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Nitrogen utilization of potato genotypes and expression analysis of genes controlling nitrogen assimilation

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Introduction: Significant differences in nitrogen use efficiency (NUE) were detected previously among potato cultivars. Exploration of the genetic background may facilitate the breeding of cultivars with highly effective nitrogen use. *Methods:* Expression of NUE genes was analyzed at three different N-supply levels in five potato genotypes. Correlations of NUE gene expressions and agronomical parameters with such indices as the nitrogen uptake efficiency, nitrogen utilization efficiency, NUE, and harvest indices were analyzed. *Results:* The correlations between expression level of the nitrate–reductase, nitrite–reductase, ammonium transporter, and asparagine synthase genes and different agronomically important parameters were detected. *Discussion:* Our results contribute to more rational, genotype-dependent nitrogen use in potato production and have relevance in breeding of new cultivars with better nitrogen utilization, as well as in production of seed potato.

Original Article

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INTRODUCTION

In the past 40 years, the doubling of agricultural food production of the world has been associated with a sevenfold increase in the use of nitrogen fertilizers (Hirel et al., 2007). Although the harmful effects of fertilizers on the environment are known, to meet the needs of a rapidly growing mankind, a highly productive agriculture should be developed, while simultaneously the quality of the environment should be preserved (Dyson, 1999). Interactions between genotype–nitrogen level reflecting to differences in responsiveness have been reported in several species in numerous studies (Ahmad et al., 2008; Arsenault et al., 2001; Berry et al., 2010; Gallais & Hirel, 2004; Marschner, 2012; Sanford & MacKown, 1986). The differences of nitrogen utilization among cultivated plant species are variant. Genotypic differences in N-needs have been studied among potato cultivars (Kleinkopf et al., 1981; Zebarth et al., 2004) and wild tuber-bearing potato species (Errebhi et al., 1999). These studies indicate that it is possible to breed potato cultivars with better N utilization. The genetic background of nitrogen assimilation is already explored in *Arabidopsis* (Vidal et al., 2010), and major nitrogen use efficiency (NUE) genes have also been identified in potato (Li et al., 2010).

In previous field experiments, we detected significant differences in the NUE of potato cultivars (Hoffmann et al., 2013, 2014). It was found that some genotypes do not react with yield decrease even when only the half of the conventionally applied nitrogen dose is applied. Then, in a pot experiment, five potato genotypes were tested at three different nitrogen supply levels, and their different parameters were measured and evaluated (Kollaricsné et al., 2015). Based on this later experiment, in this study, the NUE of these five potato genotypes is characterized. Further purpose of this study is to analyze the expression of major NUE genes in these five genotypes at the three N-supply levels, as well as to analyze the correlation of the different parameters and of the expression of these genes.

MATERIALS AND METHODS

Plant material

Tubers with an average weight of 43.8 g of the cultivars White Lady, Katica, Hópehely, and Chipke and of the breeding line S440 were potted in N-free quartz sand in 3 L pots and grown under greenhouse conditions. The tubers of S440 were somewhat larger, with an average weight of 56.9 g. Nutrients were supplied with 500 ml/week/plant 0.5 strength Hoagland medium. After the third week in three different treatments, the following nitrogen concentrations were used: 7.50, 3.00, and 0.75 mmol in the form of nitrate. The concentration of 7.50 mmol is equal to the nitrogen dose used generally in potato production, and is equal to the nitrogen concentration of the 0.5 strength Hoagland medium. The concentrations 3.00 and 0.75 mmol are considerd as medium and weak nitrogen supply, respectively. In each treatment, in three replicates, 20 plants/cultivar/replicate were grown in randomized arrangement. The Hoagland medium was weekly supplied in 500 ml ion-exchanged water (IEW) and depending on the water demand of the plants IEW was provided daily.

Analyzed parameters

Chlorophyll content was measured on the 0th day and then on all 7th day with a SPAD-502 (Konica Minolta, Osaka, Japan) chlorophyll meter. On the adaxial surface of the leaves, 10 measurements per plant were performed always between 9:00 and 11:00 a.m. according to the method of Jongschaap and Booij (2004).

During and after harvesting, the following parameters were measured and calculated:

Leaf area: The complete foliage of the plants was detached and immediately measured with an AM300 leaf area meter (ADC BioScientific, UK).

Fresh weights: The surface of roots and tubers was dried with paper towel and their weight was measured using an analytical balance.

Dry weights: All parts of the plants were dried at 65 °C for 24 hr and their weight was measured.

Harvest index: Two harvest indices were calculated according to the publications of Vos (1997) and Belanger et al. (2001). HI_1 is generally used for potato, but the pot experiment also allowed the calculation of HI_2 where the root weight is also included in the calculation:

$$HI_1 = \frac{\text{Dry weight of tubers}}{\text{Dry weight of tubers} + 1.25 \times \text{dry weight of foliage}},$$

$$HI_2 = \frac{\text{Dry weight of tubers}}{\text{Dry weight of tubers} + \text{dry weight of foliage} + \text{dry weight of roots}}.$$

Nitrogen content. The nitrogen content of tubers, foliage, and roots was measured separately. Then, for each, the dried plant material of the three repeats of each treatment was taken together and comminuted to fine powder. Each batch was divided into three to measure the N-content of each samples three times. N-content was measured with the Kjeldahl method (Persson et al., 2008).

To characterize nitrogen utilization of the cultivars, the nitrogen uptake efficiency (NUpE; Li et al., 2015; Zebarth et al., 2004), the nitrogen utilization efficiency (NUtE; Zebarth et al., 2004), and the NUE (Hirose, 2012; Kleinkopf et al., 1981; Ospina et al., 2014; Zebarth et al., 2004) have been calculated.

$$NU_pE = \frac{\text{Total N content of plant (g)}}{\text{Total avaible N (g)}},$$

$$NU_tE = \frac{\text{Total dry matter of plant (g)}}{\text{Total N content of plant (g)}},$$

$$NUE = \frac{\text{Total dry matter of plant(g)}}{\text{Total available N (g)}}.$$

Expression analysis of nitrogen assimilation genes

For gene expression analyses from each plant, one leaflet of a potato leaf was sampled on the 0th day (the last day before treatment), as well as on the 7th, 14th, 28th, and 42nd day. Total RNA was isolated with RNAzol® RT (Sigma-Aldrich, USA), and after DNase treatment (RNase Free DNase Set, New England Biolabs, UK), cDNA synthesis was performed with the High Capacity cDNA Reverse Transcription Kit (Life Technologies, USA). Expression of the nitrate–reductase (NR), nitrite–reductase (NiR), ammonium transporter (AMT), and asparagine synthase (AS) genes was studied as described by Li et al. (2010). For reference, the cytochrome-oxidase I (coxI) was used. The qPCR experiment was performed in a StepOne Real-Time PCR System (Applied Biosystems, USA). For detection, the Power Sybr Green Mix (Applied Biosystems) was used. The reaction was evaluated using "StepOne Software v2.3" (Applied Biosystems).

