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Cultivar Differences in Response to Reduced Nitrogen Fertilization in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Nitrogen fertilization is a key factor in wheat crop management, but it has environmental impacts [through nitrate leaching, use of fossil fuels for manufacture and application, as well as important greenhouse gas (N_2O) emissions] and represents a significant cost for the growers. Therefore, there is presently an increasing concern to reduce the excessive use of N fertilizers.

We analyzed results of seven yield trials with winter wheat cultivars grown on two levels of Nitrogen fertilization, performed during the years 2021-2023 at five sites, where the average yield difference between the two levels of N fertilization was higher than 5%. The analyzed trials included a large variation for grain yield and protein concentration, as well as for the index of tolerance to Nitrogen deficiency.

ANOVA for grain yield and protein concentration showed significant effects of cultivars and environments for both yield and protein concentration, but for tolerance index only environments had a significant effect. The effect of lower N availability was stronger and more variable on protein content than on yield. Correlation between grain yield and protein concentration was not significant, but manifested the known negative trend, similar both under recommended and reduced N fertilization. Deviations from regression of protein concentration on grain yield were similar in most cultivars under recommended and reduced N fertilization, but in cultivar FDL Consecvent were higher under lower N availability, suggesting a better adaptation to this stress. Deviations from the regression of performance under Nitrogen deficiency stress on the performance with better N availability, for yield and protein percentage were not significantly correlated, and only three cultivars (FDL Consecvent, Voinic and FDL Miranda) showed positive deviations for both traits.

Performance under N deficiency stress is influenced by the potential manifested under no stress and by the response to stress, described by the tolerance index. Analyzing the effect of these two components, we found that, for the analyzed germplasm and environments, performance under no N stress showed higher correlation with performance under reduced N fertilization than the tolerance index. The implications for breeding programs are that (1) genetic progress in grain yield and protein concentration under normal N fertilization, which should be easier to achieve, will be reflected in the performance under reduced fertilization, and (2) a wider diversity of germplasm should be explored, to identify possible differences in the tolerance index, which could further improve performance under reduced fertilization.

Deviations from the regressions that describe the general relationship between the levels of N fertilization highlighted a few cultivars with better efficiency in using less available nitrogen, and are worth exploiting in breeding for improved performance under reduced fertilization.

Keywords: grain yield, protein concentration, nitrogen deficiency stress.

INTRODUCTION

Nitrogen (N) is an essential nutrient for plants as it constitutes a wide range of fundamental biomolecules such as amino acids, proteins, enzymes, chlorophyll, and nucleic acids (Novoa and Loomis, 1981). In wheat, N is necessary to stimulate plant growth for maximizing yields and for the synthesis of gluten proteins, which are essential for bread and pasta quality.

In recent decades, due to intensive

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cultivation cycles, there has been a decreasing trend in soil organic matter, leading to a decline in nitrogen content in the soil, and this decline has promoted the development of nitrogen fertilizers to remedy the deficits of this plant nutrient. N fertilizers have then become a key agricultural practice for the increased yields achieved by modern agriculture (Valente et al., 2024).

This evolution was due to the development of the Haber-Bosch artificial N-fixing process in 1909, which enabled the large-scale industrial production of ammonia (Paull, 2009). According to the International Fertilizer Association's "World Ammonia Capacities 2023", global production capacity in 2023 reached about 192.3 million tons of nitrogen corresponding to ~233.9 million tons of ammonia (David et al., 2024), and this had a considerable impact on agricultural production.

However, this is a highly energy-demanding process, it uses fossil fuels, and it is currently considered one of the largest greenhouse gas emitters, responsible for 1.2% of CO_2 emissions globally (Smith et al., 2020).

Additionally, excessive nitrogen fertilizer usage causes significant environmental problems related to nitrous oxide (N_2O) emissions, associated with the denitrification process, N_2O being a greenhouse gas with approximately three hundred times the global warming potential of carbon dioxide (CO_2) (Valente et al., 2024).

Excessive nitrogen fertilizer usage can also lead to problems related nitrate pollution in superficial and underground water, due to leaching (Chen et al., 2016). To limit the loss of nitrates to aquifers, European directives promote Good Agricultural Practices, including the reduction of both organic and mineral nitrogen fertilization.

On the other hand, Nitrogen fertilizers represent a significant cost for farmers, and fertilizer prices are increasing faster than grain prices (Foulkes et al., 1998).

