

## Wheat genotypes under reduced nitrogen supply: changes in chlorophyll fluorescence parameters

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**Abstract:** Lowering chemical fertilizer input and breeding plants with better nitrogen use efficiency is one of the main goal of research on plant nutrition. It is therefore of major importance to identify the critical steps controlling plant nitrogen use efficiency (NUE). Based on identifying nitrogen sensitive genotypes with every trait, which is participates in this features, we can step forward to have breed crop lines regarding NUE. NUE is largely influenced by the plant's photosynthetic efficiency. Chlorophyll fluorescence (Chl-fl) is connected to the primary reactions of photosynthesis. Parameters of Chl-fl induction curve provide parameters which are used to characterise plant conditions *in vivo*. The Chl-fl parameters were measured at tillering of 31 wheat genotypes under reduced nitrogen content. Several parameters of Chl fluorescence including potential (Fv/Fm) and actual photochemical activity (Yield), non-photochemical quenching (NPQ), vitality index (RFD), etc. were detected with a PAM 2001 modulated light fluorometer. Dry weight of root and shoots and number of tillers also were investigated. Based on our results, some genotypes under reduced nitrogen supply are characterized by similar photochemical activity than under optimal nitrogen nutrition. These genotypes may have the basis of new breeding lines after detailed investigations. Chl-fl parameters's sensitivity for detecting nitrogen deficiency is different, but some of them are really applicable for describing nitrogen lack.

**Keywords:** wheat, nitrogen, chlorophyll fluorescence

### Introduction

Nitrogen is important in supporting growth and providing high yields. Insufficient nitrogen application causes reduction in plant production, but nitrogen in excess means environmental, health and economic problems. The increased nitrogen-input and global nitrogen-flows pose environmental (Galloway et al., 2008) and nutrient imbalance problems in agricultural development (Vitousek et al., 2009). Beside the question of sustainable agriculture, the demand for fertilizers remains strong. The world nitrogen-fertilizer consumption had decupled in a last 50 years (IFA Statistics, 2010). Two crops, wheat and rice, which have key roles in feeding people, consume 70% of the chemical fertilizers. Unfortunately, the utilization efficiency of nitrogenous fertilizers of these plants under field conditions is around 50% and 25-30%, respectively. A crucial challenge for intensive but green agriculture is to improve nitrogen use efficiency (NUE) so that yields can be improved or maintained with reduced N inputs. Nitrogen use efficiency can be defined as the amount of biomass or yield produced per unit of nitrogen fertiliser applied (Vitousek, 1982). Several commonly used practices have been documented to improve nitrogen use efficiency, like selecting a variety or hybrid with a high harvest index (ratio of grain yield to total plant mass; Bufogle et al., 1997); selecting the proper nutrient rate through soil testing or realistic yield expectations and expected crop removal; matching application timing with crop uptake patterns (Scharf and Alley, 1993); banding fertilizer sources (Eghball and Sander, 2001). Nitrogen use efficiency is largely influenced by the plant's photosynthetic activity. The main objective of our research is to establish the relationships between nitrogen-use efficiency and photosynthetic parameters, like chlorophyll fluorescence, in different wheat genotypes under controlled conditions. We

investigate parameters, which are able to use detecting different N supply conditions of plant.

### Materials and methods

The experimental plant was wheat (*Triticum aestivum* L.), 31 different genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltói, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai) were investigated using two different nitrogen supply (optimal and ¼ part of optimal). The environmental conditions of climate-room (University of Debrecen, Department of Agricultural Botany, Crop Physiology and Biotechnology) were controlled. All of the measurements were in tillering growth stage with three replicates. Dry weight of roots and shoots were determined separately with thermogravimetric method under 65°C. The numbers of shoots were also counted. The photochemical activity was established by *in vivo* chlorophyll fluorescence (Chl-fl) method described by Schreiber et al., 1986 with chlorophyll fluorometer (PAM-2001, WALZ GmbH, Germany). Several parameters of Chl-fl including potential (Fv/Fm) and actual photochemical activity (Yield), non-photochemical quenching (NPQ), vitality index (RFD), electron transport rate (ETR) were detected. For preparing results Microsoft Office Excell 2007 and SigmaPlot for Windows Version 12.0 programmes were applied. Significant differences between treatments were signed with star (\*):  $p < 0,05^*$ ,  $p < 0,01^{**}$ ,  $p < 0,001^{***}$