Statistical evaluations

The data recorded during the experiments were statistically evaluated using the IBM SPSS Statistics for Windows, version 20.0 software (IBM Corp., Armonk, NY, USA). Analysis of variance was performed to check the significance of differences in treatments and genotypes. The grand means were compared using least significant difference and Duncan's *post hoc* test ($p < .05$). Two-sample *t*-test was used ($p < .05$) for the analysis of the Ct values of qPCR ($\Delta\Delta CT$ method), with this the difference from the N7.50 treatment was evaluated at the given measurement time.

The correlation between the examined parameters was analyzed by Microsoft Office Excel 2007 program. Guilford's (1950) method was used to interpret the Pearson's correlation coefficient.

RESULTS

Analysis of agronomically important parameters

Most parameters, such as the area of the foliage, tuber parameters, fresh and dry weights, and nitrogen contents, could be measured only at or after harvesting, and the harvest indices as well as NUpE, NUtE, and NUE could

be calculated when data of these parameters were already obtained. The results are shown in Table 1.

Effect of N-supply on soil–plant analysis development (SPAD) values

Nitrogen deficiency of leaves can be detected with a chlorophyll content meter. The technology developed by the Minolta company measures in so-called SPAD units to express chlorophyll content. In this experiment, except the cultivars White Lady and Katica, significant differences have been recognized among the SPAD values of the compared five potato genotypes (Table 2).

Expression of major NUE genes

Except for White Lady, the NR gene expression pattern was similar in the three treatments, and it was different among all the cultivars (Fig. 1A). In Katica, in the N0.75 treatment, a pronounced upregulation peak was observed at the 7th treatment day (TD).

Except Katica, the expression pattern of the NiR gene was similar at medium and high N-supply in the genotypes, although it was different among all of them. At low N-supply, NiR expression pattern was different from that of the other two treatments (Fig. 1B).

Expression pattern of the AMT gene was different among the cultivars (Fig. 1C). In White Lady at N0.75, the AMT expression pattern was different from the other two treatments. For S440 unfortunately, the 42nd TD sample was lost. In Katica, in all treatments, a pronounced upregulation peak of the AMT gene was observed on the 7th TD.

The AS gene expression pattern was different among the cultivars, and at low N-supply, it was different from the other two treatments (Fig. 1D). In S440, in all treatments, very different expression pattern was obtained for the 0th–28th TD period. In the N0.75 treatment on the 7th TD, it was downregulated in Katica and Chipke, whereas upregulated in S440 and Hópehely.

Correlation analysis of time series data

Chlorophyll content (SPAD values) and expression of major NUE genes were measured several times during 42 days of experimental period. Differences among NUpE, NUtE, and NUE indices of the cultivars are shown in Figs 2–4, respectively. Correlation analysis was performed and the results are presented in Table 3.

Pronounced positive correlation was found between the expression of the NR gene with the NiR, AMT, and AS genes before the treatments were started, i.e., on the 0th TD. Similarly, strong correlation was observed between NR and NiR, as well as NR and AMT expression on the 7th TD and then this correlation gradually weakened. The correlation between the AS and AMT gene expressions was moderately strong on the 42nd TD.

The SPAD values, i.e., the chlorophyll content of the leaves, showed on the 0th TD moderately strong correlation with the expression of the NR, AMT, and AS genes and a very weak correlation with the NiR gene expression. The correlation between SPAD values and NR expression was detectable until the 14th TD. Between SPAD values and AMT expression correlation could be revealed throughout the experiment but at very different levels.

Correlation analysis of major NUE gene expressions and agronomical parameters

Correlation data on the 42nd TD are shown in Table 4.

Expression of the NR gene showed moderate positive correlation with root N-content and moderate negative correlation with tuber N-content. Weak negative correlation of NR gene expression was observed with the leaf area, the plant total N-content, the tuber dried to fresh ration, the total tuber weight, and positive with the foliage N%.

Expression of the NiR gene showed moderate negative correlation with the root N, and moderate positive with the plant total N-content. Weak negative correlation was found with the leaf area, and positive with the tuber N-content and the tuber N%.

Expression of the AMT gene showed moderate negative correlation with the SPAD value, the leaf area, the foliage N-content, and the foliage N%. Weak negative correlation was found with the plant total N-content, the tuber N%, the total tuber weight, the average tuber weight, and positive with the tuber dried to fresh weight ratio.

Expression of the AS gene showed pronounced positive correlation with the average tuber weight, moderate negative correlation with the tuber number and weak negative correlation with the leaf area, the total N-content, the N% of the tuber, and with the dried to fresh weight ratio of the tubers.

Among the agronomically important parameters strong positive correlation was found between the SPAD values and leaf area, between the foliage N-content and foliage N%, and between the foliage N-content and tuber N%. Strong negative correlation was detected between the tuber N-content and the SPAD values, the foliage N-content and the root N-content.

Correlational relations of NUE indices

Correlation data are shown in Table 5.

Strong positive correlation was detected between NUpE and tuber dried to fresh ratio, between NUtE and NUE, between NUtE and the root to shoot weight ratio, and between the Harvest Index 2 and Harvest Index 1. Strong negative correlation is detected between the SPAD values and HI_1.

