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EVALUATION OF BIOMASS PRODUCTION IN DIFFERENT POTATO GENOTYPES UNDER VARIOUS NITROGEN LEVELS IN VITRO

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Abstract
Different potato genotypes vary in nitrogen uptake and biomass production at different available nitrogen levels. In vitro experiments offer a controlled environment to further explore these variations. The study aimed to evaluate the morphological traits of various potato genotypes in a two-factorial experiment, analysing microplant responses depending on nitrogen availability in the growth medium in vitro. The study, conducted in 2022 in Priekuli, Latvia, tested 19 potato genotypes under varying nitrogen levels in an in vitro medium. The experiment was carried out in three time-shifted series each with four replications. The Murashige and Skoog basal medium used for the experiments included N concentrations of 60 mmol L^{-1} (N60), 20 mmol L^{-1} (N20), and 7.5 mmol L^{-1} (N7.5). The morphological traits analyzed in the potato microplants included plant height, fresh weight (FW) and dry weight (DW) of the total plant, as well as FW and DW of the shoots and roots. Additionally, calculated traits included the root % share, root-to-shoot ratio and the nitrogen stress tolerance coefficient (NstC) of the genotypes. Results showed significant differences in biomass production between different N levels ($p<0.05$) as well as significant differences between genotypes grown under the same N level were identified. NstC differed between genotypes.
Keywords: potato microplants, nitrogen stress tolerance, nitrogen availability.

Introduction

Potato (*Solanum tuberosum* L.) is an important crop for global food and nutritional security. As a crop it requires a large amount of nitrogen (N) (Haverkort et al., 2003; Vos, 2009; Hirel et al., 2007; Lammerts van Bueren et al., 2014; Tiwari et al., 2018). To comply with environmentally friendly and sustainable agricultural practices, the fundamental goal of crop production is to optimise high nutrient efficiency in crops, thereby ensuring that economic benefits increase and environmental pollution due to nitrate loss is reduced (Getahun et al., 2020; Stefaniak et al., 2021). One approach is the development of enhanced-efficiency fertilizer and optimised nutrient management (Hopkins et al., 2008), but the second approach is the breeding of cultivars with improved nitrogen use efficiency (NUE) (Schum & Jansen, 2014).

Although field trials are crucial, pre-screening of genotypes under controlled environmental conditions can be helpful in the breeding and selection process (Schum & Jansen, 2014). *In vitro* trials for NUE evaluation are rare, only a few of them are described by Hajari et al. (2014, 2015), Shum & Jansen (2014) and Schum et al. (2017). *In vitro* trials allow us to investigate many plants under controlled conditions in a short period; nevertheless, this also allows us to observe root development, which is usually impossible in field trials (Rabante-Hane et al., 2022).

Knowledge about the key traits affecting NUE is essential to investigate plant growth. NUE is connected with N metabolism in potato plants, affecting N uptake, assimilation, utilization and remobilization in different parts of the plant (roots, stems, leaves, stolons/tubers) (Foulkes et al., 2009). High N supply can ensure more shoot growth, resulting in higher plant (Tiwari et al., 2018).

Roots are an important part of plants and provide uptake of water and nutrients (White et al., 2013). Potato has a relatively shallow root system, which results in a deficient acquisition of nutrients (Wishart et al., 2013). According to Ospina et al. (2014) physiological and molecular mechanisms are involved in N uptake, transport, assimilation, and utilization in potato plants. Various genotypes have different responses to nitrogen levels (Dimante et al., 2024). Many physiological and molecular mechanisms among different potato cultivars influence N uptake, transport, assimilation and utilization (Zebarth et al., 2004; Ospina et al., 2014). Potato genotypes may perform better under reduced N availability and different meteorological conditions (Skrabule et al., 2023).

The objective of this study was to assess the biomass production, root-to-shoot ratio, and nitrogen stress tolerance in 19 potato genotypes grown *in vitro* under three nitrogen levels.

