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Characterization of 4 winter wheat cultivars with different Nitrogen Use Efficiency (NUE): A Lysimeter study

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Summary

In Hungary, the most important cereal is the winter wheat, the yield of which could be significantly affected by the amount of applied nitrogen (N) fertilizers. Unfortunately, the physiological and genetic background of N-use related traits (NUE, NUpE, NUtE) is only poorly understood. Moreover, overuse of fertilizers can be resulted in negative environmental impacts. Therefore, a lysimeter study was conducted at the NAIK ÖVKI Lysimeter Research Station (Szarvas, Hungary) in 2014/2015. In our experiment, 4 winter wheat varieties ('Kinachi-97', 'Sultan-95', 'Euclide', 'Mv Toborzó') were tested for NUE in 64 backfilled gravitation lysimeters. Four different rates of N fertilizer were utilized (N0, N60+60, N120, N120+60) in four replications. Single and split N application were also described and timed to the tillering and jointing stages. In Hungary, winter wheat is usually not irrigated, but equal amount of water was applied six times during the growing season to maintain plant development during the drought periods and simulate the effect of heavy rains. Development of plants and changes in chlorophyll contents (non-destructive Minolta SPAD-502) were recorded on a weekly basis. Shortly, among the four cultivars, 'Euclide' can be characterised by the highest NUE in two N treatments.

Keywords: winter wheat, nitrogen use efficiency, N leaching, gravitation lysimeter

Abbreviations: N – nitrogen, NUE – nitrogen use efficiency

Introduction

The application of chemical fertilizers had a great role in the improvement of cereal production worldwide. However, the application of nutrients alone could not lead to higher yields. New plant types suitable for intensive agriculture were developed by the plant breeders in the second half of the 20th century (Fageria et al. 2008). In Hungary, winter wheat is an economically important crop grown on more than 1 million hectares. The yield of varieties can be increased significantly by the application of N containing fertilizers. However, the physiological and genetic background of N-use related traits (eg. NUE), especially in case of Hungarian ones is only poorly understood. Improving NUE is very important task for breeding because of many aspects of cereal production: (1) it increases profitability through greater yields or reduced fertilizer costs, (2) reduces the greenhouse gas (GHG) emissions and (3) reduces the nitrate leaching to the groundwater reservoirs (Gaju et al. 2011). Significant effects of genotypes, environment and soil N content on NUE were determined (Dobermann 2005, Gaju et al. 2011, Monostori et al. 2016). Therefore, nutrient efficient plants will play a vital role in increasing crop yields per unit area (Fageria et al. 2008). Plant breeders and cereal producers need cost and time effective methods to determine plants' N demand. One of those methods to follow N uptake of plants is the indirect measurement of plant chlorophyll content by using of hand-held SPAD chlorophyll meter. Linear relationship was determined between leaf chlorophyll content analysed in laboratories and SPAD-readings (Wood et al. 1993, Wang et al. 2004).

Field lysimeters are effective scientific instruments to specify the effects of different nutrient and irrigation regimes on the development of crop plants (Szaloki and Szaloki 2003). Lysimeters are also used to evaluate nitrogen losses through leaching and significant variability was observed between years due to the variations in crop rotation, fertilization rates and yield of crops planted (Eder et al. 2015). Unravelling of the N uptake of wheat and the possible effects of groundwater pollution is essential for a sustainable agricultural practice.

Therefore, the objectives of this study were (1) to evaluate and determine the N-related traits, especially NUE and agronomic parameters of winter wheat varieties, selected in earlier studies and (2) to monitor the N leaching in lysimeters over the entire growing season.

Material and Methods

Site

The Lysimeter Research Station of the National Agricultural Research and Innovation Centre is located in Szarvas (south-east Hungary, latitude 46°86'N, longitude 20°52'E). The Station was built in the year of 1971 when 320 non-weighable backfilled gravitation lysimeters were set up on a 1 hectare experimental field. The lysimeters are built up

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Table 1. **Means of soil parameters in the lysimeters at the beginning of the study.**

(0-30 cm depth) (Szarvas, 2014)					
pH (KCl)	K_A*	Total salt m/m%	$CaCO_3$ m/m%	Humus m/m%	KCl- $NO_{2,3}$-N mg/kg
7.14	48.81	0.04	2.23	1.95	3.01

*K_A – Soil plasticity according to Arany (MSZ-08-0205:1978)

in 5 blocks that are connected to 5 measuring cellars. The lysimeters were built in a “4in1 design” that means 320 lysimeters in 5 blocks, 2x32 lysimeters in one side of a measuring cellars and 4 lysimeters with side connections (in one of the 8 parcels in one side). The dimensions of a lysimeter are 1m x 1m x 1m. In our experiment, each vessel was filled with Meadow Chernozem soil with a physical properties of clay loam (clay content is 50%) that contains high level of potassium and phosphorus but the nitrogen level was low (*Table 1*). The soil depth is 0.8 m above a fine gravel drainage zone (0.1 m) that has an outlet to the lysimeter cellar. The percolated water can be collected in 20 l tanks separately from each lysimeter for further analysis.

