



Relation between nitrogen nutrition index and production of spring malting barley

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Received 29 January 2017; Accepted after revision 3 June 2017; Published online 1 July 2017

Abstract

Although the nitrogen nutrition index (NNI) is a widely used indicator of plant nitrogen status, no model of NNI calculation for spring barley (*Hordeum vulgare*) reflecting also specific malting requirements of grain has been published. The aim of this study was to determine an optimal range of the nitrogen nutrition index (ratio of nitrogen concentration in shoot biomass to critical nitrogen concentration) with respect to optimal grain protein content ($N \times 6.25 = 9.0 - 11.5\%$), plump grain (grain > 2.5 mm) yield and lodging of spring malting barley during 7-year (2007 – 2013) strict field experiments realized under the conditions of three experimental sites in the Czech Republic. A dose of 80 kg N/ha and 130 kg N/ha, respectively, was applied in mineral fertilizers. The nitrogen nutrition index was determined at the BBCH 30 (beginning of stem elongation) and BBCH 45 (late boot stage) growth stages. The most suitable indicators for evaluation of the nitrogen concentration in shoot biomass of the spring malting barley in our experiments proved to be Justes et al.'s model $N_c = 5.35 \text{ DM}(-0.442)$ designated for winter wheat and Zhao's model $N_c = 4.76 \text{ DM}(-0.39)$ designated for winter barley, where DM is shoot dry matter in t/ha. The nitrogen nutrition index for spring malting barley should not exceed a value of $\text{NNI} = 0.80$ and $\text{NNI} = 0.90$ using the Justes et al.'s model and the Zhao's one during the BBCH 30 – 45 growth stages.

Keywords: Dilution curve; Lodging; Plumpness; Protein content; Quality; Yield.

Introduction

Barley is the second most grown cereal in the European Union (Bozek et al., 2016) and nitrogen supply has a crucial influence on grain yield (Shejbalova et al., 2014). On the other hand, high nitrogen rates lead to, for example, greater lodging, reduced grain plumpness and higher grain protein content (O'Donovan et al., 2011) resulting in an inadequate malting quality (Marconi et al., 2011). High rates of nitrogen also decrease most of the efficiency indicators (Szmigiel et al., 2016) increasing risk of nitrogen losses (Gaju et al., 2011).

A proper diagnosis of plant nitrogen status is also an important prerequisite for drought resistance because according to Kreck et al. (2008) nitrogen fertilization alleviates adverse effects of drought stress on the yields of spring barley grain due to higher activity of nitrate reductase. In addition, Sedlar et al. (2014) recorded an

increased resistance of spring barley to a low precipitation amount during grain filling period depending on technique of nitrogen fertilization. Nitrogen fertilization is then especially important because of frequent drought occurrence and higher temperatures during the growing season in conditions of changing climate in Central Europe as stated by Neugschwandtner et al. (2015) and Kren et al. (2015).

During the vegetative growth period of crops nitrogen concentration decreases monotonically as the crops grow (Lemaire et al., 2008). The critical nitrogen concentration represents the minimum nitrogen concentration required for maximum crop growth (Justes et al., 1994). Using a critical nitrogen dilution curve enables then to quantify the nitrogen status for many crop species (Debaeke et al., 2012; Hu et al., 2014; Sadras and Lemaire, 2014; Reyes et al., 2015; Sedlar et al., 2015). However, for practical use of the critical nitrogen dilution curve in malting barley production, evaluation in terms of other parameters is necessary. Furthermore, no model of calculation of the critical nitrogen dilution curve for spring barley is nowadays available.

Another uncharted issue in terms of the nitrogen nutrition index is a higher risk of lodging due to high supply of nitrogen as O'Donovan et al. (2011) stated, which is one of the most important factors determining the yield of barley (Spunar et al., 2002) and mycotoxins level in barley grain (Nakajima et al., 2008).

The aim of this study was to determine which of models of calculation of the critical nitrogen concentration in shoot biomass is appropriate for spring malting barley production and to determine the optimal range of the nitrogen nutrition index reflecting grain yield, grain plumpness, grain protein content and lodging of malting barley grown under Central European field conditions.