DISCUSSION

SPAD values can predict nitrogen deficiency some weeks before the symptoms would be visible. Except the tuber total weight, average tuber weight, and tuber number the SPAD values showed at least moderate correlation with all analyzed parameters relevant in nitrogen utilization. Our results are similar to the findings of Minotti et al. (1994) and Busato et al. (2010) who observed in the Allegheny and Castile cultivars, as well as in the Atlantic, Agata, Monalisa, and Asterix cultivars, respectively, significant chlorophyll content increase in correlation with growing N doses. At the

Table 1. Nitrogen utilization of potato genotypes and expression analysis of nitrogen assimilation genes

Genotypes and treatments		Leaf area	Fol N rat	Root N rat	Tuber N rat	Plant N%	Foliage N%	Tuber N%	Tub D–F rat	Tuber FW	Aver. tub W	Tuber no.	NUpE	NUtE	NUE	R–S ratio	HI_1	HI_2
LSD $p < .05$		758.60	0.07	0.11	0.02	0.14	0.08	0.14	0.050	18.52	5.80	1.73	0.22	3.75	13.44	90.11	11.65	13.00
White Lady	7.50	7491.00	0.26	0.37	1.76	0.43	1.91	1.76	0.17	185.10	10.89	17.00	0.60	56.26	34.39	143.81	59.37	40.78
	3.00	4961.89	0.20	0.44	1.58	0.42	1.65	1.58	0.18	146.95	7.35	20.00	0.86	66.69	57.65	223.43	60.39	35.90
	0.75	3111.89	0.16	0.23	1.52	0.23	0.90	1.52	0.18	150.70	8.37	18.00	0.73	83.67	61.27	201.24	67.58	50.78
S440	7.50	4325.33	0.23	0.25	1.98	0.51	1.85	1.98	0.15	276.65	30.74	9.00	0.64	41.76	26.69	108.63	61.07	49.91
	3.00	4162.78	0.19	0.18	1.90	0.48	1.61	1.90	0.19	260.15	26.02	10.00	0.88	45.99	40.45	94.41	68.91	58.84
	0.75	4552.11	0.17	0.25	1.84	0.40	1.34	1.84	0.25	224.70	13.22	17.00	0.88	55.70	49.49	168.29	73.63	54.05
Katica	7.50	5172.00	0.27	0.53	2.04	0.39	1.72	2.04	0.18	108.40	7.74	14.00	0.62	56.37	34.49	186.55	41.49	24.95
	3.00	5085.11	0.20	0.34	1.58	0.32	1.16	1.58	0.20	142.01	15.78	9.00	0.75	75.68	56.88	173.12	57.48	37.78
	0.75	4320.33	0.13	0.38	1.23	0.32	0.74	1.23	0.20	135.00	13.50	10.00	0.67	108.28	73.17	330.75	65.28	37.06
Hópehely	7.50	4270.33	0.29	0.54	2.34	0.25	2.07	2.34	0.12	49.30	6.16	8.00	0.40	47.45	18.73	216.52	44.80	27.38
	3.00	3200.56	0.25	0.38	1.51	0.22	1.81	1.51	0.14	118.85	14.86	8.00	0.51	59.39	30.70	150.01	54.75	40.68
	0.75	2846.33	0.17	0.46	1.38	0.18	1.46	1.38	0.16	101.55	11.28	9.00	0.56	69.86	39.17	277.86	63.48	38.67
Chipke	7.50	4521.33	0.22	0.44	1.48	0.38	2.04	1.48	0.18	141.10	7.84	18.00	0.60	55.32	33.12	211.20	63.08	40.78
	3.00	4152.00	0.21	0.36	1.45	0.28	1.70	1.45	0.17	147.70	10.55	14.00	0.65	63.27	41.29	175.30	66.30	35.90
	0.75	3299.44	0.18	0.25	1.33	0.19	1.21	1.33	0.20	136.30	12.39	11.00	0.57	78.41	44.67	166.76	72.14	55.95

Note. Leaf area: total leaf area of the plants in mm^2; fol N rat: ratio of foliage N compared to the total plant N; root N rat: ratio of root N compared to the total plant N; tuber N rat: ratio of tuber N compared to the total plant N; Plant N%: N content of the total plant in %; foliage N%: N content of the foliage in %; tuber N%: N content of the tuber in %; Tub D–F rat: dried and fresh weight ratio of tubers; Tuber FW: fresh weight of tubers/plant; aver. tub W: average tuber weight; Tuber no.: tuber number/plant; NUpE: total N content of plant (g)/total available N (g); NUtE: total dry matter of plant (g)/total N content of plant (g); NUE: total dry matter of plant (g)/total available N of plant (g); R–S ratio: root–shoot ratio; HI_1 = tuber dry weight/(tuber dry weight + 1.25 × foliage dry weight); HI_2 = tuber dry weight/(tuber dry weight + foliage dry weight + tuber dry weight); LSD: least significant difference.

Table 2. Nitrogen utilization of potato genotypes and expression analysis of nitrogen assimilation genes

		SPAD values						
Genotypes	N treatments	TD0	TD7	TD14	TD21	TD28	TD35	TD42
White Lady	N0.75	47.8^{cd}	45.6^{cd}	39.4^{cd}	36.5^{cde}	36.5^{ef}	27.6^{cd}	23.3^{b}
	N3.00	48.9^{d}	49.1^{g}	43.1^{ef}	38.4^{ef}	39.2^{gh}	36.7^{h}	29.7^{f}
	N7.50	50.4^{d}	52.4^{h}	47^{g}	43.5^{h}	44.2^{i}	41^{i}	33.7^{h}
LSD			0.6	0.7	1.0	0.4	0.6	0.7
S440	N0.75	38^{a}	35.3^{a}	35^{a}	34.3^{a}	31.3^{a}	21.3^{a}	21^{a}
	N3.00	39.8^{a}	35.5^{a}	37^{ab}	35.8^{abc}	31.9^{ab}	25.9^{bc}	25.5^{cd}
	N7.50	37.5^{a}	35.5^{a}	36.9^{ab}	36.9^{cde}	35^{de}	28.9^{de}	28.7^{f}
LSD			1.0	1.4	0.5	0.7	0.8	0.6
Katica	N0.75	48.6^{d}	44.8^{cd}	39.2^{bcd}	35.3^{ab}	33^{bc}	31^{f}	20.1^{a}
	N3.00	50^{d}	48.8^{fg}	44.8^{fg}	38.3^{def}	40^{h}	37.1^{h}	27.3^{e}
	N7.50	50^{d}	48.5^{g}	44.9^{fg}	42.4^{gh}	43.2^{i}	43.5^{j}	32.4^{g}
LSD			1.0	1.1	1.1	1.0	0.4	0.5
Hópehely	N0.75	44.8^{bc}	41^{b}	38.2^{bc}	34.1^{a}	30.8^{a}	30.5^{ef}	24.4^{bc}
	N3.00	44.8^{bc}	40.7^{b}	40.3^{cd}	34.5^{ab}	34.3^{cd}	32.8^{g}	26.8^{de}
	N7.50	44.5^{b}	44.1^{c}	43.1^{ef}	36.9^{cde}	40.5^{h}	36.2^{h}	32.1^{g}
LSD			0.9	0.8	0.6	0.5	0.7	0.4
Chipke	N0.75	45.4^{bc}	45.9^{cde}	40.9^{de}	36.4^{bcd}	33^{bc}	22.2^{a}	21^{a}
	N3.00	47.8^{cd}	48.1^{efg}	45.4^{fg}	39^{f}	38^{fg}	24.5^{b}	23.1^{b}
	N7.50	45.5^{bc}	46.7^{def}	44.3^{f}	41^{g}	37.8^{fg}	26.2^{bc}	26.1^{de}
LSD			1.2	0.7	0.5	0.8	0.8	0.5

