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Genetic variation and QTLs for ^{15}N natural abundance in a set of maize recombinant inbred lines

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Abstract

The meaning of variation in $^{15}N/^{14}N$ isotope ratio in plants grown in the field is better known when variation is due to environment than when it is due to plant genotype. To study the physiological and genetic meaning of variation of such a ratio, a set of 99 recombinant inbred lines of maize were evaluated at low and high N-input and organ ^{15}N abundances were correlated to agronomic and physiological traits. At the level of means, at high N-input there appeared no difference in ^{15}N partitioning according to plant organs, with the same abundances for blades, stalks + sheaths and kernels. However, at low N-input blades and kernels were ^{15}N-enriched, whereas stalks were significantly ^{15}N-depleted with an abundance close to that observed for high N-input. ^{15}N abundance of whole-plant and organs showed significant genotypic effects and genotype by nitrogen input interaction, varying according to the organ and the stage, silking and grain maturity. Genetic variation for ^{15}N abundance and correlations involving ^{15}N abundance were always lower at high N-input than at low N-input. ^{15}N abundances of blades and stalks + sheaths were negatively related to silking date whatever the stage (silking or maturity) and N-fertilization whereas kernel ^{15}N abundance was not affected by silking date. At low N-input, whole-plant ^{15}N abundance at maturity was positively correlated to whole-plant and kernel protein content whereas at high N-input such correlation disappeared. Whole-plant ^{15}N abundance at silking was negatively related to root fresh weight and to glutamine synthetase activity measured in young plants grown in hydroponics. Twelve QTLs for ^{15}N abundance were detected, mainly at high N-input; among them, 10 coincided with QTLs involved in nitrogen use efficiency (grain yield, N-uptake and N remobilization) and the root system. Interpretation of all results leads to the conclusion that two mechanisms could explain genetic variation in ^{15}N discrimination ability: morpho-physiological differences, in particular in the root system, and activities of the first enzymes of nitrogen metabolism, with a positive relationship between enzyme activity and discrimination abilities.

Keywords: ^{15}N abundance; Nitrogen use efficiency; Maize; QTLs

1. Introduction

Variation in $^{15}N/^{14}N$ isotope ratio in plants grown in the field can have several origins: environment, plant stage and plant genotypes (species and genotypes within species). First, it can be due to the origin of the absorbed nitrogen, from soil or from nitrogen fertilizer. Indeed, due to the industrial process used for its production, nitrogen fertilizer has a lower level of ^{15}N isotope than do air and soil. Thus, a low isotope ratio could mean that plants have mainly absorbed nitrogen from fertilizer whereas a high ratio could mean an uptake of nitrogen mainly from the soil. This is why variation in the isotope ratio in leaves or whole-plant has been thought to reflect the origin of N used by the plants. A variation among species has also been shown by Kohl and Shearer (1980) and Mariotti et al. (1980). The latter authors showed an enrichment factor decreasing with the age of plants, as has since been observed in many other studies (Evans, 2001; Kolb and Evans, 2003). Isotope ratio within the plant also depends on the amount of nitrogen at the

Abbreviations: GOGAT, ferredoxin-dependent glutamate synthase; GS, glutamine synthetase; N, nitrogen; ^{14}N, ^{15}N, nitrogen isotopes; N0, low N-input; N1, high N-input; NR, nitrate reductase; QTL, quantitative trait locus; RILs, recombinant inbred lines

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plant's disposal. In pearl millet, Mariotti et al. (1982) showed that discrimination against ^{15}N takes place only when nitrogen is not limiting. This discrimination was related to nitrate reductase activity and the decrease in discrimination observed with age was then related to a non saturation of nitrate reductase activity due to a decrease in the nitrate uptake or an increase in nitrate reductase activity.

