

FOLIAR APPLICATION OF ^{15}N -UREA AND ALLOCATION INTO GLUTEN OF DIFFERENT WHEAT CULTIVARS

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Abstract

Assessment of fertilizer nitrogen uptake into wheat grains and proteins is an important issue for selection of N efficient cultivars. The ^{15}N dilution technique is the most reliable way to follow the flow and fate of N for studies examining the path of N into wheat grains. Studies on wheat cultivar responses to late-season urea application in terms of grain quality traits are scarce. To assess the effect of a late N fertilization, known as quality effective, classified German spring wheat cultivars and two cultivars from the Mediterranean territory were examined in a field experiment in northeast Germany. At flag leaf sheath opening, 20 kg N ha⁻¹ were given as foliar application (urea solution; to which ^{15}N labelled urea was added; 10 atom-% ^{15}N enrichment; correspond to 8.3 mg ^{15}N m⁻²). At maturity, 35 days after ^{15}N labelling, the gluten protein content, the ^{15}N -recovery, and the ^{15}N content of grains and of gluten were determined. Forty (Gönen) to 58% (Picolo) of the applied ^{15}N was recovered in the grains per m², which means an effective translocation of the late applied N, however no significant differences between the cultivars occurred. The ^{15}N content in grains was markedly higher in Thasos, Melissos, Taifun, Picolo, Triso (mean 14.7 $\mu\text{g } ^{15}\text{N g}^{-1}$ dry matter⁻¹) compared to Tybalt, Monsun, Golia and Gönen (mean 11.6 $\mu\text{g } ^{15}\text{N g}^{-1}$ dry matter⁻¹). The ^{15}N content of the gluten fraction was not influenced by cultivar and lies in the range between 14.7 and 19.4 $\mu\text{g } ^{15}\text{N g}^{-1}$ dry matter⁻¹. The applied ^{15}N is after 35 days at harvest to 16% involved in the gluten synthesis, and this independently of the spring wheat cultivars and classification.

Keywords: *Triticum aestivum* L., Spring wheat, Urea, ^{15}N , Foliar application, Gluten

1. INTRODUCTION

Cereals possess a whole range of proteins involved in nitrogen uptake and partitioning for nitrate and ammonium, urea, amino acids, peptides, nucleotides and their degradation products. Nitrogen uptake from soil and re-allocation of N containing resources from vegetative plant structures, like leaves, stems and the grain surrounding ear tissues during the development of the grain (endosperm and embryo) is important for optimal nitrogen utilisation. Compared to other N fertilizer, urea (<http://faostat.fao.org>) is the most widely used nitrogen fertilizer in agriculture. Plants possess dedicated urea transporters, hydrolyse urea very efficiently and can use urea as sole nitrogen source. In the meantime, it is known that plants have special urea transporters that hydrolyse urea and use it as sole nitrogen source. Plants possess a high affinity secondary active urea transporter (DUR3) that is involved in taking up environmental urea but may also mediate internal urea transport. A role in internal urea transport is indicated by the expression of AtDUR3 near the root xylem and in the shoot. Passive urea transport is mediated by major intrinsic proteins (MIPs) also called aquaporins. These proteins conduct selected low molecular solutes along a concentration gradient through a channel. *Arabidopsis thaliana* contains 35 MIPs grouped into 4 subclasses: the plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), Nodulin 26-like membrane intrinsic proteins (NIPs), and small, basic membrane intrinsic proteins (SIPs) (Witte 2011 and references therein). The metabolic source of urea is the catabolism of arginine by arginase in the mitochondria, generates also ornithine. Mitochondrial ornithine metabolism converts this compound to glutamate. Urea is transferred to the cytosol possibly by an aquaporin, and then hydrolysed by urease, and the ammonium is re-assimilated by (cytosolic) glutamine synthetase using glutamate (from ornithine catabolism) as substrate. All nitrogen of arginine is incorporated into glutamine by these reactions while urease is required to mobilize half of

the nitrogen stored in arginine. This is the only firmly established role of urease in plant metabolism (apart from hydrolysing root-imported urea) and arginase is the only enzyme in plants known to generate urea *in vivo*. The arginine catabolism is central to the mobilization of nitrogen from source tissues and arginine is the most important metabolite for nitrogen storage in many plant seeds (Witte 2011 and references therein).