Note. Different letters after the values indicate significant differences. SPAD values represent chlorophyll content; SPAD: soil–plant analysis development; TD: treatment day; LSD: least significant difference.

42nd day of our experiment significant difference in the relative chlorophyll content could be observed in each cultivar at the different N-dose treatments, which correlates with the results obtained on other cultivars by Jongschaap and Booi (2004).

The level of N-supply affects the root–shoot ratio in shrubs (Lloret et al., 1999) and wheat (Shangguan et al., 2000). We obtained similar results in potato. Furthermore, our results indicated pronounced positive correlation between the root–shoot ratio and the NUtE index (Table 5).

Among the tested genotypes cultivar White Lady produced the highest yield, while medium yield was produced by Katica and Chipke and the smallest by Hópehely and S440 (Table 1). White Lady and Katica cultivars have the best nitrogen utilization parameters among the tested potato genotypes, in point of NUpE and absorbed nitrogen utilization (NUtE) as well. Analysis of the effect of N-supply on total tuber yield verified the results of Hoffmann et al. (2013) obtained in plough-land experiments, indicating that in cultivar Katica and Hópehely the medium N-supply results significantly higher yield than the conventionally used high N-supply. In cultivar Chipke, total tuber yield did not differ significantly among the three N-supply levels. In cultivar White Lady, the highest yield was obtained at high N-supply. Although all genotypes utilized nitrogen with higher efficiency in N0.75 treatments, smaller yield was obtained than in the other two treatments where N-supply was higher.

According to our results, pot experiments are applicable for detection of differences in nitrogen utilization between genotypes, including pre-selection as well. Chlorophyll content, root–shoot ratio, yield parameters and harvest indices correlated the best with NUE indices in this pot experiment.

The expression pattern of major NUE genes in the examined genotypes was different. In N-assimilation, NR is the first enzyme that reduces the nitrate to nitrite (Bertero et al., 2003). Overexpression of the NR gene reduces the nitrogen accumulation. NiR is the second enzyme in N-assimilation that reduces the nitrite to ammonia (Takahashi et al., 2001). In our experiment, similar expression patterns of the NR and NiR genes were obtained. On analyzing expression of the NR and NiR genes, Li et al. (2010) observed also similar expression patterns in potato cultivars Shepody, Red Pontiac, and Russet Norkotah.

The AMT has a role in ammonia uptake, and is controlled by the available nitrate (Babourina et al., 2007). During nitrogen assimilation, the ammonium ion is further modified partially by the glutamine-dependent asparagines synthase (Gaufichon et al., 2010). In our experiment, the expression pattern of the AMT gene was different among the cultivars. The AS gene expression pattern was very different among the cultivars, and at low N-supply, it was different from the other two treatments.

In the present analysis, strong correlation between the expression of the studied genes and nitrogen utilization parameters was found. The NUpE index, which represents the efficiency of nitrogen uptake, showed a moderate, negative correlation with the expression of asparagine synthase, and a positive correlation with the expression of NiR genes. The NUtE index indicating the utilization efficiency of uptaken nitrogen showed a moderately strong negative correlation with NiR expression and a medium positive correlation with the expression of the AMT.

