisted of cut winter triticale straw, ploughed in after harvesting, in an amount of about 5 t ha⁻¹. In addition, 1 kg N 100 kg⁻¹ of straw was supplied, and the green mass of white mustard stubble intercrop in the amount of 15-16 t ha⁻¹ served as a fertilizer added in the autumn. Phosphorus was supplied as enriched superphosphate (18% P) at a dose of 43.6 kg ha⁻¹ P; potassium was added to soil as potassium chloride (50% K) at a dose of 99.6 kg ha⁻¹ K; different levels of mineral nitrogen fertilization with nitro chalk (27% N) were also applied. Mineral phosphorus

and potassium fertilization was performed in autumn before pre-winter ploughing. Nitrogen fertilization on plots up to 100 kg ha⁻¹ N was applied in spring before planting tubers, while on plots with 150 and 200 kg ha⁻¹ N there was a supplementary dose 50 and 100 kg ha⁻¹ N added just before the emergence of potato plants. Tubers were planted in the last ten days of April, at a spacing of 75 x 33 cm, and harvested in the last ten days of September. The plot size was 14.85 m² and the number of plants per plot was 60.

Every year, at two-week intervals starting from the early crop cover in inter rows (phase BBCH 30) to fruit setting on plants (phase BBCH 70), i.e. on dates: 28, 42 and 56 days after emergence DAE (Days After Emergence), 3 plants were harvested from each plot to determine the nitrogen nutritional index of the plants with an NNI test. The dry mass was determined in the leaves, stems, roots and tubers of each sample, after two-step drying of fresh material: pre-drying at 60°C, then drying to dry matter at 105°C. Dry material was mineralized with concentrated sulfuric acid and hydrogen peroxide, after which nitrogen was determined with the Kjeldahl method on an automatic distillation device Kjeltec 2200 (Foss Tecator). The nitrogen nutrition index (NNI) was calculated according to the equation (LEMAIRE, GASTAL 1997):

$$\text{NNI} = N_a / N_c,$$

where:

N_a – actual nitrogen content in the dry matter of plants (g kg⁻¹);

N_c – critical (optimal) nitrogen content in the dry matter of plants (g kg⁻¹).

The critical nitrogen content (N_c), called the dilution curve, was calculated from the relation between the maximum dry matter yield (W) and the nitrogen content in plants (N_a) on this plot, according to the power function equation (GREENWOOD et al. 1990):

$$N_c = aW^{-b},$$

where:

N_c – critical content of total nitrogen in the plant (g kg⁻¹ DM);

W – dry matter of plants: leaves, stems, tubers (t ha⁻¹);

a – nitrogen content with the dry matter yield of 1 t ha⁻¹;

b – nitrogen content depending on the increase in dry matter.

The nitrogen nutrition index of 1 means optimal nutrition of plants with nitrogen, while the NNI below 1 – a deficit of nitrogen, and values exceeding 1 NNI – an excess of nitrogen. Immediately prior to collecting potato plants to determine the NNI index, the SPAD (Soil Plant Analysis Development) index was measured at any given time using a Minolta Camera SPAD 502 (scale 0 - 80). On each plot, 30 measurements were taken on the fourth and fifth leaf counting from the top of the stem growth, i.e. 10 measurements on each of the three plants. Based on the SPAD measurements and the NNI values, the dependences between the results obtained were determined.

The relationship between SPAD measurements and NNI indices is described by a linear regression model:

$$Y = a + bx,$$

where:

Y – SPAD values,

a – minimum value of the component,

b – increase of the component's value under the influence of the nitrogen dose,

x – NNI value

The experimental results were statistically analyzed by regression and variance, using an ANOVA statistical program. Comparisons of the means were carried out using the Tukey test at $p = 0.05$.