The grain yield of six winter wheat cultivars (Varga and Svečnjak, 2006) was not influenced by an additional late-season urea treatment of 30 kg ha⁻¹ at low or high basal N rate, of 67 and 194 kg ha⁻¹, respectively. Indeed, late foliar urea application similarly improved grain quality at both low and high N rates by average 5% for protein content, 12% for Zeleny sedimentation and 29% for wet gluten. These quality increments were consistent in three growing seasons regardless of significant variations in grain yields and protein concentrations. At low N rate quality parameter associated with the urea treatment were relatively small compared to those achieved by high basal N rate. Significant cultivars x urea interactions were examined for most quality traits, which were due to the cultivar differences in the magnitude of responses.

Wheat it is the most widely grown cereal, being adapted to a broad range of temperatures, water regimes and fertilization levels. Assessment of fertilizer nitrogen uptake into wheat grains and proteins is an important issue for selection of N efficient cultivars. The ¹⁵N dilution technique is the most reliable way to follow the flow and fate of N in natural systems and, therefore also suited for studies examining the path of N into wheat grains. Internal grain quality, like crude protein and storage proteins have major impact on the end use properties of the grain. The major consideration is the impact of the grain proteins on functional properties for food processing. Studies on wheat cultivar responses to late-season urea application in terms of grain quality traits are scarce. To assess the effect of a late N fertilization, known as quality effective, classified German spring Quality (A) and Elite wheat (E) cultivars and two cultivars from the Mediterranean territory, were examined in a field experiment in northeast Germany.

In crop production relying on urea fertilization, it is widely accepted that urea is more than 50% degraded by soil microbes and taken up by plants as ammonia and nitrate. Such losses would probably reduce within a certain framework if plants absorbed urea efficient. An improved understanding of urea uptake and effect bears the potential of reducing nitrogen losses from agricultural systems.

2. MATERIAL AND METHODS

In 2010 the field experiment with ¹⁵N, as a part of the examinations by Bräsemann (2015), were carried out from March to July at the Humboldt- University in Berlin-Dahlem (latitude: 52° 28'N; longitude: 13° 18'E; altitude: 51 m above sea level). The prevailing soil type is parabrown soil with weak marks of pale soil, FAO-Classification: Albic Luvisol. This is a silty to medium-loamy sand (surface soil) and a silty-loamy sand to sandy clayey loam (sule-soil). The long-term (1981–2010) average annual air temperature and precipitation are 9.9 °C and 562 mm, respectively. The experiment was designed as a 1-factorial randomized block system with nine cultivars, with four replications each (36 plots 6.50 x 1.50 m = 351 m²).

To assess the effect of the late N fertilization, known as quality effective, classified German spring wheat cultivars: Quality wheat (A): Monsun (KWS SAAT SE & Co. KGaA), Melissos (Strube D&S GmbH), Picolo (Dr. J. Ackermann Saatzeit), Tybalt (W. von Borries-Eckendorf via Saaten Union; Elite wheat (E): Taifun (KWS SAAT SE & Co. KGaA), Thasos (Strube D&S GmbH), Triso (DSV Saaten) and two cultivar from the Mediterranean territory Golia, Gönen, not classified, were examined.

The nitrogen fertilization (total 140 kg ha⁻¹) was divided into three splits. The first split of 90 kg N ha⁻¹ (ammonium sulphate nitrate (ASA)) was applied before sowing; the second split of 30 kg N ha⁻¹ (ASA) at stem elongation was applied to the plots by hand. At booting (flag leaf sheath opening, mean of 9 cultivars: (Eucarpia, decimal Code for growth stages (EC), EC 47 ± 1 (SE)) 20 kg N ha⁻¹ were given as foliar application by urea (SKW Stickstoffwerke Piesteritz GmbH, Germany) solution to

which ^{15}N labelled urea (Campro Scientific GmbH, Germany) was added (10 atom-% ^{15}N enrichment; corresponds to $8.3 \text{ mg } ^{15}\text{N m}^{-2}$), using a mobile crop protection sprayer. Some cultivars were already beyond the ears pushing, while others had not quite reached it.