Materials and Methods

Plant material

A set of 19 potato (*Solanum tuberosum* L.) genotypes was used for *in vitro* trials, including ten Latvian cultivars from various maturity groups: 'Agrie Dzeltenie', 'Madara', 'Monta', 'Rigonda' (early) 'Prelma' and 'Lenora' (medium early), 'Magdalena', 'Brasla', 'Imanta', 'Jogla' (medium late), as well as, five breeding clones with high potential from AREI: S 03067-33 and S 01085-21, S 11161-85, S 11152-7. Additionally, four popular cultivars from external breeding companies were included: German varieties 'Vineta', 'Jelly', 'Verdi' and 'Kuras'; the latter is widely known in starch production and was bred in the Netherlands.

Stock cultures of virus-tested *in vitro* microplants were kept in glass test tubes on agar-solidified Murashige & Skoog basal medium (MS medium) (Murashige & Skoog, 1962) supplemented with 30 g L^{-1} sucrose and no growing regulators. Cultures were incubated at 22±1 °C, photoperiod 16/8 h; light intensity 15.5±2.5 W m^{-2} and subcultured every four to six weeks until the necessary number of microplants was obtained.

Shoot tips of approximately 1.5 – 2.0 cm in length were excised from newly developed microplants and used for *in vitro* trials in different nitrogen levels.

In vitro testing

For *in vitro* testing, ten shoot tips were placed in perforated polypropylene plates within Magenta™ vessels (575 mL), each containing 50 mL of liquid MS medium supplemented with 30 $g\,L^{-1}$ sucrose and 1.5 $mL\,L^{-1}$ PPM™. The pH (measured in KCl) of the medium was adjusted to 5.7 ± 0.1. The experiments included a standard N level of 60 mmol L^{-1} (N60) and two reduced N levels of 20 mmol L^{-1} (N20), and 7.5 mmol L^{-1} (N7.5). Media was adjusted with respective concentrations of NH_4NO_3, KNO_3 and KCl. Cultures were incubated for 24 days according to the environmental conditions of the stock culture.

The *in vitro* trial was repeated in three independent time-shifted trials, each series comprised four replications per N level. Overall, the experiment included two factors: genotype and nitrogen concentration in the MS medium. A total of 19 genotypes and 3 nitrogen levels were tested, resulting in 57 treatment combinations. Each replicate included 10 plants, which meant 40 plants per treatment per series. With 3 series, that totals 120 plants for each treatment, according to genotype and nitrogen level. In total, 360 microplants per genotype were used in the study.

Morphological traits and measurements

Morphological traits of microplants were measured for each unit of 10 plantlets, and data were averaged across replications and experimental series. To compare and analyse differences between the N levels, the morphological traits assessed in the potato microplants were plant fresh weight (FW) and dry weight (DW), and FW and DW of the shoots and roots. To compare the differences between the root and shoot systems development, the root percentage share of total FW was determined as well as the root-to-shoot ratio (R:S ratio) was calculated using the formula developed by Wilson (1988), Equation (1):

$$R{:}S\ ratio = \frac{DWroots}{DWshoots} \qquad (1)$$

DW roots – the dry weight of roots;
DW shoots – the dry weight of shoots.

Nitrogen stress tolerance coefficient (NstC) of the genotypes

$$NstC = \frac{(1 - Ps \div Pc)}{(1 - meanPs \div meanPc)} \qquad (2)$$

Ps – Total DW under stress conditions (N7.5);
Pc – Total DW under control conditions (N60);
meanPs – mean of all genotypes under N7.5;
meanPc – mean of all genotypes under N60.

Potato genotypes were evaluated based on their stress susceptibility index (NstC) (2), calculated using total plant dry weight according with the formula established by Fisher and Maurer (1978), Equation (2).