Environmental and economic reasons, as well as the fact that only a third of nitrogen inputs to cereal crop worldwide is estimated to be recovered in grain for consumption, resulting in a significant resource waste with major negative environmental impacts (Hawkesford, 2017), have caused an increasing concern and stimulated research to reduce N dependency of wheat roduction and improve N use efficiency (Hawkesford, 2014; Awaad et al., 2024).

Reducing nitrogen fertilizer application could be used both as a climate change mitigation strategy and for improving the economic efficiency of wheat crop (Stuart et al., 2014). Zörb et al. (2018) analyzed the effects of reducing N availability on grain yield and quality, and Nadeem et al. (2022) demonstrated that in certain conditions N fertilizer doses can be reduced without risking to reduce yields, while having positive effects on N use efficiency (NUE) and on reducing N losses.

Sylvester-Bradley and Kindred (2009) recommended that breeding and variety testing should be conducted at some sites with more than one level of applied N, to have the chance of identifying cultivars with improved nitrogen use efficiency. A significant research effort has been directed towards identifying and understanding the genetic basis of NUE and tolerance to N deficiency (Le Gouis et al., 2000; Ivić et al., 2021; Zhang et al., 2024a, 2024b).

The wheat breeding program at the National Agricultural Research and Development Institute (NARDI) Fundulea has organized for many years cultivar testing in several locations, both with the recommended and at a lower level of N fertilization. Marinciu et al. (2018) reported results obtained in 4 years, at three locations from South Romania. Here we present new results, obtained with newer wheat cultivars and in environmental conditions that reflect recent climate changes, to explore genetic possibilities to reduce the negative effects of lower N availability on grain yield and protein concentration.

MATERIAL AND METHODS

We analyzed results of yield trials conducted during the years 2021-2023 with 25 winter wheat cultivars grown on two levels of Nitrogen fertilization at five sites for which data for yield and grain protein concentration were available:

- Agricultural Research and Development Station (ARDS Valu lui Traian) - Lat. 44º16' N, Long. 28º51'E, on vermic chernozem soil, with pH=7.42 and humus content in arable layer 3.62%.
- National Agricultural Research and Development Institute Fundulea (NARDI Fundulea) - Lat. 44°30`N, Long. 26°51`E, on cernoziom soil with pH=5.8 and humus content 3.4%;
- Agricultural Research and Development Station Teleorman (ARDS Teleorman - Drăgănești Vlașca) - Lat. 44°07'N, Long. 25°45'E, on aluvial chernozem soil with pH=6.5 and humus content 3.0%;
- Agricultural Research and Development Station Caracal (ARDS Caracal) - Lat. 44º11'N, Long. 24º37'E, on chernozem soil with pH=7.6 and humus content 3.04%;
- Agricultural Research and Development Station Turda (ARDS Turda) - Lat. 46º58' N, Long. 23º78'E, on argiloluvial vertic chernozem soil with pH=6.45 and humus content 3.02%.

Weather conditions varied at the sites during the three years of testing. Here we only present the rainfall data, as this has the largest potential effect on wheat response to N fertilization (Table 1).

Table 1. Rainfall during the testing period (mm)

	Valu lui Traian	Fundulea	Teleorman	Caracal	Turda
Rainfall 1.09-28.02					
2021	254.2	288.0	344.5	212.6	194.0
2022	288.6	142.0	232.4	219.7	135.4
2023	172.2	152.0	235.0	302.0	272.6
Rainfall 1.03-30.06					
2021	339.4	282.2	315.9	283.3	192.5
2022	117.0	149.3	149.9	149.8	175.5
2023	165.2	159.8	164.0	212.6	219.0

Crop management, including Nitrogen doses applied in the autumn and spring, differed between sites, according to usual practices in the regions and to conditions of the year, as seen in Table 2.