Materials and Methods

Strict field experiments with spring barley (*Hordeum vulgare* L.) of malting cultivar Jersey were realized during a seven-year period (2007 – 2013) at three sites with different soil-climatic conditions in the Czech Republic (Central Europe): Hněvčeves (S₁, 50°18'46.269" N, 15°42'51.552" E) with an annual mean temperature of 8.4°C and annual mean precipitation of 560 mm, Humpolec (S₂, 49°32'49.604" N, 15°21'6.405" E) with an annual mean temperature of 6.7 °C and annual mean precipitation of 678 mm and Ivanovice na Hané (S₃, 49°18'34.209" N, 17°5'18.753" E) with an annual mean temperature of 9.3 °C and annual mean precipitation of 554 mm. The annual mean precipitation and temperature constitute 30-year averages (1971 – 2000). Detailed characteristics of experimental sites are given by Kulhanek et al. (2011) and Sedlar et al. (2014).

The experimental scheme is given in Table 1. A dose of 80 and 130 kg N/ha, respectively, was applied by two commonly used techniques of nitrogen fertilizers application (basal application before sowing (+ top dressing when 130 kg N/ha was applied) and injection at the end of tillering, respectively). The application of nitrogen fertilizers containing sulphur (20 and 27 kg S/ha, respectively) was further included because of the connection of nitrogen metabolism with sulphur metabolism. Each treatment had four replications.

Table 1. Fertilizer treatment, nitrogen amounts and fertilization timing. CAN – Calcium Ammonium Nitrate, UAN – Urea Ammonium Nitrate, AS – Ammonium Sulphate (20.5 % S), UAS – Urea Ammonium Sulphate (6 % S).

| Treatment | Dosage of added N per ha (fertilizer form) | | Total N dosage per ha |
|-----------------------------------|--|------------------|-----------------------|
| | before sowing | end of tillering | |
| basal application 80 | 80 kg (CAN) | | 80 kg |
| injection 80 | | 80 kg (UAN) | 80 kg |
| basal application (+topdress) 130 | 80 kg (CAN) | 50 kg (CAN) | 130 kg |
| injection 130 | | 130 kg (UAN) | 130 kg |
| basal application + S | 23 kg (AS) + 57 kg (CAN) | | 80 kg |
| injection + S | | 80 kg (UAS) | 80 kg |

The sowing density was 450 seeds per m², the size of each plot was 39 m², of which 15 m² was harvested. Samples of shoot biomass at the BBCH 30 growth stage (beginning of stem elongation) and BBCH 45 growth stage (late boot stage: flag leaf sheath swollen) were taken from a 0.25 m² area. The proportion of plump grains was determined by estimating the amount of seed retained on a sieve with 2.5 by 22.2 mm slots. Grain yield and thousand grain weight were converted to 12 % moisture, i.e. usual moisture of grain at harvest and multiplied by the proportion of plump grains to express plump grain yield. Lodging was visually determined on a 1 to 9 scale just before harvest with 1 considered maximum lodging. Harvest index was calculated as a ratio of grain yield and weight of shoot biomass at harvest. Straw yield was determined by weighing of straw on harvested field plot. Length of 50 stems from each plot was determined prior to harvest.

Nitrogen concentration in plant biomass was determined by the Kjeldahl method on the Vapodest 50s (Gerhardt, Germany). Grain protein content was calculated by multiplying the nitrogen concentration in dry matter of grain with the 6.25 coefficient ($N \times 6.25$) (Andersson et al., 1999). The set of our results was divided according to FAO (2009) into two categories: samples with optimal ($N \times 6.25 = 9.0 - 11.5$ %) and excessive ($N \times 6.25 > 11.5$ %) grain protein content.

In the experiments three models of calculation of the critical nitrogen concentration (N_c) were compared:

Justes et al. (1994) designated for winter wheat: (Eq. 1) $N_c = 5.35 DM^{0.442}$

where DM is shoot dry matter (t/ha). For $DM < 1.55$ t/ha, the constant value $N_c = 4.4$ % is used.

Zhao (2014) designated for winter barley: (Eq. 2) $N_c = 4.76 DM^{0.39}$

where DM is shoot dry matter (t/ha). For $DM < 1.79$ t/ha, the constant value $N_c = 3.77$ % is used.

Ziadi et al. (2010) designated for spring wheat: (Eq. 3) $N_c = 3.85 DM^{0.57}$

where DM is shoot dry matter (t/ha). For $DM < 0.98$ t/ha, the constant value $N_c = 3.89$ % is used.