Statistical analysis

To analyse the impact of factors, a factor analysis of variance (ANOVA) was conducted. Tukey's post hoc test, with a significance level set at $\alpha = 0.05$, was used to identify groups with significant differences. The statistical analysis was carried out using Jamovi software (version 2.6.23) (Jamovi The Jamovi), which operates in conjunction with R version 4.4.

Results and Discussion

Biomass production

Nitrogen reduction in medium affected morphological traits of potato genotypes. The two-factor analysis showed that there was significant effect of Genotype and N level on dependent variables such as total FW, FW of shoots and roots, shoot length and total DW ($p<0.005$), and that there was an interaction between genotype and N level concerning the dependent variables, ($p<0.005$) (Table 1).

The total FW per replication (10 microplants) was determined for each treatment, and average values across four replications are presented. These ranged from 2.21 g to 19.01 g at N60, 3.55 g to 14.09 g at N20, and 2.05 g to 8.04 g at N7.5 (Table 1). Cultivars 'Rigonda', 'Monta', 'Agrie Dzeltenie', and breeding line S 01085-21 showed the highest total FW under N60 (Figure 1). Additionally, these genotypes demonstrated a significant reduction in biomass as nitrogen availability decreased in the media. However, genotypes such as S 03067-33, S04065-2, S 11161-85, 'Jogla', and 'Kuras' did not show significant differences between the N60 and N20 treatments. The 'Madara' cultivar showed higher total FW with N20 compared to N60. The difference between N20 and N7.5 resulted in a total FW of 2.89 g.

The average shoot FW per 10 microplants ranged from 2.11 g to 11.99 g at N60, 0.7 g to 12.05 gat N20, and 1.36 g to 7.39 g at N7.5 (Table 1). The shoot FW of most genotypes decreased with reduced nitrogen supply (Figure 2). However, in three genotypes (S 03067-33, 'Magdalena', 'Madara'), we observed that shoot FW was higher at N20 compared to N60, but only for 'Madara' difference between N20 and N60 was significantly higher. These tendencies were not sustained at N7.5.

The average root FW per 10 microplants ranged from 0 g to 8.91 g in N60, 0.27 g to 5.07 g at N20, and 0 g to 1.62 g at N7.5 (Table 1). Root FW decreased under lower nitrogen supply in all genotypes except 'Prelma', which showed higher root FW at N20 compared to N60.

Root percentages of the total plant fresh mass

The root percentage share of total FW ranged from 0.00% (S 11152-7) to 46.87% ('Rigonda') at N60 (Table 1). In the reduction of available nitrogen, two different responses were observed among genotypes after 24 days in the media. Most genotypes showed a constant decrease in root FW percentage with increasing nitrogen deficiency stress, while some genotypes showed improved root development under N20 and N7.5 conditions. For example, 'Prelma' showed a 6.93% FW root percentage at N20 compared to N60, but 'Magdalena' showed by a 3.98% better root percentage at N7.5, than observed at N20.