RESULTS AND DISCUSSION

Nitrogen fertilization was the factor significantly differentiating the dry matter yield, nitrogen content and nitrogen nutritional status as determined by NNI and SPAD indicators of the potato cultivars during the growing period (Tables 1, 2, 3). A significant increase in the dry matter yield at each

Table 1

The influence of a cultivar and nitrogen dose (kg ha^{-1}) on indicators of growth and nitrogen nutritional status of potato plants determined at day 28 after emergence

Cultivar	N dose	W*	N**	NNI index	SPAD index
Gwiazda	0	1.01c	36.37c	0.61d	40.9c
	50	1.40b	37.15c	0.73c	42.0b
	100	2.24a	39.64c	0.97b	43.4b
	150	2.15a	44.18b	1.07a	44.3a
	200	2.04a	45.93a	1.09a	44.8a
Etiuda	0	0.99c	41.50c	0.72d	41.5c
	50	1.30b	41.54c	0.86c	42.1c
	100	2.01a	42.12c	0.99b	43.5b
	150	1.91a	47.16b	1.09a	44.8a
	200	1.81a	48.57a	1.10a	44.9a
Gustaw	0	1.46d	35.38b	0.71c	41.8d
	50	2.00c	37.73b	0.80c	42.5c
	100	2.74a	36.60b	0.99b	43.5b
	150	2.52b	40.64a	1.08a	44.2a
	200	2.40b	42.38a	1.10a	44.4a

Mean values marked in columns with the same letters do not differ significantly.

* dry mass of plants (t ha^{-1}); ** nitrogen content in dry mass (g kg^{-1}).

Table 2

The influence of a cultivar and nitrogen dose (kg ha^{-1}) on indicators of growth and nitrogen nutritional status of potato plants determined at day 42 after emergence

Cultivar	N dose	W*	N**	NNI index	SPAD index
Gwiazda	0	2.71c	25.22c	0.64d	41.3d
	50	3.53b	26.39b	0.81c	42.2c
	100	4.89a	28.45b	1.02b	43.8b
	150	4.88a	30.95a	1.11a	45.0a
	200	4.75a	32.23a	1.14a	45.3a
Etiuda	0	2.77d	21.97c	0.62d	41.4d
	50	4.25c	23.27c	0.81c	42.2c
	100	6.84a	27.02b	1.02b	43.7b
	150	5.02b	31.71a	1.17a	45.6a
	200	4.81b	33.84a	1.22a	46.0a
Gustaw	0	3.20e	22.11c	0.66d	41.7c
	50	4.33d	23.77b	0.79c	42.3c
	100	5.28c	24.66b	1.02b	43.6b
	150	6.64a	28.15a	1.16a	45.1a
	200	6.05b	29.70a	1.18a	45.3a

Mean values marked in columns with the same letters do not differ significantly.
* dry mass of plants (t ha^{-1}); ** nitrogen content in dry mass (g kg^{-1}).

Table 3

The influence of a cultivar and nitrogen dose (kg ha^{-1}) on indicators of growth and nitrogen nutritional status of potato plants determined at day 56 after emergence

Cultivar	N dose	W*	N**	NNI index	SPAD index
Gwiazda	0	6.12e	18.30d	0.61e	41.5e
	50	7.13d	19.32d	0.80d	42.4d
	100	11.26a	20.87c	1.03c	43.7c
	150	10.78b	24.20b	1.17b	44.5b
	200	8.26c	28.62a	1.28a	46.7a
Etiuda	0	7.34d	16.71c	0.72d	42.1c
	50	8.59c	18.02b	0.84c	42.6c
	100	12.85a	18.72b	1.05b	43.9b
	150	12.44a	20.55a	1.14a	45.3a
	200	11.23b	22.81a	1.21a	45.6a
Gustaw	0	6.19c	14.58d	0.58d	41.4d
	50	9.83b	16.88c	0.85c	42.3c
	100	10.95a	19.41b	1.03b	43.8b
	150	10.62a	21.05a	1.10a	44.5a
	200	10.24b	22.27a	1.14a	44.7a

Mean values marked in columns with the same letters do not differ significantly.
* dry mass of plants (t ha^{-1}); ** nitrogen content in dry mass (g kg^{-1}).