The nitrogen nutrition index was calculated as a ratio of measured total nitrogen concentration and the critical nitrogen concentration in shoot biomass (Sadras and Lemaire, 2014).

$$\text{NNI} = \frac{N_m}{N_c} \quad (\text{Eq. 4})$$

where N_m is total nitrogen concentration in shoot biomass (%) and N_c is the critical nitrogen concentration in shoot biomass calculated according to the respective model. For $\text{NNI} > 1$ the crop nitrogen status can be considered non-limiting, so any increase in nitrogen supply would not increase crop biomass and for $\text{NNI} < 1$ the crop nitrogen status can be considered limited by nitrogen supply (Sadras and Lemaire, 2014).

Differences in the NNI values between optimal and excessive grain protein contents (Tables 3 – 5), differences in the NNI values among lodging occurrences (Table 6) and differences in the plump grain yields between optimal and excessive grain protein contents (Table 3) were evaluated using one-way standard analysis of variance (ANOVA). Fisher LSD test was used for analysis of the data variance which was expressed using letter indices in tables. Nevertheless, for easier interpretation, the results were expressed as intervals of least squares means. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be statistically significant. Spearman's rank correlations were used to analyse relations between studied parameters of spring barley because studied variables were not normally distributed; each variable consists of 472 cases. The statistical analysis of data was carried out using the Statistica 13 (Dell Inc., USA).

Results

As stated in Table 2, the nitrogen nutrition index at the BBCH 30 growth stage calculated by the Justes et al.'s model and the Zhao's model significantly correlated moderately with grain protein content ($r = 0.46 - 0.50$). It means that 21 – 25 % of the variation in the grain protein content is explained by the NNI value. The nitrogen nutrition index calculated by the Ziadi et al.'s model also significantly correlated with the grain protein content; this correlation, though, was weak ($r = 0.26$). Nitrogen nutrition index determined at the BBCH 30 growth stage significantly correlated also with plump grain yield ($r = -0.32 - (-0.36)$) and lodging ($r = -0.26 - (-0.31)$) using both the Justes et al.'s model and the Zhao's one for calculating of the critical nitrogen concentration (N_c). The NNI value determined at the BBCH 45 growth stage significantly correlated with the grain protein content ($r = 0.35 - 0.36$) as well as lodging ($r = -0.35$) using both the N_c calculation models. This means that ca. 12 % of the variation in both grain protein content and lodging is explained by the NNI value determined at the BBCH 45 growth stage. Very weak correlations were recorded between the NNI determined at both studied growth stages and many other parameters such as grain yield, straw yield, thousand grain weight, harvest index or length of stem (data not shown).

Table 2. Spearman's rank correlations between the traits determined. The r-values marked with asterisks are significant at levels of significance * $P < 0.05$ and ** $P < 0.001$, respectively. GPC – grain protein content, PGY – plump grain yield.

| Variable | NNI 30 Justes | NNI 45 Justes | NNI 30 Zhao | NNI 45 Zhao | NNI 30 Ziadi | NNI 45 Ziadi | GPC | PGY | lodging |
|----------------|------------------|------------------|----------------|----------------|-----------------|-----------------|---------|--------|---------|
| NNI 45 Justes | 0.34** | | | | | | | | |
| NNI 30 Zhao | 0.97** | 0.29** | | | | | | | |
| NNI 45 Zhao | 0.35** | 1.00** | 0.31** | | | | | | |
| NNI 30 Ziadi | 0.76** | 0.53** | 0.61** | 0.51** | | | | | |
| NNI 45 Ziadi | 0.31** | 0.99** | 0.25** | 0.98** | 0.54** | | | | |
| GPC | 0.46** | 0.35** | 0.50** | 0.36** | 0.26** | 0.35** | | | |
| PGY | -0.32** | -0.16** | -0.36** | -0.16** | -0.13* | -0.17** | -0.57** | | |
| lodging | -0.26** | -0.35** | -0.31** | -0.35** | -0.15* | -0.35** | -0.34** | 0.23** | |
| grain > 2.5 mm | -0.26** | -0.22** | -0.28** | -0.21** | -0.17** | -0.23** | -0.54** | 0.59** | 0.57** |

The set of our results was then divided according to FAO (2009) into two categories: samples with optimal ($N \times 6.25 = 9.0 - 11.5\%$) and excessive ($N \times 6.25 > 11.5\%$) grain protein content. The nitrogen nutrition index in both growth stages was compared among the two categories of the grain protein content separately for each experimental site (Tables 3 and 4) due to significant differences in the grain protein content among the experimental sites (data not shown).