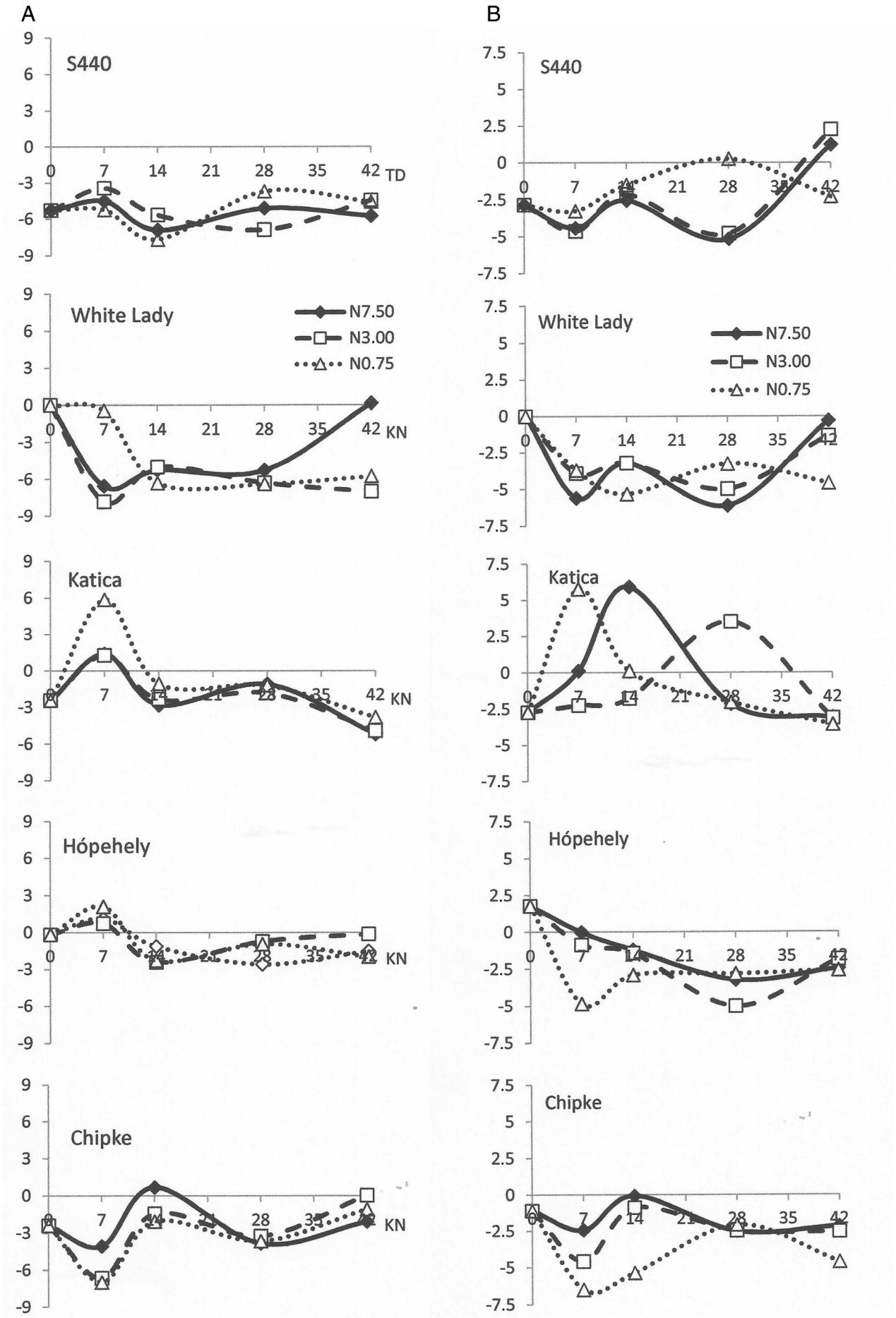

Fig. 1. Expression of the nitrate–reductase (A), nitrite–reductase (B), ammonium transporter (C), and asparagine synthase (D) genes in the four potato cultivars. Axis X indicates the days of treatment. Axis Y indicates the $\log_2$ values

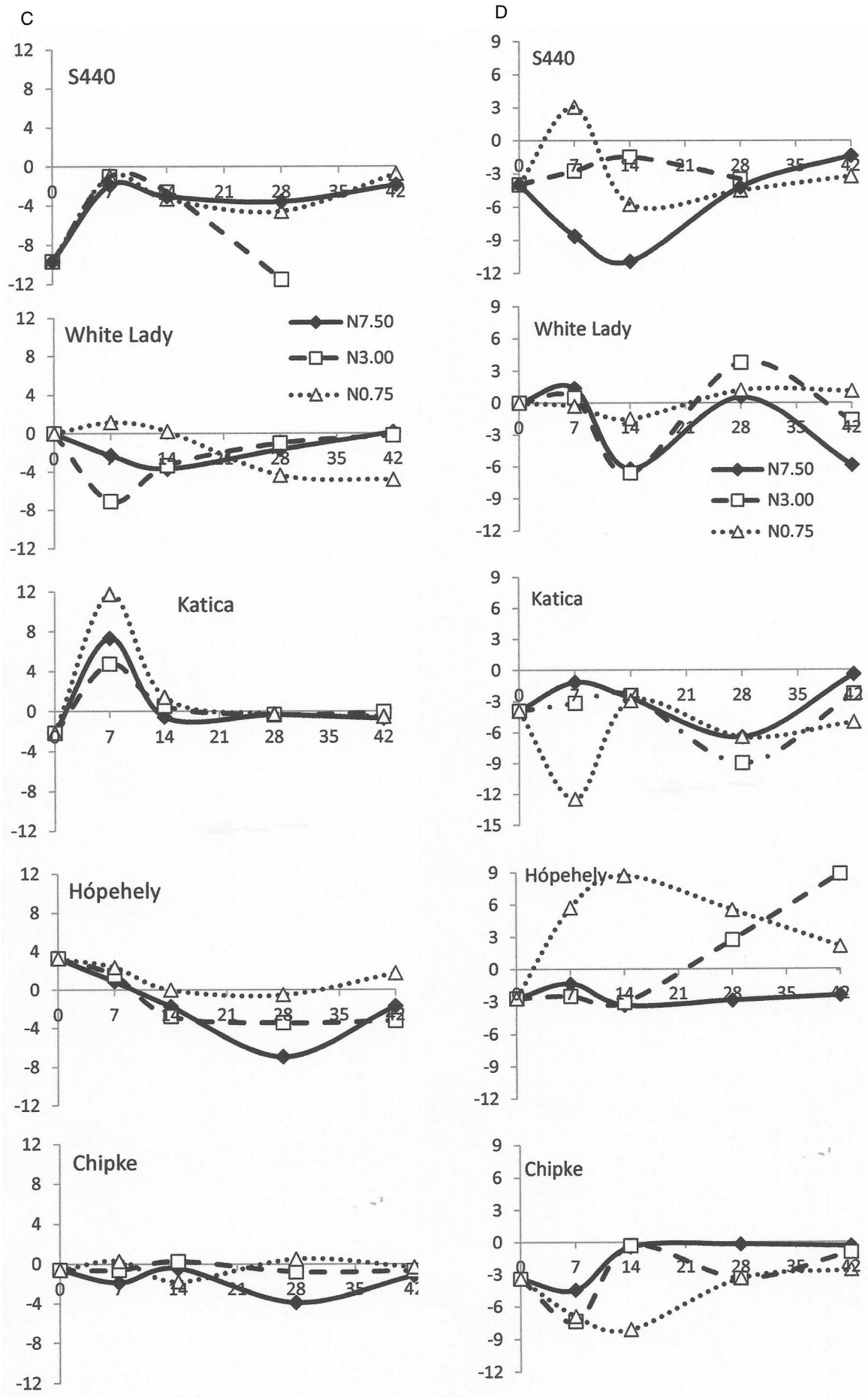

Fig. 1. (Continued)