Table 2. Rainfall during the testing period (mm)

	Valu lui Traian	Fundulea	Teleorman	Caracal	Turda
Preceding crop					
2021	Peas	Peas	Peas	Peas	Peas
2022	Maize	Peas	Cicer	Peas	Peas
2023	Canola	Peas	Peas	Canola	Peas
Recommended N fertilization kg N/ha					
2021	143	128	84	136	100
2022	143	100	84	136	100
2023	143	82	84	136	100
Reduced N fertilization kg N/ha					
2021	27	36	30	50	50
2022	27	36	30	50	50
2023	27	0	30	50	50

As the result of different weather and management conditions, the yield difference between the reduced and the recommended levels of N fertilization, averaged over all cultivars in each trial, varied from -1154 to +552 kg ha^{-1}, i.e. -13.4% to +8.2% of the yield at recommended N fertilization (data not shown). To estimate the cultivar specific response to lower N availability, we only analyzed the results of seven trials where the average yield difference between the two levels of N fertilization were higher than 5%. This way we avoided the confounding effects of many factors, such as: more severe water stress induced by more vegetative growth of fertilized plants, by exposure to higher temperatures because of prolonged vegetation related to better N nutrition, by differences in lodging or disease attacks at the two levels of N fertilization or by reduced accessibility of fertilizers applied during dry periods, which caused higher or equal yields at reduced N fertilization in many trials.

Out of the twenty-five cultivars tested in each trial, we analyzed eleven that were common to all sites and years.

We analyzed grain yield and grain protein percentage of each cultivar under recommended and reduced N fertilization in the analyzed trials, as well as a tolerance index to insufficient N availability for both grain yield and protein concentration, defined as the difference between results obtained with reduced fertilization and those with the recommended one, according to Rosielle and Hamblin (1981):

$$TOL = X_{N\ reduced} - X_{N\ recommended}$$

Based on this definition, the results obtained with reduced Nitrogen fertilization can be described as the sum of two components: (1) the potential expressed by results under recommended fertilization and (2) response to stress (the tolerance index):

$$X_{N\ reduced} = X_{N\ recommended} + TOL$$

To estimate the relative importance of these two components, we analyzed their correlation with the results obtained under reduced N fertilization.

RESULTS AND DISCUSSION

All analyzed parameters showed large variations under the influence of environments and cultivars. Grain yield with recommended fertilization varied from 2908 kg ha^{-1} for Bezostaya 1 at Caracal in 2023 to 9565 kg ha^{-1} for FDL Consecvent at Turda in 2021, and with reduced N fertilization from 2621 kg ha^{-1} for Bezostaya 1 at Caracal in 2023 to 8811 kg ha^{-1} for FDL Consecvent at Turda in 2021. Grain protein concentration with recommended fertilization varied from 10.6% for FDL Miranda at Caracal in 2023 to 16.8% for Bezostaya 1 at Fundulea in 2022, and with reduced N fertilization from 8.5% for FDL Miranda at Turda in 2023 to 17.3% for Bezostaya 1 at Fundulea in 2022.

The tolerance index of grain yield to reduced N availability varied from -1501 kg ha^{-1} for FDL Miranda at Turda in 2021 to +498 kg ha^{-1} for FDL Miranda at Valu lui Traian in 2023, while the tolerance index for grain protein concentration varied from -5.8% for Otilia at Valu lui Traian in 2023 to +0.6% for FDL Miranda and Bezostaya 1 at Fundulea in 2022 and Bezostaya 1 at Valu lui Traian in 2023.

Taking into account only trials where averaged over all cultivars effect of reduced N fertilization on yield was greater than 5%, performance under reduced N fertilization for grain yield varied in the seven trials from 86.6% to 94.9%, while for protein concentration it varied from 76.9% to 100.8%. This suggests that the effect of lower N availability was on average stronger and more variable on protein content than on yield (Table 3).

Table 3. Average effects of reduced N fertilization on grain yield and protein concentration in trials where the effect of reduced N fertilization was more than 5%

Yield trial	Performance under reduced N fertilization (% of recommended)	
	Grain yield	Protein concentration
Fundulea 2022	91.1	100.8
Valu lui Traian 2023	90.8	79.5
Caracal 2021	94.9	86.2
Caracal 2023	88.7	93.4
Turda 2021	90.6	82.9
Turda 2022	91.7	82.4
Turda 2023	86.6	76.9
All cultivars average	90.63	86.01

ANOVA for grain yield and protein concentration in the eleven cultivars tested in the seven trials, where the average yield difference between the two levels of N fertilization was higher than 5%, showed significant effects of cultivars and environments for both yield and protein concentration, but for tolerance index only environments had a significant effect (Table 4).