assessment date was recorded in response to a dose of 100 kg N ha⁻¹. Under the influence higher fertilization levels, 150 and 200 kg N ha⁻¹, a tendency of decreasing or a proven decrease in the dry matter yield of plants was usually observed. However, a significant increase in the nitrogen content in the dry matter of plants was found up to the dose of 200 kg N ha⁻¹. Regardless of a cultivar and evaluation date, the yield of plant dry matter was 6.41 t ha⁻¹ under the impact of 100 kg N ha⁻¹, decreasing by 11.9% after the application of 200 kg N ha⁻¹. In turn, the nitrogen content in the yield of dry plant matter after the application of 200 kg N ha⁻¹ was higher by 31.3% in relation to the object where nitrogen was not applied (Table 4). With the growth of potato plants, from the phase of plant cover in inter rows (28 DAE) to the fruit setting phase (56 DAE), a significant increase in the dry matter yield of plants and a significant reduction in the nitrogen content were demonstrated (Table 4). It was also shown that the cultivars Etiuda and Gustaw were characterized by a significantly higher dry matter yield during the growing season than the early cultivar Gwiazda, whereas cv. Gustaw had a significantly reduced nitrogen content in the dry matter compared to the other cultivars (Table 4). Such dependences of the yield and nitrogen content in relation to nitrogen fertilization and those associated with the growth and development of potato genotypes during the growing season were consistent with the studies carried out thus far (DE LA MORENA et al. 1994, JOERN, VITOSH 1995, VOS 1997, LOVE et al. 2005, KUMAR et al. 2007).

Table 4

Total yield of dry mass (t ha⁻¹) and indices of the nitrogen nutritional status of potato plants in depending on a nitrogen dose (kg ha⁻¹), cultivar and date of assessment

Factors	W*	N**	NNI index	SPAD index
Dose of N (kg ha ⁻¹)				
0	3.56d	25.60c	0.66d	41.5d
50	4.73c	27.00b	0.82c	42.3c
100	6.41a	28.47b	1.01b	43.6b
150	6.13a	31.89a	1.12a	44.8a
200	5.65b	33.69a	1.15a	45.2a
Cultivar				
Gwiazda	4.76b	30.46a	0.93a	43.4a
Etiuda	5.60a	29.63a	0.97a	43.6a
Gustaw	5.51a	27.90b	0.95a	43.3a
Date of assessment				
28	1.87c	41.12a	0.92a	43.2a
42	4.66b	27.29b	0.96a	43.6a
56	9.06a	20.48c	0.97a	43.5a

Mean values marked in columns with the same letters do not differ significantly.

* dry mass of plants (t ha⁻¹); ** nitrogen content in dry mass (g kg⁻¹).

Determinations of the dry matter yield of whole potato plants and the dry matter nitrogen content enabled us to assess the nutritional status of nitrogen using an NNI test, i.e. by expressing the ratio of the current nitrogen content to the critical, which is the minimum amount required to achieve the maximum yield. In this study, the critical curve determined for the yield of potato plants at an optimal dose of nitrogen was in the form: $N_c = 5.43W^{0.42}$ (Figure 1). The coefficients a and b of the critical curve for

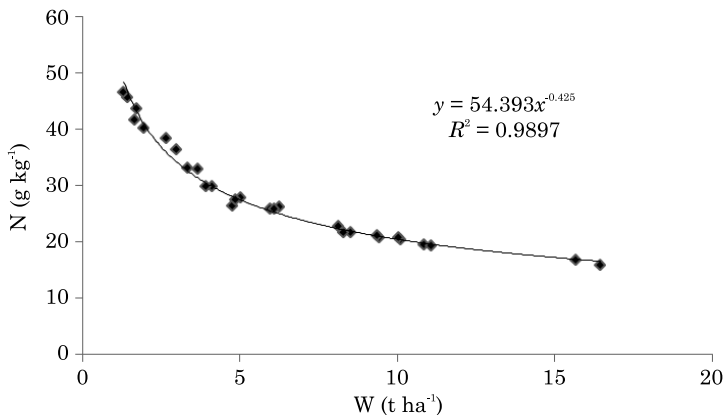


Fig. 1. The relationship between the nitrogen content and dry matter potato plants in the treatment characterized by the highest yield