### Results and discussion

Root has a high influence on plant production by its uptaking activity. Besides this activity root size and architecture also has a crucial role in efficient nutrient absorption in the case of nitrogen as well (Xu et al., 2012). Root allows nitrogen enter into the cell by transporters of root cell plasma membrane, on the other hand responses with the balance of growth and development for local signals of nitrogen (Krapp et al., 2014). Following of dry weight changes by the effect of reduced nitrogen amount may have an important data in the differences of genotypes. The figure 1. shows the alternation in root's dry weight by the effect of lower nitrogen supply in different wheat genotypes. In the most investigated genotypes the reduced nitrogen application induced higher root dry weight. Serious nitrogen deprivation leads to short primary roots and decline the ratio of lateral roots. Babona (0.75g ±0,09) and Hyfi (0.74g ±0,091) have relatively high values of root dry weight under low nitrogen condition. Medium nitrogen deficiency can force auxin production which induces the growth of lateral roots (Gruber et al., 2013). Nitrogen, which was taken up by roots will be transported almost immediately to the shoot. Shoot has also an important role in plant development, thus its advancement, size determinant in yield. The figure 2. shows the values of shoot's dry weight by the effect of optimal and lower nitrogen supply in different wheat genotypes. According to our results the reduced nitrogen supply did not caused dry weight loss in Mulan, Mv. Karéj and Mv. Lucilla. No significant differences was experienced in these genotypes, the reduced nitrogen amount did not means deficiency. Optimal N nutrition caused generally higher number of shoots, but in some genotypes the reduced N amount resulted in similar number of shoots, than the optimal supply (Babona, Bánkúti Marquis, HB-04, Sirtaki) (data not showed).

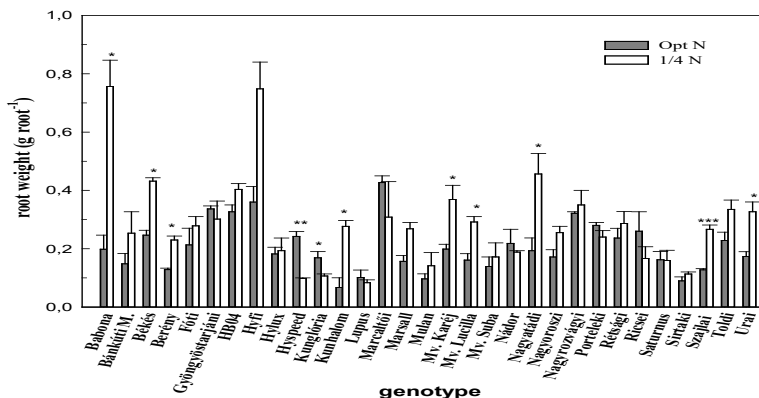


Figure 1 Changes of dry weight of roots ( $\text{g root}^{-1}$ ) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal,  $\frac{1}{4}$  part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltói, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai).  $n=3$ ,  $\pm$ s.e. (N effects compare to the control value  $p \leq 0,05^*$ ,  $p < 0,01^{**}$ ,  $p < 0,001^{***}$ )