Table 4. ANOVA (F values) for grain yield and protein concentration under recommended and reduced N fertilization and for the tolerance index

			Grain yield			Grain protein concentration		
			Reduced N	Recomm.	TOL	Reduced N	Recomm.	TOL
Source of Variation	df	F crit	F	F	F	F	F	F
Cultivars	10	1.99	**14.15**	**10.76**	0.59	**4.91**	**4.58**	1.12
Environments	6	2.25	**227.29**	**150.74**	**4.24**	**124.4**	**31.65**	**21.42**
IA cultivars*env.	60							

F values written in **bold** are significant at P<5%.

Correlation between grain yield and protein concentration was not significant, but manifested the known negative trend, similar both under recommended and reduced N fertilization (Figure 1).

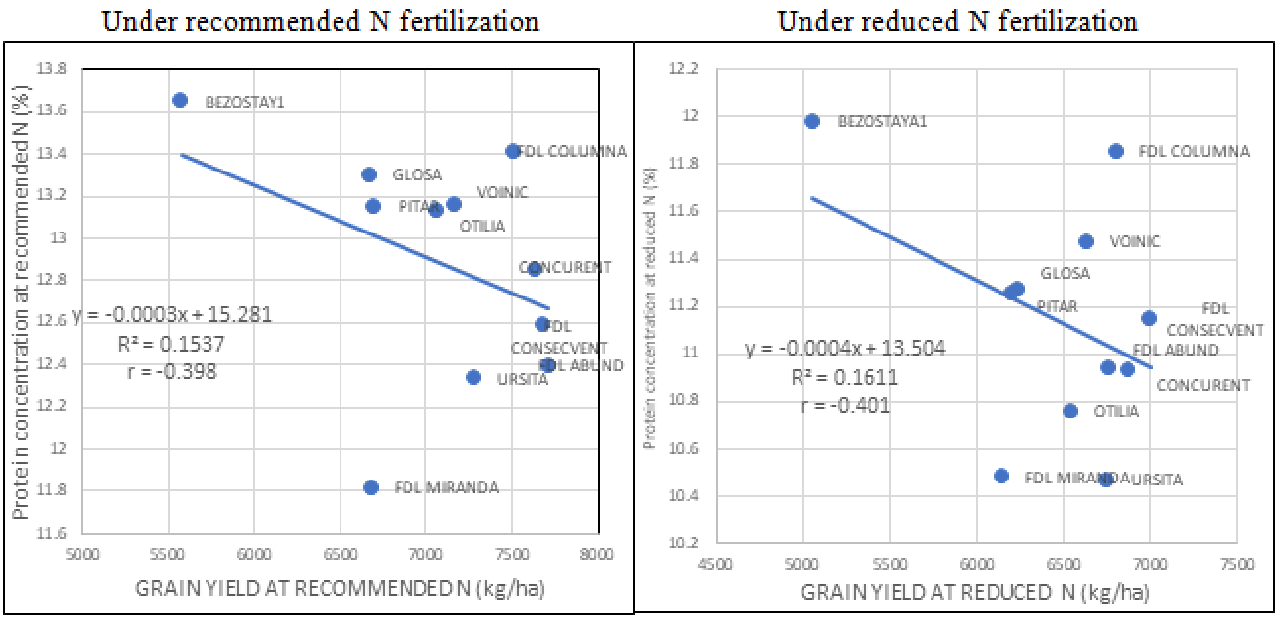

Figure 1. Relationship between grain yield and protein concentration averaged on seven trials

Along with the similar correlation coefficient and slope of regression, we observed in most cases a similar behavior of cultivars. However, some cultivars had different performance at the two levels of fertilization. These differences can be easier analyzed based on deviations from regression (Figure 2). Cultivars, such as FDL Columna, Voinic and Bezostaya 1, showed similar positive deviations under both levels of N fertilization, and others, such as FDL Miranda and Ursita, showed large negative deviations in both conditions. On the other hand, cultivar FDL Consecvent had better protein content with reduced N fertilization than expected based on the regression on yield. This could suggest that this cultivar can have better efficiency in using less available Nitrogen.