Meteorology

Meteorological data was collected automatically by a WS-GP1 Compact Weather Station (Delta-T Devices, UK) during the growing season (*Table 2*). Average annual rainfall in the experimental site is 550mm. During the autumn and winter (September-January period), precipitation was beyond the average, available water was sufficient to the optimal plant growth. However, from February to April, rainfall was measured as 56.2 mm, what caused drought-related yield reductions in conventional wheat production of Hungary. To avoid that, irrigation was applied at a uniform rate (10mm per irrigation) six times during March, April and May.

No serious frost damages were observed during the winter months. In February, mean minimum temperature was -1.23°C. Minimum temperature below -10°C was measured only on two days in December (-10.8°C) and January (-12.2°C). Vegetation period was started from the last pentad of February (mean temperature 6.84°C).

Varieties

Four winter wheat varieties (‘Euclide’, ‘Kinachi-97’, ‘Mv Toborzó’ and ‘Sultan-95’) previously screened in field trials (Martonvásár, Keszthely) for different NUE characteristics were used in our experiment (Monostori et al. 2016). ‘Euclide’ is a French variety characterised by relative long duration and good lodging resistance. ‘Mv Toborzó’ is a Hungarian wheat variety bred in Martonvásár for short duration and extra quality. ‘Kinachi-97’ and ‘Sultan-95’ were released in Turkey as a result of International Winter Wheat Improvement Program (Hede et al. 2004).

Maintenance of experiment

Date of sowing was 8th of October, 2014. The seedling number was set to 500 after the 2-leaf developmental stage in every lysimeter. Parcells around the lysimeters were also used for winter wheat production. Crops were protected against weeds, pests and diseases as required by manual weeding and conventional chemical compounds. As already mentioned, irrigation was applied at a uniform rate (10 mm per applications) six times during March, April and May to maintain optimal crop growth. Border plots were not irrigated which has a result of very poor utilization of fertilizers and grains of N-fertilizer were observable even after a month.

Nitrogen application

Because of the “4in1 design” of the lysimeters, the experimental plots were basically rotated by the varieties and furthermore, N application was also randomized. N-regimes were set to (N0) as no N fertilizer application, (N120) 120 kg ha^{-1}, (N60+60) 60+60 kg ha^{-1} and (N120+60) 120+60 kg ha^{-1} N. N-fertilizer, in form of calcium ammonium nitrate (27 % N), was applied two times during the spring period, at tillering stage on 4th of March, 2015 (Zadock’s scale: 20-26); in the split treatments (N60+60, N120+60) plots were top-dressed secondary at jointing stage on 23rd of April, 2015 (Zadock’s scale: 39) (Zadoks et al. 1974).

Growth related parameters and nitrogen use efficiency

Beside the agronomic parameters (yield, grain weight, thousand grain weight, plant height) changes in chlorophyll contents using SPAD-502 hand-held chlorophyll meter (Minolta Co. Ltd., Osaka, Japan), soil parameters, amount of the percolated water during the growing period, N content of the grain and shoot and different N-related parameters were also recorded or determined. Analytical measurements were done by the NAIK ÖVKI’s accredited Laboratory for Environmental Analytics as described in the Hungarian Standards.

SPAD-measurements were taken on the last fully developed leaves (after heading on flag leaves) of ten randomly selected plants within each lysimeters.

Specific NUE was determined after harvest of the varieties as Dobermann (2005) described as follows:

$$NUE=(Y_0/F_N)+AE_N$$

where Y_0 – crop yield in the control treatment, F_N – amount of N applied, AE_N – agronomic efficiency of applied N. Statistical analysis was performed by using of MS Excel and SPSS 22 statistical programme. LSD is the least significant difference (5%) for all comparisons.

Table 2. **Monthly rainfall in the cropping season (mm) in Szarvas in 2014–2015, total precipitation for the season and 6-year averages (Avg., 2005–2010) are also shown.**

Year	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Total
2014/2015	184.2	86.4	26.4	70.6	61.4	16.8	29.2	10.2	61.0	36.2	582.4
Avg.	67.2	35.8	39.8	35.9	25.2	40.1	34.1	41.8	64.2	88.9	473.0

Results

During our lysimeter experiment, effect of N-applications on four winter wheat varieties was observed by using of SPAD-502 chlorophyll meter. Measurements were taken once a week from the application of N-fertilizer to the end of the season.