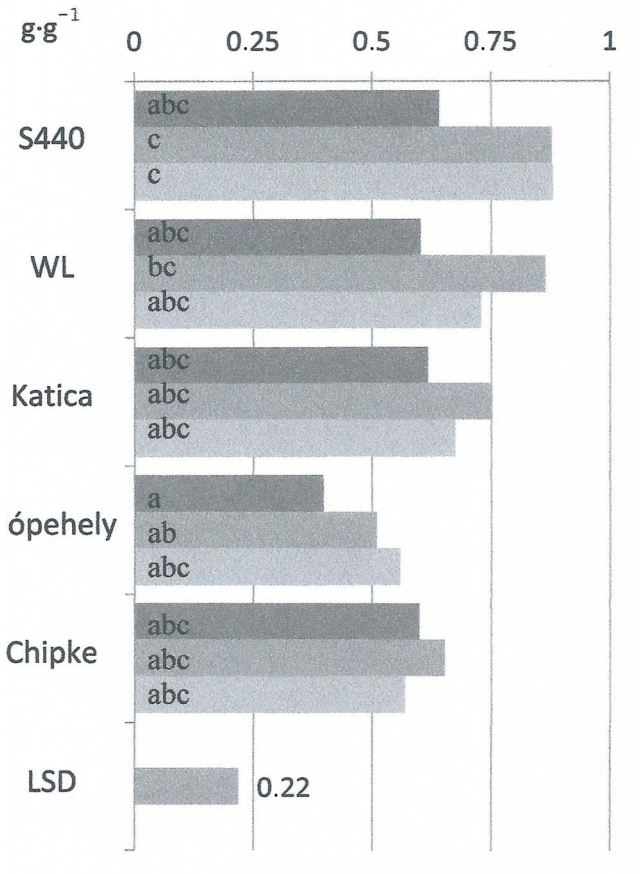

Fig. 2. NUpE indices of the analyzed genotypes. Different letters after the values indicate significant differences. LSD: least significant difference

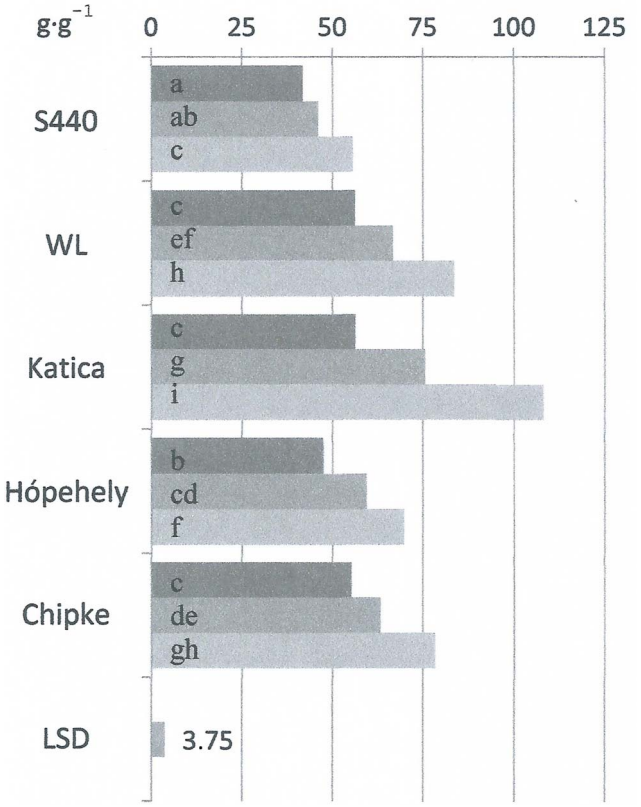

Fig. 3. NUtE indices of the analyzed genotypes. Different letters after the values indicate significant differences. LSD: least significant difference

All these results indicated that there are huge differences in optimal nitrogen requirement of potato cultivars. Information on NUE of potato cultivars could support farmers in more economic production and could easy the unnecessary environmental pollution by N-fertilizers. The detected genotype-dependent effect of N-supply on tuber number and weight could be utilized in more economic seed potato production and in breeding practice.

CONCLUSION FOR FUTURE BIOLOGY

The use of more than optimal nitrogen fertilizers increases the costs of potato production and endangers the environment. Optimization of the nitrogen supply of the different potato cultivars may alleviate these problems. However, pot tests are a good model for the characterization of NUE of potato cultivars; production tests under field conditions at different production sites and during several years should be performed to understand the real NUE of the different cultivars. Furthermore, the genetic background of NUE should be explored for breeding and development of new potato cultivars with high NUE and other desired characteristics.

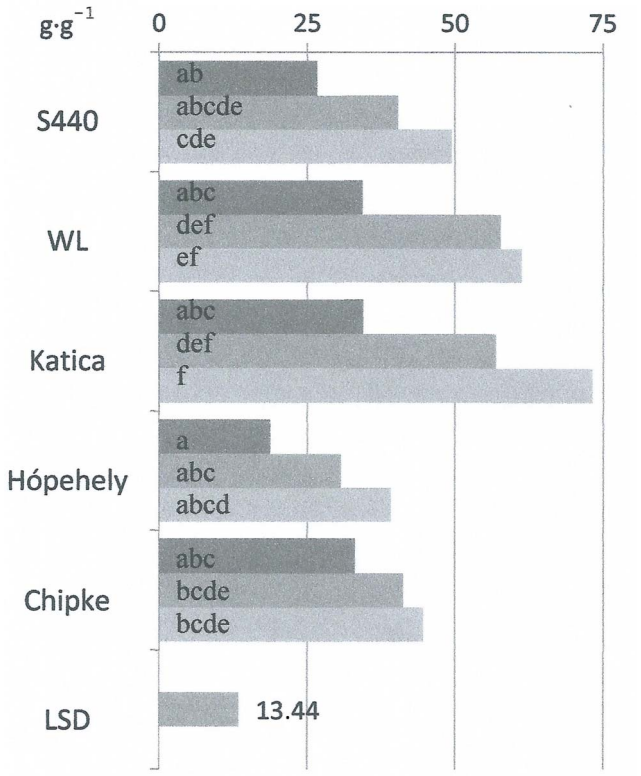

Fig. 4. NUE indices of the analyzed genotypes. Different letters after the values indicate significant differences. LSD: least significant difference

Table 3. Correlation analysis between the activity of the major NUE genes and SPAD values