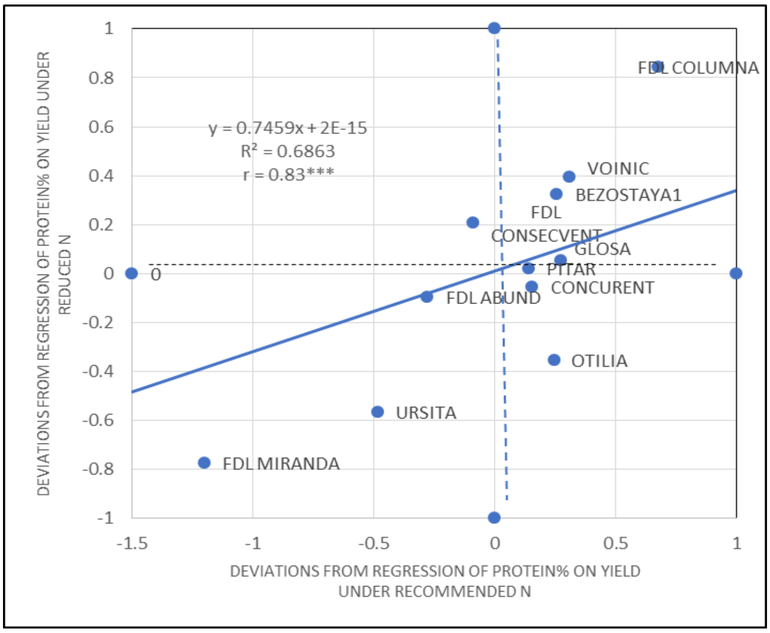

Figure 2. Comparison of deviations from regression of protein concentration on grain yield under recommended and reduced N fertilization

To optimize breeding strategies for increasing performance under reduced fertilization it is important to analyze the relative influence of these two components on cultivar performance under nutritional stress.

Regarding the first component, grain yield under recommended fertilization was closely correlated with yields under reduced N fertilization, with correlation coefficient (r) of 0.98*** (Figure 3). However, cultivars Glosa and Ursita gave a little higher yield under reduced N availability than expected based on the regression, while Bezostaya 1 and FDL Abund reduced their yield under reduced fertilization, more than other cultivars.

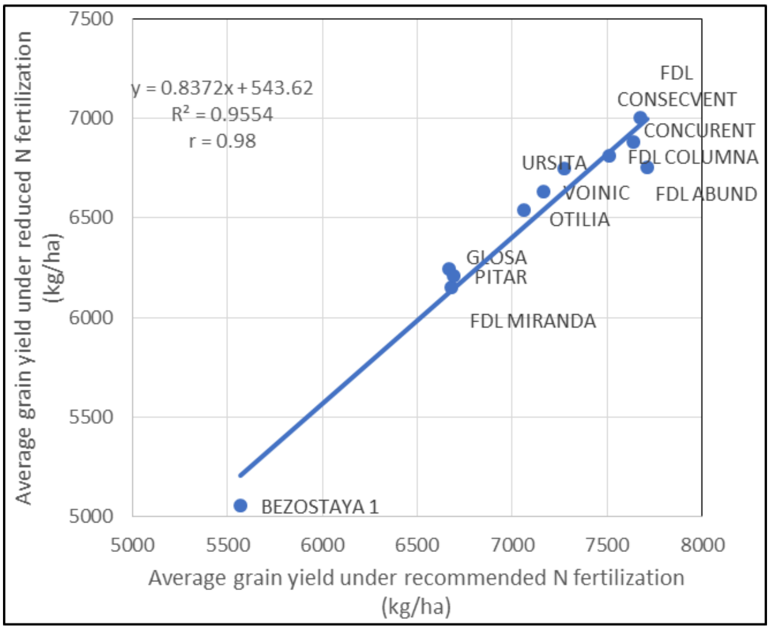

Figure 3. Relationship between yields under recommended and reduced N fertilization, averaged over seven trials where difference between fertilization levels was significant

For grain protein concentration, performance under less stress, was also closely correlated with performance under stress, but deviations from the regression were larger (Figure 4). Cultivars FDL Columna, Bezostaya 1, FDL Consecvent and Voinic showed large positive deviations, while Otilia and Ursita had largest negative deviations.

