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Genetic variability for post-anthesis nitrogen metabolism in winter wheat varieties grown under limiting and non-limiting nitrogen conditions

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SUMMARY – In wheat, the post-flowering period largely controls both yield and grain protein content. Our objective was to characterize genotypic differences for nitrogen (N) absorption and remobilisation under limiting an non limiting fertilization conditions. Five winter wheat cultivars were grown in the field for two years at two N levels. ^{15}N was applied at flowering to evaluate N absorption and remobilisation. Marker enzyme activities for N assimilation and recycling and N metabolite contents were monitored on the flag leaf and the upper internode. We showed that the use of ^{15}N- labelling was a reliable method to assess post-flowering N absorption and remobilisation. This approach revealed that on average 71.2% of grain N came from remobilisation with significant genotypic differences for the amount of N absorbed and remobilised. Significant differences were found for N remobilisation efficiency estimated as plant N at anthesis on N remobilised to the grain (from 72.2 to 86.8%) and for N translocation efficiency which corresponds to N absorbed after flowering that is translocated to the grain (from 90.0 to 93.4%). Significant genotypic differences were also found for all enzymes activities and metabolites used as representative markers for N assimilation and recycling. Glutamine synthetase activity was highly correlated to remobilised N and to grain yield, while nitrate reductase activity was correlated to absorbed N and to grain N content. These results indicates that a number of agronomic and physiological markers could be used to breed for varieties exhibiting a better N use efficiency.

Introduction

In wheat, both grain yield and grain nitrogen (N) content are largely dependent upon the amount of N remobilized to the grain and to pre-anthesis and post-anthesis N uptake. Sixty to 95% of grain N comes from remobilisation of N stored before anthesis (Van Sanford and Mc Kown, 1986). A less important fraction of seed N comes from post-flowering N uptake and translocation to the grain. Leaf N remobilisation also interfere with photosynthesis and as such with carbon assimilates production. Due to increasing costs and pollution risks, the use of N fertilizer needs to be rationalized. Since, both the size and the N content of the grain can be significantly reduced under N deficient conditions, our objective was to characterize genotypic differences for N absorption and remobilisation under limiting an non-limiting N conditions.

Material and methods

Five registered wheat cultivars ('Arche', 'Récital', 'Renan', 'Shango', 'Soissons') commonly cultivated in France and differing for their response to N deficiency (Le Gouis *et al*., 2000) were sown on 16.10.2001 and 15.10.2002 at INRA Estrées-Mons (Somme, Northern France). In 2002, the plots received either 140 or 50 kg N ha^{-1}. In 2003 they received either 180 or 80 kg N ha^{-1}. Samples were taken up at flowering, 14 days after flowering (DAF), 28 DAF and at final harvest as described by Kichey *et al* (2006). ^{15}N was applied at flowering to evaluate post-anthesis N absorption (Nabs) and remobilisation to the grain of N stored before anthesis (Nrem). N translocation efficiency (NTE) was calculated as the proportion of Nabs that was translocated to the grain. N remobilisation efficiency (NRE) was calculated as Nrem divided by N absorbed at flowering. On the second year we measured on the flag leaf and the upper internode at each sampling date the content in total N, ammonium, nitrate, protein, chlorophyll and the activities of the nitrate reductase (NR), glutamine synthetase (GS) and aminating glutamate dehydrogenase (NADH-GDH).

Results and discussion

Post-anthesis N absorption and remobilisation estimated either by the balance method between flowering and harvest or using ^{15}N-labelling were almost identical but the estimation using the ^{15}N-labelling method was more repeatable. On average 71.2% of grain N came from remobilisation of N stored before anthesis. Significant genotypic differences were found for Nabs and Nrem and the genotype x N level interaction was also significant (Table 1). Nrem was the highest for 'Arche' and Nabs was the highest for 'Soissons'. The interaction with the N level was mainly due to 'Arche' and 'Récital'. Significant differences were found for NRE and NTE. On average NRE was equal to 78.5% with the highest efficiency observed for 'Soissons' and the lowest for 'Renan' and 'Shango'. NTE was very high with more than 90% of N absorbed after flowering being further translocated to the grain. 'Renan' had the highest NTE and 'Shango' the lowest.

Table 1. Post-anthesis N absorption and remobilisation estimated using ^{15}N-labelling at flowering (averaged on two years)

	'Arche'		'Récital'		'Renan'		'Shango'		'Soissons'		LSD
	N-	N+	N-	N+	N-	N+	N-	N+	N-	N+	
Nrem (g m^{-2})	7.74	13.77	5.98	9.51	6.74	11.16	6.31	11.08	6.99	11.09	0.82
Nabs (g m^{-2})	3.52	3.81	3.13	5.57	3.60	4.54	3.03	3.39	3.92	5.42	1.09
NRE (%)	72.7	80.2	82.8	86.1	72.4	73.1	74.5	69.8	88.8	84.8	12.1
NTE (%)	92.8	91.6	91.7	90.1	93.4	93.4	90.3	89.7	91.7	90.6	1.1

Significant genotypic differences were found for all enzymes activities and metabolites contents. Correlations between the physiological and agronomic traits were searched using values for the five genotypes at the two N levels in 2003. Significant correlations were found between grain yield and Nrem (r=0.90), GS activity (r=0.83) and EDTA-NR activity (r=0.78). Grain N content was correlated with Nrem (r=0.67), Nabs (r=0.85) and Mg-NR activity (r=0.87).

In wheat (Kichey *et al*., 2006) and in maize (Hirel *et al*., 2005), GS activity was shown to be one of the best physiological markers to depict the plant N status whatever its developmental stage and N nutrition conditions. The strong correlation observed between GS activity, Nrem, and GY in five wheat cultivars strengthens this hypothesis and confirms that in cereals the enzyme is one of the main components that may contribute through N metabolite assimilation and translocation to plant biomass production and yield. Grain N was strongly correlated with both Nabs and Nrem. We confirmed with five genotypes that Nabs is tightly correlated to NR activity (Kichey *et al*., 2006). In addition, we showed that NR activity is also strongly correlated to grain N, which suggests that NR is involved in grain N supply after flowering.

Conclusion

This study showed that physiological markers such as GS and NR activities were correlated to the proportion of N remobilized and N absorbed respectively, both N sources being further used for grain yield elaboration or grain storage protein synthesis. These markers could be used to assist breeders for selecting wheat cultivars exhibiting higher N use efficiency.

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