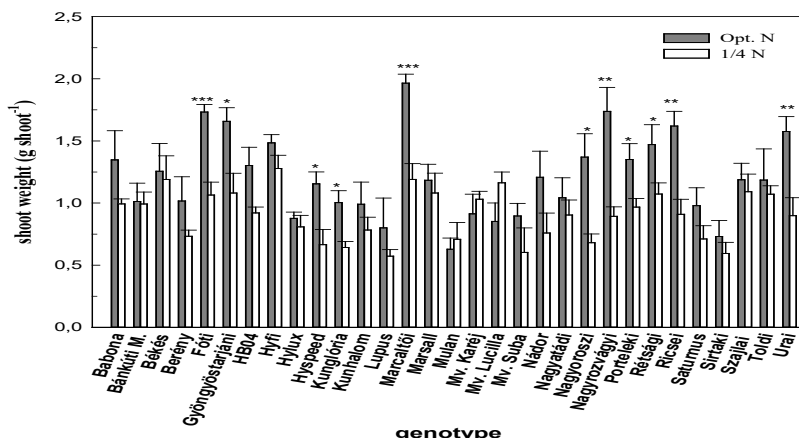


Figure 2 Changes of dry weight of shoots ( $\text{g shoot}^{-1}$ ) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal,  $\frac{1}{4}$  part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Fóti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marcaltói, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyrozvágyi, Porteleki, Rétsági, Ricsei, Saturnus, Sirtaki, Szajlai, Toldi, Urai).  $n=3$ ,  $\pm$ s.e. (N effects compare to the control value  $p \leq 0,05^*$ ,  $p < 0,01^{**}$ ,  $p < 0,001^{***}$ )

Nitrogen nutrition influences the plant photosynthetic capacity through the decrease of synthesis of several key photosynthetic enzymes, especially of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), thus affecting the carbon assimilation, and subsequently also the photochemical processes in thylakoid membranes (Harbinson et al., 1990). Chlorophyll fluorescence measurements give us several useful parameters for characterizing photochemical activity of leaf. The optimal value of Fv/Fm according to Björkmann and Demming-Adams (1987) is  $0.832 \pm 0,004$ . In the case of Bánkúti M., Békés, Fóti, Kunglória, Rétsági, Szajlai, Toldi and Urai genotypes no significant differences were expressed between the two treatments. It means, that the reduced N supply did not caused incline in the maximal photochemical efficiency of PSII in these genotypes.

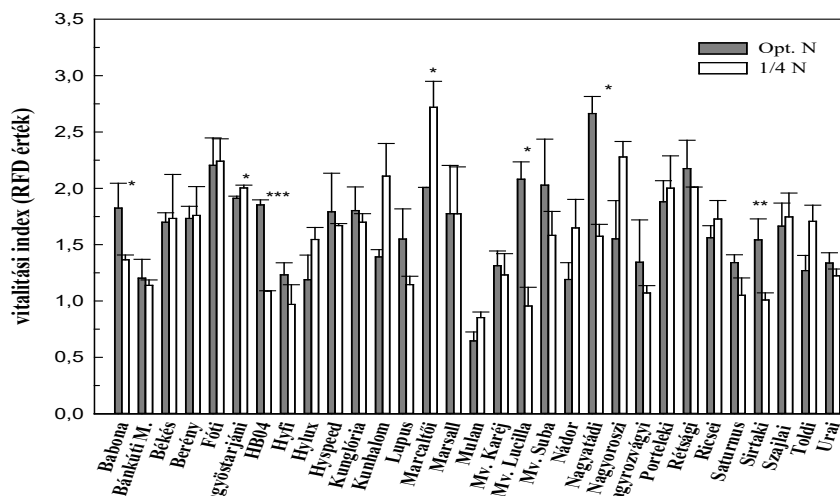


Figure 3 Changes of potential photochemical activity (Fv/Fm) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, 1/4 part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Főti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marsaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyorozvágyi, Porteleki, Rétsági, Ricsai, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3,  $\pm$ s.e. (N effects compare to the control value  $p \leq 0,05^*$ ,  $p < 0,01^{**}$ )