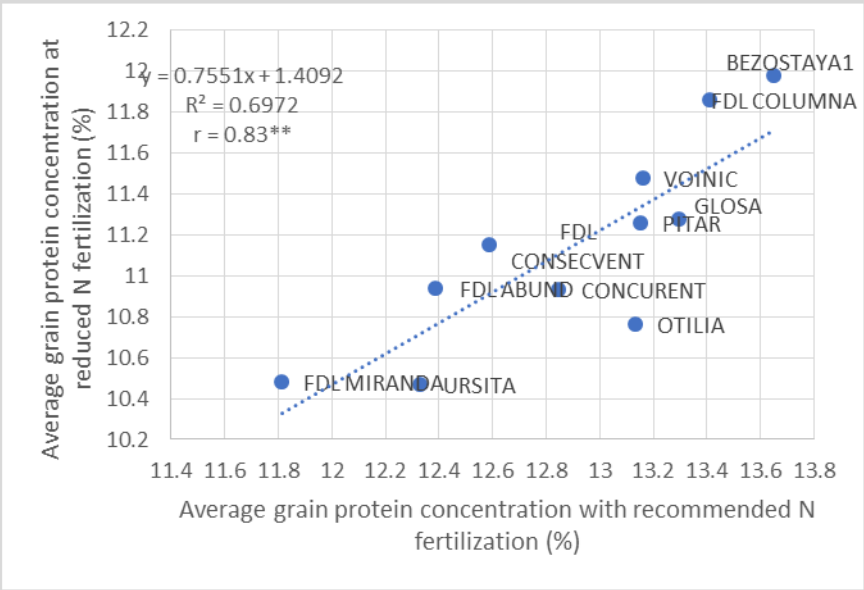

Figure 4. Relationship between grain protein concentrations under recommended and reduced N fertilization, averaged over seven trials

Comparing cultivar responses to reduced N availability for grain yield and grain protein percentage, as described by the deviations from the general regression, one can observe major differences between the analyzed cultivars (Table 5).

Table 5. Deviations in yield and protein concentration from the regression of reduced on recommended N fertilization

Cultivar	Yield deviations from the regression of reduced on recommended N fertilization	
	kg/ha	% of trial average
Glosa	115	1.63
Ursita	108	1.53
Voinic	85	1.21
Otilia	83	1.17
Pitar	59	0.84
FDL Consecvent	29	0.41
FDL Miranda	11	0.16
FDL Columna	-25	-0.35
Concurent	-65	-0.92
Bezostaya 1	-155	-2.19
FDL Abund	-246	-3.49

Cultivar	Protein % deviations from the regression of reduced on recommended N fertilization	
	% protein	% of trial average
FDL Columna	0.32	2.48
Bezostaya 1	0.26	2.03
FDL Consecvent	0.23	1.81
FDL Abund	0.17	1.34
FDL Miranda	0.15	1.15
Voinic	0.12	0.97
Pitar	-0.09	-0.68
Glosa	-0.17	-1.35
Concurent	-0.18	-1.38
Ursita	-0.25	-1.97
Otilia	-0.57	-4.41

Deviations from the regression of performance under Nitrogen deficiency stress on the performance with better N availability, for yield and protein percentage were not significantly correlated, and only three cultivars (FDL Consecvent, Voinic and FDL Miranda) had positive deviations for both traits (Figure 5).

This special behavior of cultivars FDL Consecvent and Voinic, which utilized Nitrogen more efficiently under low availability, having at the same time superior performance at both levels of fertilization, deserves attention. The behavior of cultivar FDL Columna is also worth noticing, since it had a much better protein concentration than expected based on regression and only a small deviation from the regression for grain yield.

Most cultivars had the positive deviation for grain yield compensated by negative deviation for protein percentage, or vice-versa, and only one cultivar (FDL Concurent) had negative deviations for both traits.