potato plants were therefore lower than those determined by GREENWOOD et al. (1990). Smaller values of the coefficients of critical equations than demonstrated by GREENWOOD et al. (1990) were also reported by other researchers (DUCHEUNE et al. 1997, LEMAIRE, GASTAL 1997, BÉLANGER et al. 2001). In this experiment, it was shown that the nitrogen nutrition status (NNI) for potato cultivars increased in the analyzed development phases along with the increase in the mineral nitrogen fertilization dose (Tables 1, 2, 3). Optimal nitrogen nutrition, that is an NNI index value in the range from 0.95 to 1.05, was obtained at the dose of 100 kg N ha⁻¹, while nitrogen applied in the dose of 50 kg N ha⁻¹ indicated NNI values well below 1, suggesting malnutrition, while doses of 150 and 200 kg N ha⁻¹ led to NNI values above 1.05, indicating excessive nutrition with this element (Tables 1, 2, 3). Our previous research and studies reported by others have also confirmed that a nitrogen dose of 100 - 120 kg N ha⁻¹ corresponded to NNI values oscillating around unity, and a dose of 200 kg N ha⁻¹ limited the plant growth with NNI values between 1.1 and 1.4 (BÉLANGER et al. 2001, WIERZBICKA, TRAWCZYŃSKI 2015). On average, the nitrogen nutrition index ranged from 0.66 on the plots without the use of nitrogen to 1.15 for the plots with the highest dose this element, i.e. 200 kg N ha⁻¹ (Table 4). In turn, the cultivars and dates of assessment (plant development phase) had a statistically unproven effect on the nutritional status of potato plants with nitrogen (Table 4). Thus, from the values of the NNI indices presented in Tables 1, 2, 3, 4, it appeared that

no particular attention should be paid to the selection of the plant growth phase when determining their nutritional status with nitrogen. The variability of the nitrogen status indicator (NNI) depending on the nitrogen dose used indicates that the latter can be used as a test to assess the nutrient demand for this element by a plant. In the NNI test, however, a sample is required to determine the total dry matter yield of the plants and the total nitrogen content. A significant simplification in the use of plant tests to assess the nutritional status of plants with nitrogen may be by applying portable optical devices which – by measuring the emission of light of different wavelengths passing through a leaf – help to determine the so-called SPAD, an index of its greenness or chlorophyll content. Practical application of the SPAD test to assess the nutrition of plants with nitrogen must be connected with its calibration, that is the determination of critical value of SPAD corresponding to the optimal nitrogen nutritional status of the plants. In the tests performed, the calibration of the SPAD for a potato test was based on the NNI index. Calibration of tests to assay values of the leaf greenness SPAD index with respect to the plants' nitrogen nutritional status expressed by the NNI value has been performed mainly on cereals (FOTYMA, BEZDUSZNIK 2000, PECIO, BICHOŃSKI 2003). For potato plants, MINOTTI et al. (1994), when using an N-Tester graduated in the range of 0-80, obtained critical readings greater than in the current research, that is in the range of 49 - 56 SPAD units. Higher values of the SPAD index, i.e. from 49 to 60 units, were reported by PUIU et al. (2012). According to BOOIJ et al. (2000), average values achieved by SPAD chlorophyll were between 40 and 41 units in 7 to 8 weeks after the emergency of potato crop. However, GIANQUINTO et al. (2004), in studies on different potato cultivars where a SPAD-502 meter was employed, found critical values of plant nitrogen nutrition within the limits of 40 - 45 SPAD units, that is similar to our results. In turn, RYKACZEWSKA (2005), when analyzing nitrogen fertilization in a range of from 0 to 200 kg N ha⁻¹, stated that the SPAD values fluctuated within a broad range from 30 to 50 units and varied depending on a cultivar and measurement date, as the index values for developed plant potato tended to decrease. In the research by MAJIČ et al. (2008), values of the SPAD index are between 57 to 34 units at 65 to 95 day after planting of tubers. Values between 20 and 50 SPAD units in one potato cultivar fertilized in a range of doses from 0 to 250 kg N ha⁻¹ were also shown by VOS and BOM (1993). Neither VOS and BOM (1993), RYKACZEWSKA (2005) nor MAJIČ et al. (2008) indicated the critical values of SPAD units, that is referring to the optimal nutritional status of potato plants with nitrogen during the growing season. In the current study it has been shown that the relationship between SPAD readings and NNI values was that of a linear regression for each assessment date (Figures 2, 3, 4) and depending on the earliness cultivars (Figures 5, 6, 7). The coefficients R² of the linear regression between the NNI test values and SPAD readings in relation to the measurement date (values from 0.88 to 0.90) and tested cultivars (values from 0.85 to 0.92) were highly significant. Substituting the value of NNI = 1,