Grain protein content ($\text{N} \times 5.7$) was determined by Kjeldahl nitrogen analysis. The NaCl-insoluble (gluten) proteins were evaluated by modifying the standard method of the International Association of Cereal Chemistry (106/2, ICC, 1986) (Götz et al. 2008). Measurement of ^{15}N in grains and gluten was accomplished by isotope ratio mass spectrometry (IRMS) with the Tracer Mass 20–20, SerCon, Crewe, UK, and the corresponding ^{15}N content was calculated (Faust et al. 1981). Parallel to the ^{15}N measurement by IRMS, the N content of gluten was used to calculate the protein content. The data (ANOVA) were analysed using statistical software IBM SPSS Statistic 22.0.

3. RESULTS AND DISCUSSION

The mean air temperature in March, April, May, June, and July were 5.3, 10.1, 11.9, 19.0, 23.5 °C, compared to the mean of the years 1971-2000 with 4.9, 9.0, 14.3, 16.9, 18.9 °C, respectively. The mean of rain fall in March, April, May, June, and July were 36, 10, 91, 2 and 46 mm compared to the mean of the years 1971-2000 with 37, 34, 51, 66 and 53 mm, respectively.

Grain yield was under the rain-fed conditions markedly influenced by the cultivar (Tab.1). The cultivars Monsun (A) and Golia (nc) yielded about 30% higher ($398, 392 \text{ g m}^{-2}$, respectively) than the cultivars Taifun (E) and Gönen (nc) ($274, 293 \text{ g m}^{-2}$, respectively). Differences between the other cultivars were not so pronounced, since the cultivars were statistically assigned to different homogeneous groups. Although the total amount of rainfall of 240 mm (mean 1971-2000) during the growing season (March to July) for spring wheat production is sufficient, the distribution in 2010 was not regular, because there was a clear minus especially in April, June and July of 24, 64 and 7 mm, resulting in a total difference of 55 mm water for growth and development compared to the long term mean. Consequently, the respective phenological phases for yield structure, a product of three yield components, the number of ears per unit area, the number of kernels per ear, and individual kernel weight (Slafer et al. 2014) and the grain filling were influenced to a different degree depending on the cultivar.

Table 1. Grain yield, protein content of grains, protein content of gluten, ^{15}N content of grains and ^{15}N content of gluten of different spring wheat cultivars

Cultivar	Yield (g DM m^{-2})	PC Grain (% DM)	PC Gluten (% DM)	^{15}N Grains ($\mu\text{g } ^{15}\text{N g}^{-1} \text{ DM}^{-1}$)	^{15}N Gluten ($\mu\text{g } ^{15}\text{N g}^{-1} \text{ DM}^{-1}$)
Monsun (A)	398 ^a	14.95 ^c	29.28 ^b	11.44 ^{ba}	15.22 ^a
Melissos (A)	302 ^{cba}	19.61 ^{ba}	37.76 ^{ba}	15.18 ^b	14.67 ^a
Piccolo (A)	372 ^{ba}	18.84 ^{ba}	44.40 ^a	12.92 ^{ba}	15.05 ^a
Tybalt (A)	305 ^{cba}	17.93 ^{ba}	39.98 ^a	11.94 ^b	17.16 ^a
Taifun (E)	274 ^c	16.93 ^{cb}	36.95 ^{ba}	13.05 ^{ba}	17.98 ^a
Thasos (E)	292 ^{cb}	18.58 ^{ba}	38.89 ^{ba}	16.28 ^a	16.21 ^a
Triso (E)	295 ^{cb}	19.94 ^a	44.93 ^a	12.40 ^{ba}	19.37 ^a
Golia (nc)	392 ^a	16.89 ^{cb}	35.78 ^{ba}	10.83 ^b	15.28 ^a
Gönen (nc)	293 ^{cb}	17.98 ^{ba}	38.65 ^{ba}	11.21 ^b	16.53 ^a