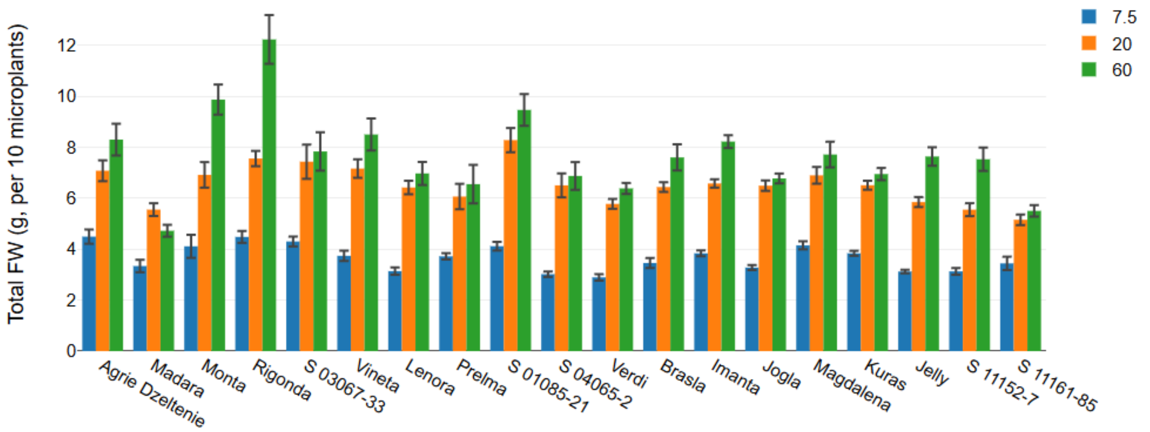
Table 1

Overview of plant performance across all genotypes at different N levels

Trait	*N60*		*N20*		*N7.5*		*F statistics*		
	Mean	*Min/Max*	*Mean*	*Min/Max*	*Mean*	*Min/Max*	*N level (N)*	*Genotype (G)*	*N x G*
Fresh weight Total (g/10 plants)	7.70	2.21/19.01	6.56	3.55/14.09	3.67	2.05/8.04	547.06^{***}	15.68^{***}	3.91^{***}
Fresh weight Shoots (g/10 plants)	5.35	2.11/11.99	4.95	0.70/12.05	2.96	1.36/7.39	321.44^{***}	12.14^{***}	1.75^{*}
Fresh weight Roots (g/10 plants)	2.35	0.00/8.91	1.62	0.27/5.07	0.71	0.00/1.62	482.97^{***}	15.59^{***}	7.38^{***}
Shoot length (cm)	8.66	2.90/13.20	4.46	1.10/9.80	3.11	1.20/9.00	1215.27^{***}	15.07^{***}	5.04^{***}
Dry weight Total (g/10 plants)	0.59	0.23/1.10	0.63	0.28/1.05	0.46	0.23/0.83	111.86^{***}	11.35^{***}	2.02^{***}
Dry weight Shoots (g/10 plants)	0.47	0.14/0.86	0.53	0.17/0.95	0.40	0.21/0.80	66.86^{***}	10.40^{***}	1.81^{***}
Dry weight Roots (g/10 plants)	0.12	0.00/0.40	0.10	0.01/0.21	0.06	0.00/0.15	280.31^{***}	18.31^{***}	4.82^{***}
R:S ratio	0.44	0.00/0.88	0.36	0.07/7.24	0.25	0.00/0.55	25.54^{***}	1.48^{ns}	1.52^{**}
Root percentage (%)	29.94	0.00/46.87	24.47	6.31/87.87	19.13	0.00/35.33	155.51^{***}	14.24^{***}	3.7^{***}
*Significantly different at the 0.05 probability level p<0.05; ** p<0.01; ***p<0.001; ns – not significant									

Figure 1

Total fresh weight (g, per 10 microplants) of genotypes under different N levels



The average R:S ratio per 10 microplants ranged from 0 to 0.88 in N60, 0.07 to 7.24 in N20, and 0 to 0.55 in N7.5 (Table 1) ANOVA revealed a significant effect of nitrogen levels on the root-to-shoot (R:S) ratio Tukey's post-hoc test showed the pairwise comparisons between N60 - N20, N60 - N7.5, and N20 - N7.5 that resulted in p<0.05. The highest R:S was observed for 'Prelma' in N20 (7.24) (Figure 3). Statistical analysis showed that the effect of genotype was not significant in relation to the R:S ratio within each N treatment, but there was a significant interaction between the genotype and N level (p<0.005) in relation to the R:S ratio. Most of the genotype R:S ratio reduced as the N level reduced.

However, for some genotypes - such as 'Agrie Dzeltenie', 'Monta', S 03067-33, and S 11161-85 - the R:S ratio did not differ significantly between N20 and N7.5. For 'Magdalena', which previously showed a higher root percentage at N7.5 than at N20, the difference in R:S ratio was minimal (0.07). Interesting results showed 'Prelma', whose R:S ratio didn't significantly differ between N60 (0.14) and N7.5 (0.16), but was significantly higher at N20 (0.76).