Significant effect of N on chlorophyll content was observed in all of the varieties. In N0 treatments, plants in all vessels had lower SPAD-values and these values were decreased as the season was continued (*Figure 1a-d*). Effects of split applications were observable in all the varieties what is suggested that the N-fertilizer was taken up by the plants effectively. Highest SPAD readings were measured in N120+60 in all of the varieties after the second top-dressing of the plots but 'Mv Toborzó' had even the highest (58.13) among these wheat genotypes. This value shows that N level was too high and the variety 'Mv Toborzó' has different reaction to N fertilizers compared to the other three varieties.

Yield of varieties (*Table 3*) was measured after harvest and cleaning to determine the effect of different N regimes on seed production and calculate NUE of genotypes. In our experiment, 'Euclide' was the most productive variety in N0, N60+60 and N120 treatments. However, significant difference was observed only in N60+60 and N120. In N60+60, average yield of 'Euclide' was 704.1 g. Seed production of 'Kinachi-97' and 'Euclide' was higher in N60+60 than in N120+60, and N60+60 was also superior to N120 in all the genotypes, which means time of application and effect of genotypes are important factors for an effective N fertilization.

Based on our measurements, because of superior yields, 'Euclide' was characterised by the significantly highest NUE in N60+60 and N120 treatments (*Table 4*).

During our experiment, significant nitrate accumulation was not detected in the soil. Percolated water was collected only during the winter period because in the period of N-applications, natural precipitation was limited. These results show that even a high N-fertilization (N120+60) can be utilized by the plants if water distribution is sufficient (*Table 5*). Significant N movement was not observable even in the highest N regime.

Discussion

Szaloki and Szaloki (2003) described that lysimeters are effective ways to specify the effects of different nutrient and irrigation regimes. Because side of the lysimeters prevent horizontal shift of soluble nutrients, effect of nutrients on the development of crop plants can be measured more accurate. Our results in a lysimeter study suggest that winter wheat

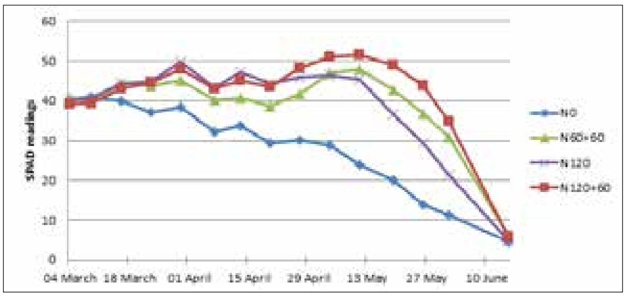

Figure 1a. **Effect of four different N regimes on average SPAD values of 'Kinachi-97'. N fertilizer was applied on 4th of March and on 23rd of April, 2015 (Szarvas, Hungary).**

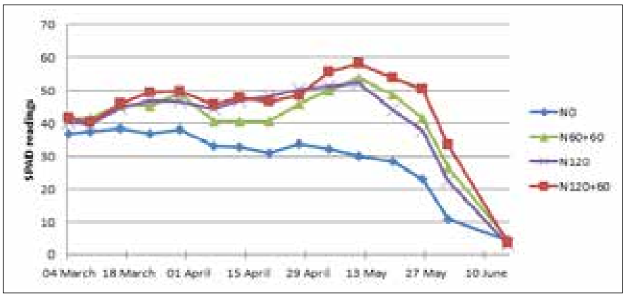

Figure 1b. **Effect of four different N regimes on average SPAD values of 'Mv Toborzó'. N fertilizer was applied on 4th of March and on 23rd of April, 2015 (Szarvas, Hungary).**

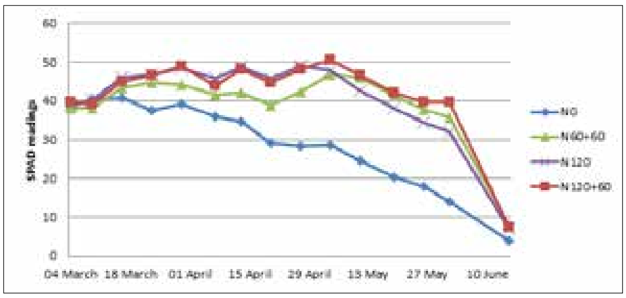

Figure 1c. **Effect of four different N regimes on average SPAD values of 'Sultan-95'. N fertilizer was applied on 4th of March and on 23rd of April, 2015 (Szarvas, Hungary).**