Quality wheat (A), Elite wheat (E), not classified (nc); DM: Dry matter, PC: Protein content; different letters indicate significant differences between cultivars, Tukey-HSD test; means, $n=4$

The protein content (PC) (Tab. 1) as one of the important components of grain quality varies between 19.94 % (Triso (E)) and 14.95% (Monsun (A)) and was for these two cultivars inversely related to yield, this known negative correlation occurs because grain yield is mainly a result of starch accumulation. As shown for grain yield, differences between the other cultivars were not so pronounced, since the cultivars were statistically assigned to different homogeneous groups.

The accumulation of the storage protein gluten, which is differently enriched in gliadins and glutenins, with molecular weights between 30,000 and 80,000, and between 80,000 and several million, respectively, occurs when cell division in the endosperm has finished and cell growth is due only to cell expansion. The gluten agglomeration capabilities depend strongly on the wheat type (Van der Borgh et al. 2005). The protein content of gluten (Tab. 1) has shown cultivar specific differences, was highest in Picolo (A), Tybalt (A), Triso (E), 44.40, 39.98, 44.93%, respectively, and compared to Monsun (A) with the lowest of 29.28%. Interestingly, the relationship between grain protein content and gluten protein content is high, $r^2 = 0.98$, if Melissos (A) and Thasos (E) excluded, and for all examined cultivars after all $r^2 = 0.69$. The gluten protein content of three wheat cultivars examined under field conditions in the Mediterranean region of western Turkey, Anapo, Negev, and Sagittario (Götz et al. 2017) was markedly lower (21%) and not different among cultivars, the sowing rate, and the phase of N fertilization, and with 91 mm rain from the beginning of the reproductive stage until harvest. The location of growth and maturation conditions serves as the main reason for the differences in distribution among the gluten storage protein glutenin polymers and in the mode of glutenin polymerization. Up to date the knowledge is still restricted regarding whether the plant N availability including remobilisation from vegetative plant parts as leaves, stems, glumes, the N taken up after anthesis and/or the storage protein synthesis itself limits grain protein composition (Kichey et al. 2007, Li et al. 2016).

The ^{15}N fertilisation by foliar application at flag leaf sheath opening resulted after 35 days (± 1 day) in the recovery of ^{15}N based on area of $4.1 \pm 0.19 \text{ mg m}^{-2}$ (mean $\pm$ SE; range from 3.3 to 4.8, Gönen (nc), Picolo (A), respectively), which corresponds to the recovery of $49.69 \pm 2.34\%$ (mean $\pm$ SE; range from 39.59 to 57.95%, Gönen (nc), Picolo (A), respectively), without statistically differences between spring wheat cultivars. In the Aegean region, Turkey, the application of ^{15}N to the soil as solution, a mixture of ^{15}N -labelled compounds (ammonium nitrate, ammonium chloride, and ammonium sulphate) at stem elongation or at flowering, the uptake of ^{15}N into mature grains was not influenced by cultivar, sowing rate, or additional water supply treatment, and revealed a recovery of ^{15}N of 25% (Götz et al. 2017), which is one half compared to foliar application in this experiment in northeast Germany. It must be taken into account that after ^{15}N -fertilization via the soil immobilization in the rhizosphere, probable leaching and volatilization, by the soil and by the leaves takes place and therefore influences the recovery of the applied ^{15}N . In case those vegetative parts of the plants were not analysed regarding the ^{15}N the recovery is underestimated. It stays a scientific challenge to understand necessary the N partitioning to reproductive structures without investigation of vegetative organs.