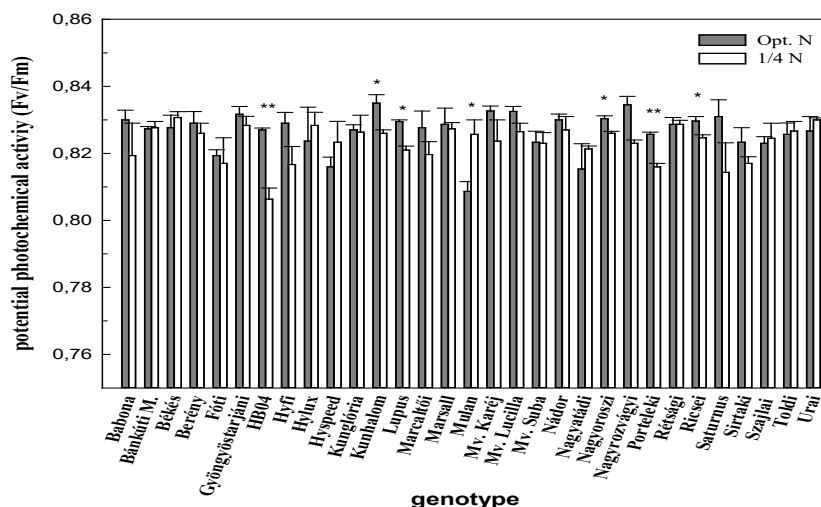


Figure 4 Changes of vitality index (RFD) by the effect of different amount of nitrogen (N) supply (Opt. N: optimal, 1/4 part of optimal: (1/4N) in wheat genotypes (Babona, Bánkúti M, Békés, Berény, Főti, Gyöngyöstarjáni, HB04, Hyfi, Hylux, Hyspeed, Kunglória, Kunhalom, Lupus, Marsaltői, Marsall, Mulan, Mv Karéj, Mv Lucilla, Mv Suba, Nádor, Nagyatádi, Nagyoroszi, Nagyorozvágyi, Porteleki, Rétsági, Ricsai, Saturnus, Sirtaki, Szajlai, Toldi, Urai). n=3,  $\pm$ s.e. (N effects compare to the control value  $p \leq 0,05^*$ ,  $p < 0,01^{**}$ ,  $p < 0,001^{***}$ )

The relative fluorescence decrease also was measured by Lichtenthaler and Rinderle (1988) who published this parameter as vitality index (RFD). When the value of RFD

decreases under 1, it indicates stress of plants. In our experiments based on this parameters the reduced N application did not cause stress in this early growth stage of 25 genotypes. In the case of six genotypes the nitrogen deprivation generate stress situation: Hyfi, Mulan, HB04, Nagyrozvágyi, Saturnus, Sirtaki 1,009 (Figure 4). From the other examined Chl-*f* parameters, the 1-*qp*/NPQ ratio was the parameter, which showed differences among genotypes in terms of treatments.

## Conclusions

In terms of root dry weight application the reduced nitrogen amount induced higher production, than in optimal nitrogen supply. Optimal nitrogen nutrition caused generally higher number of shoots, but in some genotypes the reduced nitrogen amount resulted in similar number of shoots, than the optimal supply. Interestingly, the reduced nitrogen supply did not caused dry weight loss of shoots in Mulan, Mv. Karěj and Mv. Lucilla. Among the investigated chlorophyll fluorescence parameters some are particularly adaptable for characterization of nitrogen demand of genotypes in the early growth stage. There was a genotypic variation in photochemical activity, and it was influenced by different nitrogen nutrition. The value of 1-*qP*/NPQ was found to be a suitable parameter for characterizing the intrinsic ability of PSII to balance photochemical and non-photochemical quenching under the given nitrogen supply. According to our results some genotypes's (GK. Békés, MV. Marsall, MV Toldi, Rétsági, Szajlai) photochemical activity are similar to optimal under reduced nitrogen supply. These wheats are utilizable in breeding wheat lines with sufficient productivity under relatively low nitrogen supply.

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