Stress susceptibility index (NstC)

Further analysis of biomass production depending on N concentration in the medium was conducted using the ranking method, where genotypes were ranked according to the stress susceptibility based on total DW of microplants. The NstC reflects the rate of change for each genotype and the stability of a specific parameter under stress conditions, with higher values indicating lower tolerance (Figure 4). The NstC was calculated between the N60 (standard) and N7.5 (N stress) treatments. The average NstC across genotypes was 1.02 with eight genotypes falling below average value, indicating greater tolerance to nitrogen reduction. The lowest values were detected for S 01085-21 (-0.92), 'Agrie Dzeltenie' (0.52), 'Madara' (0.53) and S 11161-85 (0.53), while genotypes S 04065-2 (1.66), 'Lenora' (1.67) and 'Verdi' (1.71) demonstrated lower tolerance to N availability changes.

Figure 2
Shoot fresh weight (g, per 10 microplants) of genotypes under different N levels

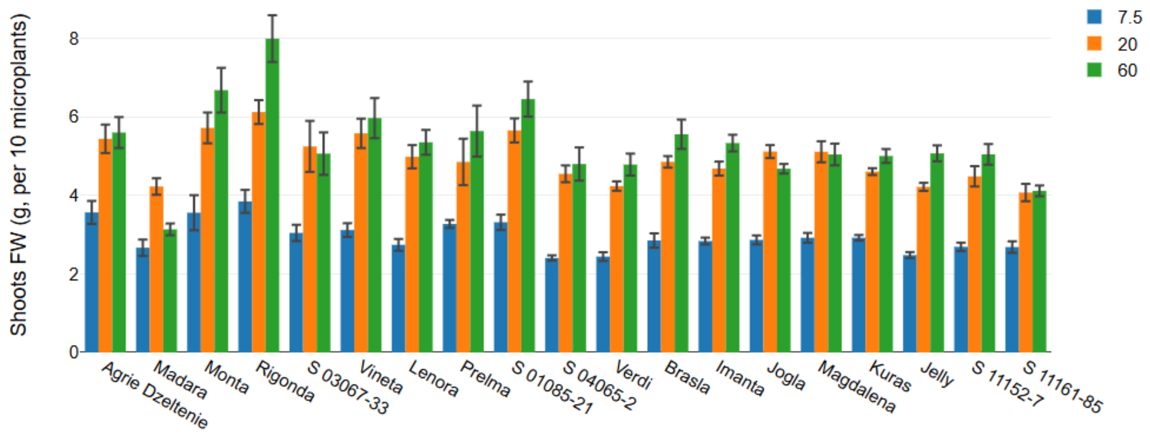
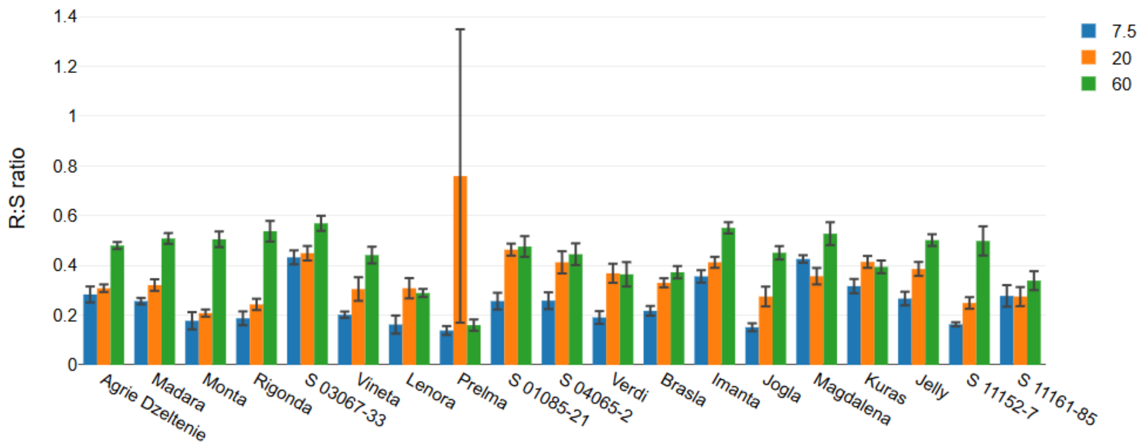