The ^{15}N uptake via the foliar application resulted in ^{15}N contents in the grains between 11 and $16 \mu\text{g }^{15}\text{N g}^{-1} \text{ DM}^{-1}$ (Tab. 1). The ^{15}N content in grains was markedly higher in Thasos (E) compared to Melissos (A), Tybalt (A) and the non-classified cultivars Golia and Gönen, 16.28, 15.18, 11.94, 10.83, $11.21 \mu\text{g }^{15}\text{N g}^{-1} \text{ DM}^{-1}$, respectively. Monsun (A), Picolo (A), Taifun (E) and Triso (E) were assigned to the same homogeneous group as Thasos (E). The ^{15}N content in the grains at maturity can be a result of different sources, as the transfer via the tonoplast, which is a semi-selective bio membrane, which separates the central vacuole of a plant cell from the cytoplasm. ^{15}N it is therefore temporarily located in source organs (leaves, glumes), and this ^{15}N can be remobilised during the grain filling phase, shifted to sink organs. Further, according to (Witte 2011) a) by high affinity secondary active urea transporter (DUR3) that is involved in taking up environmental urea but may also mediate internal urea transport, b) by internal urea transport by the expression of AtDUR3 in the shoot, and c) by passive urea transport by major intrinsic proteins (MIPs) called aquaporins. Aquaporins conduct selected low molecular solutes along a concentration gradient through a channel. *Arabidopsis thaliana* contains 35 MIPs grouped into 4 subclasses: the plasma membrane intrinsic proteins, tonoplast intrinsic proteins, Nodulin 26-like membrane intrinsic proteins, and small, basic membrane intrinsic proteins. It is also conceivable that the plants taken ^{15}N up from the soil from the possibly dripping urea solution, but the proportion is likely to be very small.

Major storage protein genes are expressed specifically in endosperm tissue, and are under strict temporal control (Dupont and Altenbach 2003). The ^{15}N content of the gluten (Tab. 1) was in the range between 14.67 (Melissos (A)) and 19.37 $\mu\text{g } ^{15}\text{N g}^{-1} \text{DM}^{-1}$ (Triso (E)) but was not different between the cultivars. An association with the grain protein content, the gluten protein content or the ^{15}N content of grains were not recognizable. However, it is worth noting that the ^{15}N content of gluten, with exception of Melissos (A) comparable, Thasos (E) similar, was about 39% larger (mean $\pm$ 5% SE) than the ^{15}N content of grains. The ^{15}N enrichment, atom-% excess (which means tracer minus natural background isotope 0.366 atom-%) of the grains was highest in Thasos (E), compared to lowest enrichment of Triso (E) and Gönen (nc), 0.04650, 0.03325, 0.03325 atom-% excess, respectively, and the mean of the other cultivars of 0.03933 atom-% excess. The ^{15}N enrichment of gluten was markedly lower; highest in Monsun (A) compared to the lowest of Picolo (A), 0.03025, 0.01975, respectively, and the mean of the other cultivars of 0.02507 atom-% excess. The reason for this might be the ^{15}N enrichment (atom-%) of easily soluble metabolites such as amino acids, urea from the ornithine cycle, but also the ^{15}N labelling of functional proteins, such as those of albumins and globulins, as representatives of the cytoplasmic proteins. Albumins and globulins are NaCl-soluble proteins and eliminated during gluten extraction.

With an average yield of the cultivars of 325 g m^{-2} , a gluten yield of 25% (dry matter basis, data not shown) and an average of the cultivars of 16.4 $\mu\text{g } ^{15}\text{N g}^{-1} \text{dry matter}^{-1}$ gluten, resulted in 1.3 $\text{mg } ^{15}\text{N}$ per square meter in the gluten fraction. Therefore, the ^{15}N applied, 8.3 mg m^{-2} , by foliar application at flag leaf sheath opening is after 35 days at harvest to 16% involved in the gluten synthesis, and this independently of the spring wheat cultivars and classification.

4. CONCLUSIONS

One possibly way to improve the economic result of wheat production is to select for cultivars with high remobilisation ability and, increase the remobilisation of N from the shoot by a better use of N in the metabolism in order to reduce the necessary dose of N-fertilizer. A foliar application of urea at flag leaf sheath opening leads to a translocation of around 50% into the grain, whereby the classification of the cultivars plays a subordinate role. The utilisation of the applied ^{15}N for storage protein synthesis of gluten until harvest, about 16%, was independent of the cultivar. This internal situation might be coordinated between the C and N metabolism because glycolysis and the TCA cycle must produce energy and metabolic precursors such as amino acids for storage protein synthesis.

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