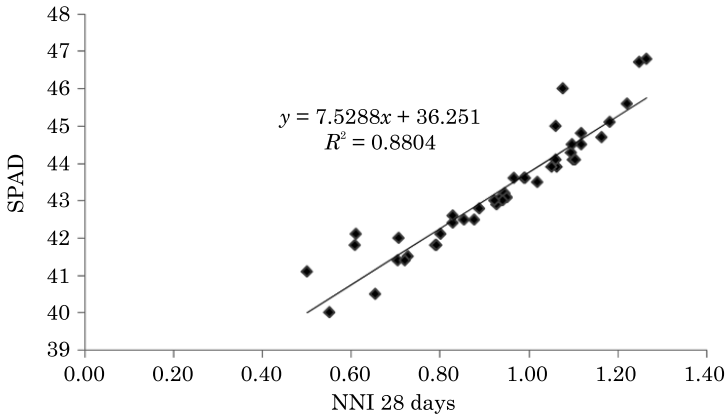


Fig. 2. The relationship between the NNI value and values of SPAD 28 days after emergence of plants

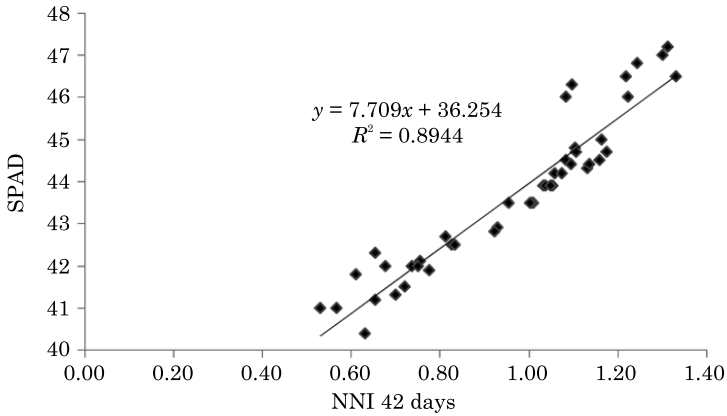


Fig. 3. The relationship between the NNI value and values of SPAD 42 days after emergence of plants

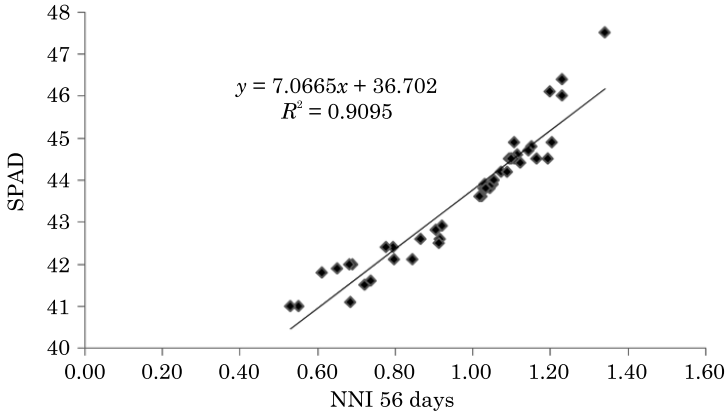


Fig. 4. The relationship between the NNI value and values of SPAD 56 days after emergence of plants

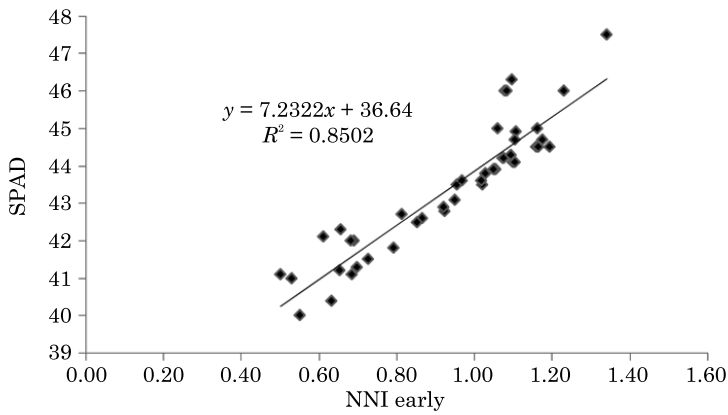


Fig. 5. The relationship between the NNI value and values of SPAD for the cultivar Gwiazda