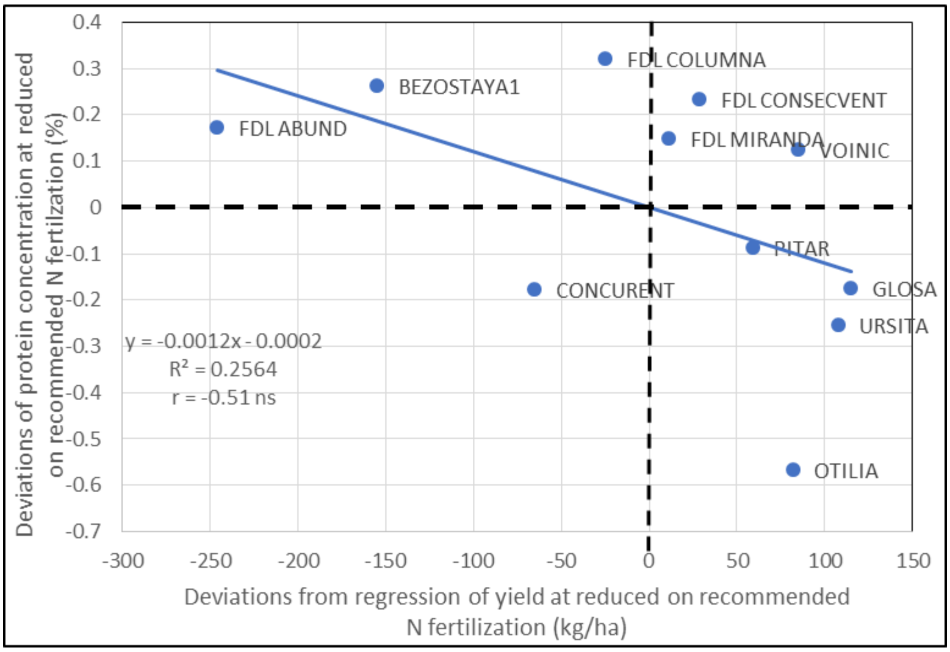

Figure 5. Relationship between cultivar responses to reduced N availability for grain yield and protein concentration

The tolerance index, which is the second component that influences the performance under stress, was not significantly correlated with cultivar performance under reduced N fertilization, for neither yield, nor protein concentration in the grains (Figures 6 and 7).

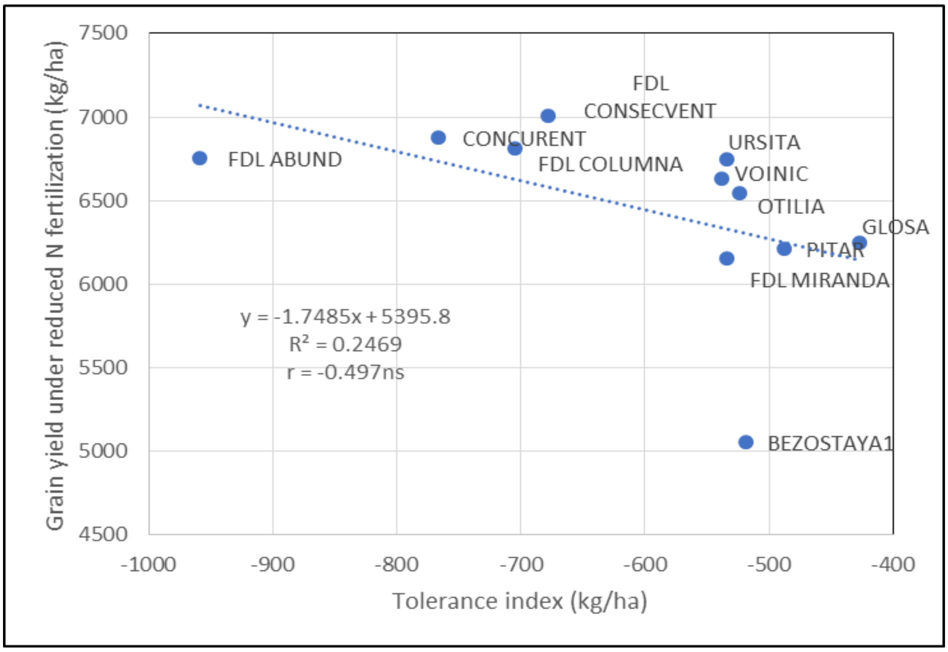

Figure 6. . Relationship between the tolerance index and performance under N deficiency for grain yield