The nitrogen nutrition index determined at the BBCH 30 growth stage achieved significantly higher values in plants with excessive grain protein content compared to plants with optimal grain protein content. If the NNI determined using the Justes et al.'s model at the BBCH 30 growth stage exceeded a value of $NNI = 0.89$, excessive grain protein content was recorded at the S_2 and S_3 experimental sites. Using the Zhao's model the excessive grain protein content occurred whenever the NNI at the BBCH 30 exceeded a value of $NNI = 1.06$, at the S_3 site the lower limit of excessive grain protein content was even $NNI = 1.00$. Although a value of $NNI = 1.04$ was an upper limit of optimal grain protein content at the S_2 site, the same value of $NNI = 1.04$ was simultaneously a lower limit of excessive grain protein content. Therefore, the NNI at the BBCH 30 growth stage not exceeding the value of $NNI = 1$ is desirable. Moreover, significant decrease in plump grain yields was recorded as a side effect of excessive grain protein content at the S_1 and S_3 experimental sites. At the S_2 experimental site these differences were not significant (Table 3).

The NNI determined at the BBCH 45 growth stage achieved significantly higher values in plants with excessive grain protein content compared to plants with optimal grain protein content at the S_1 and S_2 sites, whereas at the S_3 site this difference was not significant while using both models of N_c calculation (Table 4). If the NNI determined at the BBCH 45 growth stage did not exceed a value of $NNI = 0.68$ a $NNI = 0.73$ using the Justes et al.'s model and the Zhao's one, respectively, no excessive grain protein content was recorded at the S_1 and S_2 experimental sites.

Table 3. Nitrogen nutrition index at the BBCH 30 growth stage and plump grain yield among optimal ($N \times 6.25 = 9.0 - 11.5 \%$) and excessive ($N \times 6.25 > 11.5 \%$) grain protein content at individual experimental sites. Number of cases assessed (n).

| Parameter | grain protein content (%) | experimental site | | |
|-------------------|---------------------------|--------------------------|--------------------------|--------------------------|
| | | S ₁ | S ₂ | S ₃ |
| n | 9.0 - 11.5 | 22 | 83 | 71 |
| | > 11.5 | 146 | 77 | 73 |
| NNI 30 Justes | 9.0 - 11.5 | 0.74 - 0.86 ^a | 0.83 - 0.89 ^a | 0.79 - 0.86 ^a |
| | > 11.5 | 0.95 - 1.00 ^b | 0.89 - 0.95 ^b | 0.88 - 0.95 ^b |
| NNI 30 Zhao | 9.0 - 11.5 | 0.81 - 0.94 ^a | 0.97 - 1.04 ^a | 0.86 - 0.94 ^a |
| | > 11.5 | 1.06 - 1.11 ^b | 1.04 - 1.11 ^b | 1.00 - 1.08 ^b |
| plump grain yield | 9.0 - 11.5 | 6.29 ^a | 5.72 ^a | 6.70 ^a |
| | > 11.5 | 5.44 ^b | 6.07 ^a | 5.24 ^b |

Values within the column marked with the same letter are not significantly different at $P \leq 0.05$ for each model of NNI calculation.

Table 4. Nitrogen nutrition index at the BBCH 30 growth stage among optimal ($N \times 6.25 = 9.0 - 11.5 \%$) and excessive ($N \times 6.25 > 11.5 \%$) grain protein content at individual experimental sites. Number of cases assessed (n).

| NNI 45 | grain protein content (%) | experimental site | | |
|--------|---------------------------|--------------------------|--------------------------|--------------------------|
| | | S ₁ | S ₂ | S ₃ |
| n | 9.0 - 11.5 | 22 | 83 | 71 |
| | > 11.5 | 146 | 77 | 73 |
| Justes | 9.0 - 11.5 | 0.63 - 0.75 ^a | 0.50 - 0.57 ^a | 0.75 - 0.86 ^a |
| | > 11.5 | 0.92 - 0.97 ^b | 0.68 - 0.76 ^b | 0.82 - 0.92 ^a |
| Zhao | 9.0 - 11.5 | 0.65 - 0.78 ^a | 0.55 - 0.62 ^a | 0.77 - 0.87 ^a |
| | > 11.5 | 0.94 - 0.99 ^b | 0.73 - 0.81 ^b | 0.83 - 0.94 ^a |

Values within the column marked with the same letter are not significantly different at $P \leq 0.05$ for each model of NNI calculation.