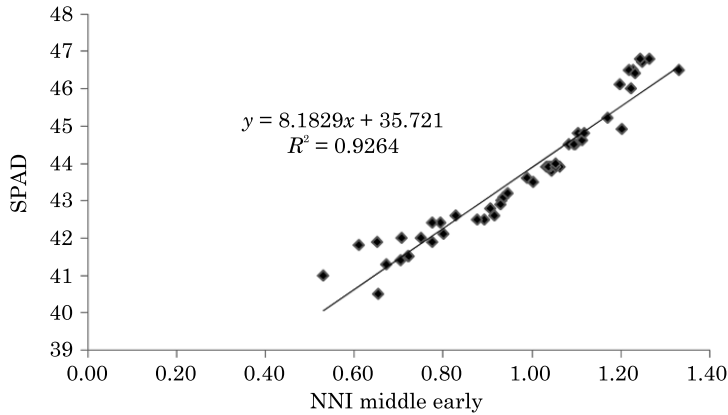


Fig. 6. The relationship between the NNI value and values of SPAD for the cultivar Etiuda

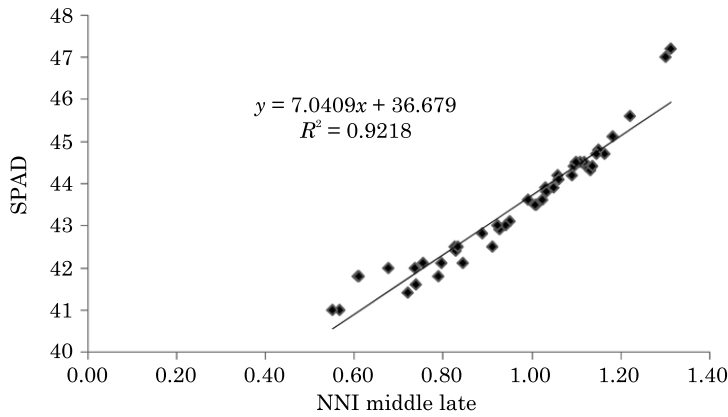


Fig. 7. The relationship between the NNI value and values of SPAD for the cultivar Gustaw

critical values of the SPAD readings were determined for the equations. It was found that the critical readings of SPAD in potato leaves at dates 28 DAE (development phase BBCH 30), 42 DAE (development phase BBCH 60) and 56 DAE (development phase 70) were similar, namely 43.8, 43.9 and 43.8 units, respectively. Slight variation in SPAD readings was also obtained in relation to cultivars with the optimal nutritional status, i.e. 43.7 for the cultivar Gustaw but 43.9 for the cultivars Gwiazda and Etiuda (Figures 5, 6, 7). With such SPAD readings, it can be assumed that potato plants are optimally nourished with nitrogen. The dependences determined in our experiment substantiate the conclusion that SPAD readings for cultivars optimally nourished by nitrogen are relatively independent of the phase of their growth and development. Hence, the values of the established SPAD green leaf index test units may refer to developmental phases, from the thinning of plants in inter rows to the fruit setting on potato plants during the growing season, during which foliar fertilization of potato plants with nitrogen is justified (MAZURCZYK et al. 2002).

CONCLUSIONS

1. Under the influence of mineral nitrogen 100 kg ha⁻¹ N, the optimal nutrition of potato plants was obtained at all dates and for each cultivar, determined on the basis of the NNI and SPAD tests.

2. The critical values of the SPAD test units were not significantly different between the measurement dates, i. e. the development phases of potato plants.

3. The SPAD value for the optimal nutritional status of potato plants with nitrogen in the growing season, from 28 DAE to 56 DAE, determined with an optical measuring instrument Minolta Camera SPAD 502, was 43.8 units on average.

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