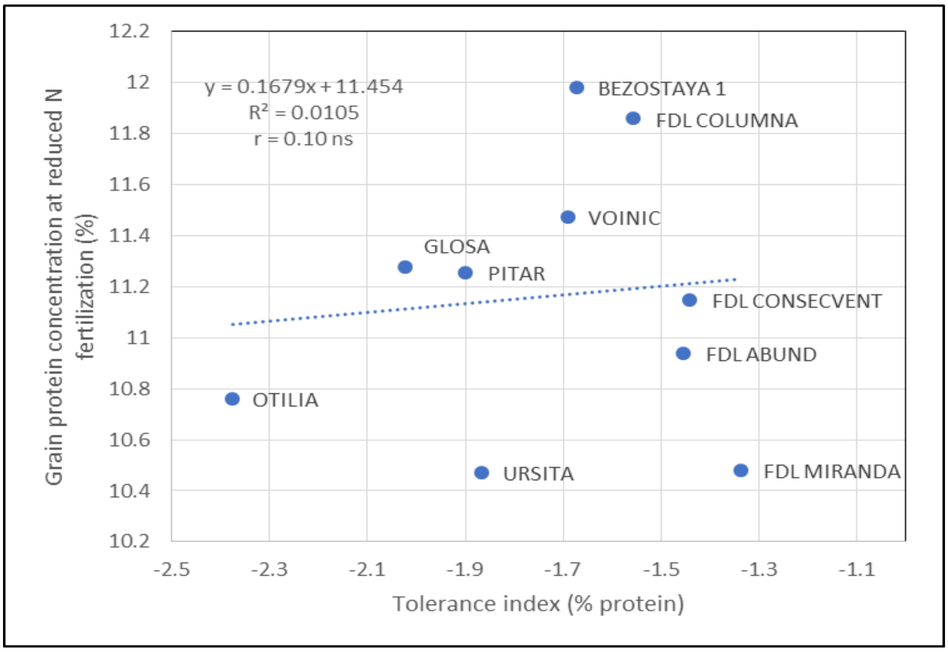

Figure 7. Relationship between the tolerance index and performance under N deficiency for grain protein concentration

Environmental and economic constraints in Europe will favor low Nitrogen input systems and wheat cultivars adapted to moderate N deficiency (Laperche et al., 2008). Therefore, improved performance under lower N fertilization should be an important breeding objective, also useful for adaptation to organic agriculture, where lower N availability is frequent. Testing cultivars under reduced N availability is a necessary step in achieving this objective, but it faces major difficulties related to the many factors involved in plant response to Nitrogen and to higher errors because of soil non-uniformities, which are usually covered by higher N fertilization. In most trials with cultivars grown with two contrasting levels of N fertilization, reduced fertilization did not cause yield decrease. We analyzed seven trials that had the average difference between the two levels of N fertilization greater than 5%, and this allowed identification of cultivar differences in the ability to perform better under N constraint.

On average, lower N availability had larger and more variable effects on grain protein concentration than on yield, indicating that breeding for improved performance under reduced N fertilization should pay more attention to ensuring the required level of bread-making quality. This agrees with Shewry et al. (2023) who showed that modern high-yielding cultivars need higher N fertilizer doses for providing the required grain protein content, than the optimum needed for grain yield.

We dissected the performance under N deficiency stress into two components and found that, for the analyzed germplasm and environments, cultivar performance under usual N availability was more important than the tolerance index for performance under N deficiency stress. This finding has important implications for breeding. First, it means that genetic progress in grain yield and protein concentration under normal N fertilization, which should be easier to achieve, will be reflected in the performance under reduced fertilization. Second, it means that wider germplasm diversity should be explored, to identify differences in the tolerance index, which might further improve performance under reduced fertilization.

The known negative relationship between grain yield and protein concentration was not substantially different under the two levels of N fertilization.

Deviations from the regressions that describe the general relationship between the levels of N fertilization are worth exploiting in breeding for improved performance under reduced fertilization. These deviations highlighted a few cultivars with better efficiency in using less available Nitrogen (such as FDL Consecvent and Voinic), that could be useful parents in breeding.

CONCLUSIONS

Our results led to the following conclusions with implications for wheat breeding programs aiming to improve performance under reduced N fertilization:

- Bread-making quality should be a priority in breeding for adaptation to lower N availability, because the effect of N deficiency was stronger and more variable on protein content than on yield.
- Dissecting the performance under N deficiency stress into two components (performance without stress and tolerance index) we found that, for the analyzed germplasm and environments, cultivar performance under no N stress was more important for the performance under reduced N fertilization, than the tolerance index. The implications for breeding programs are that:

(1) genetic progress in grain yield and protein concentration under normal N fertilization, which should be easier to achieve, will be reflected in the performance under reduced fertilization, and

(2) a wider germplasm diversity should be explored, to identify differences in the tolerance index, which might further improve performance under reduced fertilization.

- Deviations from the regressions that describe the general relationship between the levels of N fertilization are worth exploiting in breeding for improved performance under reduced fertilization. These deviations, along with the deviations from the regression of protein concentration on grain yield, recommended parents to improve the ability to cope with reduced N availability.

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