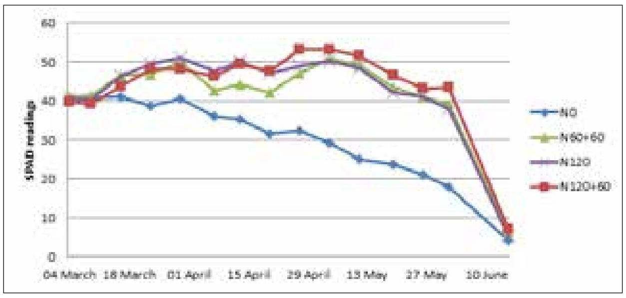

Figure 1d. **Effect of four different N regimes on average SPAD values of 'Euclide'. N fertilizer was applied on 4th of March and on 23rd of April, 2015 (Szarvas, Hungary).**

Table 3. **Yield of different winter wheat varieties grown in lysimeters with different N-applications (Szarvas, Hungary, 2014/2015).**

Treatments	Kinachi-97 g/m²	Euclide g/m²	Sultan-95 g/m²	Mv Toborzó g/m²	LSD
N0	^{a}256.1±60.0	^{a}298.0±160.0	^{a}254.5±105.1	^{a}201.0±109.9	129.4
N60+60	^{a,b}641.8±54.3	^{b}704.1±62.1	^{a}592.9±46.5	^{a}585.0±80.2	70.2
N120	^{a}559.4±121.4	^{b}659.6±40.7	^{a}532.7±84.3	^{a}472.3±74.5	96.4
N120+60	^{a}628.6±71.4	^{a}662.3±49.5	^{a}673.9±26.5	^{a}631.1±23.6	53,1

a,b – significant differences of varieties

Table 4. **NUE of winter wheat varieties grown in lysimeters with different N-applications (Szarvas, Hungary, 2014/2015).**

Treatments	Kinachi-97 kg/kg	Euclide kg/kg	Sultan-95 kg/kg	Mv Toborzó kg/kg
N60+60	53.4±4.5	58.7±5.2	49.4±3.8	48.8±6.7
N120	46.6±10.1	55,0±3.3	44.4±7.0	39.4±6.2
N120+60	34.9±4.0	36.8±2.8	37.4±1.4	35.1±1.3

varieties have different reactions to N fertilizers that resulted in different productivity. Higher NUE can be resulted in higher profitability of wheat cultivation as Gaju et al. (2011) reported. Previous study of our research consortium (Monostori et al. 2016) determined that 'Euclide' has a high NUE and it was also reproduced in a different part of Hungary. SPAD readings are sufficient tools to determine N uptake of varieties, but effect of genotypes must be considered if we use these values for selection. However overuse of N fertilizers has negative effect on the environment (Eder et al. 2015), in water-limited periods of the growing season nitrate leaching is not significant, but irrigation could be needed to maintain effective utilization of nutrients.

Conclusions

Lysimeters are effective scientific instruments to specify the effects of different nutrient and irrigation regimes on the development of winter wheat varieties. NUE is determined as a diverse character of varieties. Based on our results and on the previous studies, genotypes can be selected for that parameter by using of conventional breeding methods supplemented with analytical methods or even MAS. Under temperate climate, water-limited periods of the growing season prevent groundwater pollution by nitrate leaching, but irrigation is needed to achieve good developmental conditions and effective utilization of fertilizers.

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Table 5. **Effect of winter wheat varieties on soil N content analysed in three different dates of the growing season in N120+60 treatment (Szarvas, Hungary, 2014/2015).**

Variety	Soil sample depth in cm	Sampling after first fertilization 15.03.2015	Sampling after second fertilization 07.05.2015 KCl- $NO_{2,3}$-N mg/kg dry soil	Sampling after harvest 30.06.2015
'Kinachi-97'	0-10	116.00	7.51	6.97
	10-20	16.00	9.55	6.33
	20-30	12.20	2.54	4.44
'Sultan-95'	0-10	56.40	2.66	4.30
	10-20	15.90	2.07	3.54
	20-30	20.00	2.46	3.93
'Mv Toborzó'	0-10	43.20	9.45	7.06
	10-20	15.50	2.01	4.39
	20-30	4.20	2.61	3.22
'Euclide'	0-10	49.30	3.79	6.96
	10-20	24.20	2.11	5.07
	20-30	4.82	1.87	2.30