	AMT TD0	AMT TD7	AMT TD14	AMT TD28	AMT TD42	NR TD0	NR TD7	NR TD14	NR TD28	NR TD42	NiR TD0	NiR TD7	NiR TD14	NiR TD28	NiR TD42	AS TD0	AS TD7	AS TD14	AS TD28	AS TD42	SPAD TD0	SPAD TD3	SPAD TD7	SPAD TD14	SPAD TD21	SPAD TD28	SPAD TD35	SPAD TD42
AMT TD0	1.00																											
AMT TD7	0.11	1.00																										
AMT TD14	0.31*	0.70***	1.00																									
AMT TD28	0.41**	0.29*	0.36*	1.00																								
AMT TD42	0.01	0.04	0.04	0.60**	1.00																							
NR TD0	0.93***	−0.03	0.14	0.34*	−0.08	1.00																						
NR TD7	0.28*	0.85***	0.62**	0.05	−0.09	0.19	1.00																					
NR TD14	0.63**	0.39*	0.59**	0.32*	0.24*	0.34*	0.36*	1.00																				
NR TD28	0.43**	0.70**	0.47**	0.48**	0.29*	0.18	0.66**	0.62**	1.00																			
NR TD42	0.51**	0.00	0.00	0.11	0.15	0.32*	−0.08	0.51**	0.36*	1.00																		
NiR TD0	0.83***	−0.26*	−0.10	0.03	−0.12	0.84***	0.08	0.27*	0.16	0.50**	1.00																	
NiR TD7	0.13	0.75***	0.45**	0.08	−0.14	0.05	0.76***	0.40**	0.55**	−0.13	−0.13	1.00																
NiR TD14	−0.06	0.50**	0.25*	0.10	0.14	−0.19	0.39*	0.34*	0.52**	−0.08	−0.33*	0.58**	1.00															
NiR TD28	−0.10	0.44**	0.53**	0.29*	0.23*	−0.25*	0.29*	0.23*	0.42**	−0.18	−0.40*	0.21*	0.19	1.00														
NiR TD42	−0.54**	−0.46**	−0.62**	−0.59**	0.12	−0.43**	−0.33*	−0.43**	−0.46**	−0.10	−0.19	−0.28*	−0.01	−0.53**	1.00													
AS TD0	0.46**	−0.45**	−0.26*	0.09	−0.22*	0.73***	−0.27*	−0.27*	−0.47**	−0.02	0.56**	−0.29*	−0.48**	−0.48**	−0.07	1.00												
AS TD7	0.20*	−0.35*	−0.33*	−0.13	0.17	0.35*	−0.09	−0.33*	−0.08	−0.04	0.45**	−0.39*	−0.11	−0.03	0.03	0.44**	1.00											
AS TD14	0.43**	0.28*	0.58**	0.01	0.26*	0.32*	0.47**	0.42**	0.39*	0.23*	0.37*	0.08	0.15	0.18	−0.22*	−0.04	0.38*	1.00										
AS TD28	0.51**	−0.51**	−0.28*	−0.02	−0.05	0.62**	−0.20	−0.06	−0.19	0.21*	0.79*	−0.37*	−0.42**	−0.60**	0.07	0.67**	0.54**	0.34*	1.00									
AS TD42	0.39*	−0.04	0.00	−0.17	−0.43**	0.30*	0.23*	0.13	0.32*	0.16	0.51**	−0.03	0.02	−0.20*	−0.04	0.01	0.20*	0.36*	0.49**	1.00								
SPAD TD0	0.65**	0.40*	0.57**	0.56**	0.00	0.68**	0.30*	0.45**	0.23*	0.10	0.21*	0.32*	0.17	0.12	−0.61**	0.44**	−0.07	0.25*	0.04	−0.08	1.00							
SPAD TD3	0.61**	0.34*	0.56**	0.60**	−0.15	0.60**	0.16	0.41**	0.20*	0.21*	0.19	0.23*	0.11	0.11	−0.68**	0.40**	−0.18	0.14	−0.01	−0.11	0.93***	1.00						
SPAD TD7	0.61**	0.27*	0.52**	0.51**	−0.14	0.63**	0.13	0.42**	0.08	0.15	0.20*	0.21*	0.09	0.05	−0.64**	0.48**	−0.13	0.13	0.04	−0.19	0.94***	0.96***	1.00					
SPAD TD14	0.54**	0.34*	0.57**	0.27*	−0.34*	0.51**	0.23*	0.49**	0.12	0.25*	0.19	0.35*	0.23*	−0.08	−0.53**	0.30*	−0.29*	0.17	−0.01	−0.05	0.80***	0.88***	0.91***	1.00				
SPAD TD21	0.12	0.28*	0.51**	0.09	−0.42**	0.17	0.16	0.19	−0.20*	−0.10	−0.14	0.35*	0.08	−0.11	−0.33*	0.26*	−0.44**	0.00	−0.10	−0.26*	0.56**	0.65**	0.72***	0.83***	1.00			
SPAD TD28	0.32*	0.32*	0.38*	0.11	−0.47**	0.40**	0.28*	0.19	−0.05	−0.05	0.08	0.51**	0.21*	−0.20	−0.34*	0.38*	−0.31*	−0.08	−0.07	−0.23*	0.65**	0.71***	0.76***	0.87***	0.87***	1.00		
SPAD TD35	0.37*	0.59**	0.38*	0.11	−0.31*	0.48**	0.70**	0.12	0.23*	−0.09	0.19	0.63**	0.22*	−0.15	−0.25*	0.33*	−0.09	0.13	−0.02	−0.03	0.61**	0.52**	0.55**	0.59**	0.56**	0.77***	1.00	
SPAD TD42	0.20*	0.28*	0.14	−0.25*	−0.52**	0.32*	0.44**	−0.01	−0.09	−0.01	0.24*	0.46**	0.07	−0.48**	0.01	0.34*	−0.17	−0.01	0.12	−0.03	0.26*	0.28*	0.35*	0.56**	0.65**	0.80***	0.83***	1.00

Note. SPAD values represent chlorophyll content. NUE: nitrogen use efficiency; AMT: ammonium transporter expression; NR: nitrate–reductase exp.; NiR: nitrite–reductase exp.; AS: asparagine synthase exp.; SPAD: soil–plant analysis development; TD: treatment day.