Table 5 presents differences in the NNI determined at both growth stages (BBCH 30 as well as BBCH 45) between two categories of grain protein content. Samples with the highest lodging (lodging occurrence of 1 – 3) were removed from the data set. Excessive grain protein content was recorded if $NNI > 0.86$ at the S₁ site, $NNI > 0.79$ at the S₂ site and $NNI > 0.84$ at the S₃ experimental site while using the Justes et al.'s model and if $NNI > 0.91$ at the S₁ site, $NNI > 0.89$ at the S₂ site and $NNI > 0.90$ at the S₃ site while using the Zhao's model of N_c calculation.

The lowest lodging was recorded if $NNI < 0.87$ and $NNI < 0.67$ using the Justes et al.'s at the BBCH 30 and BBCH 45 growth stage, respectively and $NNI < 1.02$ and $NNI < 0.72$ using the Zhao's model at the BBCH 30 and BBCH 45 growth stage, respectively. Intervals of the NNI determined at the BBCH 45 growth stage reflecting optimal grain protein content ($NNI < 0.80$ Justes and $NNI < 0.90$ Zhao) achieved lower than 25 % of lodged stems (Table 6). These NNI values were significantly lower compared to the NNI values found when the highest lodging occurred.

Table 5. Nitrogen nutrition index at the BBCH 30 – 45 growth stages. Significantly lodged stands (lodging occurrence 1 – 3) were removed from dataset. Number of cases assessed (n).

| site | grain protein content | n | 95% confidence interval | |
|----------------|-----------------------|-----|--------------------------|--------------------------|
| | | | NNI Justes 30-45 | NNI Zhao 30-45 |
| S ₁ | 9.0 - 11.5 | 42 | 0.70 - 0.79 ^a | 0.75 - 0.83 ^a |
| | > 11.5 | 92 | 0.86 - 0.92 ^b | 0.91 - 0.98 ^b |
| S ₂ | 9.0 - 11.5 | 166 | 0.67 - 0.73 ^a | 0.76 - 0.83 ^a |
| | > 11.5 | 150 | 0.79 - 0.86 ^b | 0.89 - 0.96 ^b |
| S ₃ | 9.0 - 11.5 | 94 | 0.73 - 0.80 ^a | 0.77 - 0.83 ^a |
| | > 11.5 | 98 | 0.84 - 0.90 ^b | 0.90 - 0.99 ^b |

Values within the column marked with the same letter are not significantly different at $P \leq 0.05$.

Table 6. Nitrogen nutrition index at the BBCH 45 growth stage recorded for individual incidence of lodging visually determined on the 1 to 9 scale with 1 considered maximum lodging. Number of cases assessed (n).

| lodged stems (lodging) | n | NNI Justes 30 | NNI Zhao 30 | NNI Justes 45 | NNI Zhao 45 |
|------------------------|-----|--------------------------|--------------------------|--------------------------|--------------------------|
| > 75 % (1-2) | 84 | 0.95 - 1.01 ^b | 1.06 - 1.13 ^a | 0.92 - 1.01 ^d | 0.95 - 1.03 ^d |
| 25 - 75 % (3-6) | 129 | 0.92 - 0.97 ^b | 1.04 - 1.10 ^a | 0.82 - 0.87 ^c | 0.85 - 0.92 ^c |
| < 25 % (7-8) | 108 | 0.82 - 0.87 ^a | 0.91 - 0.97 ^b | 0.70 - 0.78 ^b | 0.73 - 0.80 ^b |
| 0% (9) | 127 | 0.84 - 0.89 ^a | 0.97 - 1.02 ^c | 0.60 - 0.67 ^a | 0.65 - 0.72 ^a |

Values within the column marked with the same letter are not significantly different at $P \leq 0.05$.

Discussion

Nitrogen nutrition of spring barley is strongly limited because if the nitrogen supply is too high, it has a negative impact on malting quality of grain particularly as a consequence of high grain protein content (Marconi et al., 2011). This fact indicates a necessity of precise nitrogen status determination in production of spring malting barley.