Figure 3
R:S ratio of genotypes under different N levels



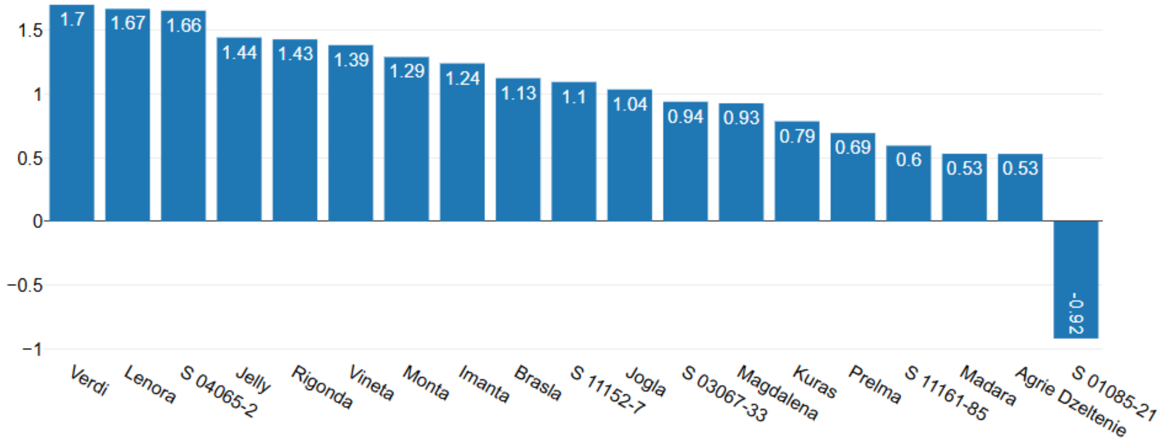
The observed significant differences between genotypes and N treatments ($p<0.005$) indicate that both genetic factors and nitrogen availability are essential for plant development and biomass production, showing that the response of a genotype under various levels of nitrogen may differ as described by Dimante et al. (2024).

In our study genotypes 'Rigonda', 'Monta', 'Agrie Dzeltenie' and breeding line S 01085-21 showed increased total FW at N60, demonstrating the significance of selecting genotypes that can improve biomass production under optimal conditions. The results align with the findings of Shum & Jansen (2014), Schum et al. (2017), and Tiwari et al. (2018), which indicate that increased nitrogen supply improves shoot growth and total plant biomass accumulation. The observed reduction in biomass related to lower nitrogen availability supports the hypothesis that decreased nitrogen levels may prevent growth and affect nutrient transport and assimilation (Tiwari et al., 2018). Still, we detected that some genotypes (S 03067-33, S04065-2, S 11161-85, 'Jogla' and 'Kuras') did not show significant differences between the N60 and N20 treatments. The 'Madara' cultivar showed higher total FW at N20 compared to N60. The analysis of shoot and root FW indicated that shoots typically formed the largest part of biomass in most genotypes. The individual response of the 'Madara', 'Magdalena' and S 03067-33, which showed higher shoot FW under N20 compared to N60, suggests that a stronger nitrogen level reduction may not negatively affect shoot FW in some genotypes.