*Existing but weak correlation. **Moderate significant correlation. ***High-pronounced correlation.

Table 4. Correlation between the expression of major NUE genes on the 42nd treatment day and agronomically important parameters which are known to correlate with nitrogen utilization

	AMT TD42	NR TD42	NiR TD42	AS TD42	SPAD TD42	Leaf area	Fol N rat	Root N rat	Tuber N rat	Plant N%	Foliage N%	Tuber N%	Tub D–F rat	Tuber FW	Aver. tub W	Tuber no.
AMT TD42	1.00															
NR TD42	0.15	1.00														
NiR TD42	0.12	−0.10	1.00													
AS TD42	−0.43**	0.16	−0.04	1.00												
SPAD TD42	−0.52**	−0.01	0.01	−0.03	1.00											
Leaf area	−0.58**	−0.30*	−0.24*	−0.24*	0.61**	1.00										
Fol N rat	−0.63**	0.20	−0.08	0.15	0.87***	0.42**	1.00									
Root N rat	0.05	0.44**	−0.40**	−0.05	0.54**	0.19	0.53**	1.00								
Tuber N	0.19	−0.41**	0.34*	−0.02	−0.71***	−0.29*	−0.75***	−0.96***	1.00							
Plant N%	−0.34*	−0.33*	0.55**	−0.37*	0.44**	0.59**	0.27*	−0.21*	0.08	1.00						
Foliage N%	−0.51**	0.39*	0.17	0.19	0.69**	0.32*	0.84***	0.43**	−0.61**	0.42**	1.00					
Tuber N%	−0.35*	−0.19	0.28*	−0.26*	0.66**	0.32*	0.70***	0.19	−0.38*	0.51**	0.56**	1.00				
Tub D–F rat	0.27*	−0.31*	−0.14	−0.38*	−0.57**	0.12	−0.63**	−0.48**	0.59**	0.17	−0.57**	−0.30*	1.00			
Tuber FW	−0.26*	−0.34*	−0.13	0.02	−0.16	0.43**	−0.35*	−0.62**	0.58**	0.41**	−0.25*	−0.55**	0.64**	1.00		
Aver. tub W	−0.21*	0.01	−0.19	0.71***	−0.36*	−0.15	−0.25*	−0.43**	0.41**	−0.35*	−0.31*	−0.54**	0.22*	0.28*	1.00	
Tuber no.	0.00	−0.06	−0.07	−0.44**	0.05	0.37*	−0.12	−0.01	0.05	0.34*	0.02	−0.12	0.40*	0.61**	−0.50**	1.00

Note. SPAD values represent chlorophyll content. NUE: nitrogen use efficiency; AMT: ammonium transporter expression; NR: nitrate reductase exp.; NiR: nitrite reductase exp.; AS: asparagine synthase exp.; SPAD: soil–plant analysis development; TD: treatment day; Leaf area: total leaf area of the plants in mm^2; Fol N rat: ratio of foliage N compared to the total plant N; root N rat: ratio of root N compared to the total plant N; Tub N rat: ratio of tuber N compared to the total plant N; Plant N%: N content of the total plant in %; Foliage N%: N content of the foliage in %; Tuber N%: N content of the tuber in %; Tub D–F rat: dried and fresh weight ratio of tubers; Tuber FW: fresh weight of tubers/plant; aver. tub W: average tuber weight; Tuber no.: tuber number/plant. *Existing but weak correlation. **Moderate significant correlation. ***High-pronounced correlation.

Table 5. Correlation between the expression of major NUE genes on the 42nd treatment day and NUE indices and some agronomically important parameters

	AMT TD42	NR TD42	NiR TD42	AS TD42	SPAD TD42	NUpE	NUtE	NUE	R–S rat	HI_2	HI_1	Tub F–R rat	Tuber FW	Aver. tub W
AMT TD42	1.00													
NR TD42	0.15	1.00												
NiR TD42	0.12	−0.10	1.00											
AS TD42	−0.43**	0.16	−0.04	1.00										
SPAD TD42	−0.52**	−0.01	0.01	−0.03	1.00									
NUpE	0.30*	−0.32*	0.46**	−0.46**	−0.29*	1.00								
NUtE	0.45**	−0.11	−0.42**	−0.09	−0.57**	0.08	1.00							
NUE	0.47**	−0.27*	−0.08	−0.31*	−0.57**	0.60**	0.84***	1.00						
R–S rat	0.61**	0.15	−0.38*	−0.18	−0.29*	−0.19	0.71***	0.48*	1.00					
HI2	−0.01	−0.32*	0.39*	0.09	−0.54**	0.47**	−0.04	0.17	−0.48**	1.00				
HI1	0.26**	−0.13	0.17	−0.02	−0.79***	0.53**	0.29*	0.48**	−0.06	0.82***	1.00			
Tub F–R rat	0.27**	−0.31*	−0.14	−0.38*	−0.57**	0.72***	0.33*	0.62**	0.01	0.47**	0.61**	1.00		
Tuber FW	−0.26**	−0.34*	−0.13	0.02	−0.16	0.64**	0.29*	0.48**	−0.28*	0.50**	0.58**	0.64**	1.00	
Aver. tub W	−0.21**	0.01	−0.19	0.71***	−0.36*	0.03	0.32*	0.21*	−0.19	0.31*	0.25*	0.22*	0.28*	1.00

Note. SPAD values represent chlorophyll content. AMT: ammonium transporter expression; NR: nitrate reductase exp.; NiR: nitrite reductase exp.; AS: asparagine synthase exp.; SPAD: soil–plant analysis development; TD: treatment day; NUpE: total N content of plant (g)/total available N (g); NUtE: total dry matter of plant (g)/total N content of plant (g); NUE: total dry matter of plant (g)/total available N of plant (g); R–S rat: root–shoot ratio; HI_2 = tuber dry weight/(tuber dry weight + foliage dry weight + tuber dry weight); HI_1 = tuber dry weight/(tuber dry weight + 1.25 × foliage dry weight); Tub D–F rat: dried and fresh weight ratio of tubers; Tuber FW: fresh weight of tubers/plant; Aver. tub W: average tuber weight.
*Existing but weak correlation. **Moderate significant correlation. ***High-pronounced correlation.

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