The Ziadi et al.'s calculation of the critical nitrogen concentration in shoot biomass (N_c) at the BBCH 30 growth stage proved to be the least suitable for diagnosing the nitrogen status of the spring malting barley due to weak correlation between the NNI and the grain protein content as well as very weak correlation between the NNI and both plump grain yield and lodging.

The strongest correlation was recorded between the NNI determined at the BBCH 30 growth stage using both models of N_c calculation and grain protein content ($r = 0.46 - 0.50$). Excessive grain protein content ($N \times 6.25 > 11.5\%$) occurred if the NNI at the BBCH 30 growth stage exceeded a value of $NNI = 0.89$ and $NNI = 1.00$ using the model of Justes et al. and Zhao, respectively. These values represent the lowest limits of occurrence of the excessive grain protein content.

In contrast to the NNI determined at the BBCH 30 growth stage, correlation between the NNI at the BBCH 45 growth stage and grain protein content was moderate

($r = 0.35 - 0.36$) which could explain insignificant differences in the NNI determined at the BBCH 45 growth stage between plants with optimal grain protein content and plants with the excessive one at the S_3 site. The excessive grain protein content occurred if the NNI at the BBCH 45 growth stage was higher than $NNI = 0.68$ and $NNI = 0.73$ with the NNI calculated by the Justes et al.'s model and the Zhao's model, respectively.

Besides the grain protein content, the NNI values determined at the BBCH 30 growth stage significantly correlated with the plump grain yield and the NNI values determined at both growth stages significantly correlated with lodging. For this reason, these parameters were also taken into account when the optimal range of the nitrogen nutrition index was searched. Moderate correlation between the NNI at the BBCH 30 growth stage calculated by both models of the N_c calculation with plump grain yield ($r = -0.32 - (-0.36)$) was found. This can explain significantly higher plump grain yield of barley plants with optimal grain protein content compared to plants with excessive grain protein content at two experimental sites. These results are in conformity with the results of Weston et al. (1993) and Sedlar et al. (2013).

Despite significant differences in the optimal range of the NNI between the BBCH 30 growth stage and BBCH 45 growth stage, common range of the NNI for both growth stages is important to find. The excessive protein content in grain of spring malting barley occurred with 95 % confidence exceeding values of $NNI = 0.79 - 0.86$ while using the Justes et al.'s model and $NNI = 0.89 - 0.91$ while using the Zhao's model of N_c calculation at both growth stages. Thus, for easier interpretation it can be assumed that the $NNI < 0.80$ using the Justes et al.'s model and $NNI < 0.90$ using the Zhao's model are upper limits for optimal grain protein content during the BBCH 30 – 45 growth stages. Thus, the Zhao's model seems to be more appropriate for the N_c calculation because the optimal NNI is closer to the value of $NNI = 1$ which is the optimum proclaimed by Sadras and Lemaire (2014). On top of that, lower limit of the excessive grain protein content occurrence was less variable among experimental sites while using the Zhao's model.

Correlation between the NNI determined at the BBCH 45 growth stage and lodging achieved the same magnitude using both models of the N_c calculation, i.e. $r = 0.35$, which means that these correlations were slightly stronger than with the NNI determined at the BBCH 30 growth stage. This is in agreement with the findings of Matusinsky et al. (2015) who stated that risk of lodging can be predicted already during stem elongation. Optimal range of the NNI with respect to grain protein content achieved lower than 25 % of lodged stems. This NNI range significantly differed from the NNI range recorded in the most lodged stands, i.e. lodging occurrence of 1 – 6. It can be assumed that the NNI complying with optimal grain protein content leads to relatively low lodging occurrence which is in accordance with the results of Lauer (1991) who recorded malting barley of an acceptable market grade while applying ethephon leading to low lodging.

The fact that the $NNI = 1$ may not be always optimal was also shown in some other crops, e.g. Debaeke et al. (2012) recorded the highest seed yields of sunflower at most experimental sites if $NNI = 0.8$ and Hu et al. (2014) found the highest tuber yields of potato if $NNI = 0.4 - 1.0$.

Acknowledgements

This study was supported by NAZV project of the Ministry of Agriculture of the Czech Republic QJ1530171.

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