This is consistent with findings by Skrabule et al. (2023), who reported that certain genotypes may perform better under reduced nitrogen conditions. This observation may also suggest adaptive mechanisms for nutrient uptake across different potato genotypes, aligning with the findings of White et al. (2013), which indicate the essential role of roots in providing nutrient acquisition.

Figure 4

Stress susceptibility index (NstC) of genotypes under different N levels



Observations of the development of roots under *in vitro* conditions are significant due to the potato's relatively shallow root system, which often limits nutrient acquisition (Wishart et al., 2013). The R:S ratio determines the growth relationship between the plant's roots and its upward structures, such as stems and leaves. This ratio provides insights into a plant's health, resource distribution, and overall growth strategy.

Under reduced nitrogen availability, two different responses were observed among genotypes. In some genotypes, the R:S ratio between N20 and N7.5 didn't significantly differ, like 'Agrie Dzeltenie', 'Monta', S 03067-33 and S 11161-85. We observed an indication that the highest R:S ratio was at N20, suggesting a change in growth strategy in response to lower nitrogen levels. Interaction between genotype and nitrogen supply under *in vitro* conditions is best described by Schum & Jansen (2014) and Schum et al. (2017), who suggest that certain genotypes may demonstrate distinct adaptive traits. Still, no significant differences in R:S ratios among genotypes ($p>0.05$) within N treatments were observed in our experiment, suggesting that although all genotypes react to nitrogen availability, their overall growth patterns are similar.

Most genotypes demonstrated a decrease in root FW under conditions of reduced nitrogen availability. However, 'Prelma' demonstrated a 6.93% increase in root weight at N20 compared to N60. This indicates a possible adaptive strategy for improving nutrient intake under unfavourable conditions, which may be essential to plant survival (Foulkes et al., 2009; Schum et al., 2017). The R:S ratio for 'Prelma' showed no significant difference between N60 and N7.5, suggesting that this cultivar may function well under nitrogen-limited conditions. In case of 'Magdalena' higher root percentage at N7.5, did not result in higher R:S ratio. The notable increase in root percentage share of total FW for specific genotypes under reduced nitrogen conditions suggests a strategic adaptation to improve nutrient absorption during stress, as previously documented (Ospina et al., 2014). Adaptive traits may improve breeding programs aimed at improving NUE in potatoes.

The NstC offers a quantitative measure of genotypic stability under stress conditions. High NstC values indicate lower tolerance to nitrogen deficiency, while lower values suggest better adaptability (Schum & Jansen, 2014). Eight genotypes (S 01085-21, 'Agrie Dzeltenie', 'Madara', S 11161-85, 'Prelma', 'Kuras', 'Magdalena' and S 03067-33) were below average values indicating that these genotypes have a higher tolerance to nitrogen reduction. When comparing NstC tendencies with additional traits, we can find some similarities that genotypes 'Prelma', 'Magdalena', 'Kuras' and 'Agrie Dzeltenie' showed good results regarding to root development under different N levels while 'Madara' had positive response in shoot FW. Still, the variations in response to nitrogen availability among genotypes, support the concept that genetic differences significantly influence nitrogen uptake and utilization (Zebarth et al., 2004; Skrabule et al., 2023).

Conclusions

1. Reduced nitrogen availability negatively impacts potato growth, as shown by the overall decrease in shoot and root FW among the majority of genotypes.
2. Certain genotypes have adaptive mechanisms for enhanced nutrient uptake, supporting the theory that specific genetic traits give advantages in nitrogen-limited environments.
3. R:S ratio presents insight into growth strategies in connection with the availability of nitrogen. The highest R:S ratio in limiting N levels shows a change in growth patterns, demonstrating adaptive responses to nutrient limitation.

4. The NstC provides valuable insights into the genotypic stability of various plant genotypes under nitrogen deficiency.
5. Genotypes with lower tolerance to nitrogen reduction are not always expressed by morphological traits that indicate complex interactions between genetic traits and nitrogen utilization.

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