The discrimination observed by Mariotti et al. (1982) for nitrate reductase is theoretically expected in all steps from nitrate uptake to amino acid synthesis, as was also mentioned in particular for glutamine synthetase activity (Handley and Raven, 1992). Such discrimination ability is clearly due to the kinetic isotope effects on enzyme-catalysed reactions measured in vitro: it appears that the ratio of the rate constant with ^{14}N to the rate constant with ^{15}N is greater than 1 (Werner and Schmidt, 2002), meaning that enzymatic reactions with ^{15}N are slower than with ^{14}N. According to Werner and Schmidt (2002) after reduction by nitrate reductase, the most central process is the GOGAT-reaction which yields ^{15}N-depleted glutamate and remaining glutamine which is the source of N-enriched amide-N in heteroaromatic compounds. As a consequence of the N-metabolism, proteins are generally ^{15}N-enriched whereas secondary products like chlorophyll, lipids, amino sugars and alkaloids are depleted in ^{15}N.

As plant parts do not play the same role in metabolism, the spatial distribution of ^{15}N-depleted and ^{15}N-enriched products within the plant could lead to variation in ^{15}N abundance according to the plant parts. Such a compartmentation also depends on the place of nitrate reduction. Yoneyama et al. (1997) showed that with nitrate reduction and assimilation confined to the shoot, shoot isotopic ratio would be similar to that of the source. However, when nitrate is partially assimilated in the root, while whole-plant isotope ratio will always be that of the source, there will be a compartmentation with root organic nitrogen depleted in ^{15}N and ^{15}N-enriched nitrate exuded by the roots (Medina and Schmidt, 1982; Yoneyama, 1995). In *Triticum aestivum*, Yoneyama et al. (1997) found that there was no compartmentation at the ear-forming stage unlike at the kernel-filling stage where there was a clear compartmentation with high isotope ratio values for ear and stem and lower values for roots and leaf blades (values for leaf sheath being intermediate between leaf blades and stem). More generally, such a compartmentation depends on the dynamics of nitrogen within the plants, in particular where there is reduction and protein synthesis, and distribution of N-compounds within the plant including remobilization towards the kernel.

Another source of variation for ^{15}N discrimination is the genotype within a species. Since variation among species exists, a genetic variation within species is expected. There are still few studies showing such variation. Kolb and Evans (2003) showed differences in discrimination for two barley genotypes with one genotype lacking the primary assimilatory enzyme. Such a genotype showed significant discrimination whatever the nutrition levels, unlike the "normal" genotype which showed discrimination for only high N-nutrition level. The discrimination for the genotype lacking root nitrate reductase was associated with a difference in the isotope composition of shoots and roots. In another study, Robinson et al. (2000) used stable isotope natural abundances for carbon and nitrogen to characterize the stress responses of 30 genotypes of wild barley. Total dry weight was negatively correlated with root ^{15}N abundance whereas it was not correlated to shoot and whole-plant abundances. Furthermore, difference in abundances between shoots and roots appeared to be a more sensitive indicator of stress response than shoot, root or whole-plant abundances alone.

Different explanations can be given for the genotypic variation in ^{15}N discrimination. For an experiment in the field, it can first be related to the root system: a dense root system will better explore the soil and absorb nitrogen from fertilizer leading to a low isotope ratio. When nitrogen has been absorbed, discrimination can take place as a result of the activities of enzymes involved in the reduction of nitrates and synthesis of amino acids. The place of reduction of nitrates, or the weight of possible places, can vary according to the genotype, leading to a variation in ^{15}N discrimination. Furthermore, several authors (Kolb and Evans, 2003; Robinson et al., 2000) consider that the main cause of discrimination could be the loss of nitrogen by the roots, by exudation, or at the level of aerial parts, by volatisation. In cereals, the dynamics of N-uptake and remobilization associated with senescence and kernel filling may be another factor affecting discrimination. Finally, a genotypic variation in enzyme activities such as nitrate reductase and glutamine synthetase could also induce a genotypic variation for discrimination according to the nitrogen nutrition level.

The aim of our study, combined with a study on the genetic and physiological basis of nitrogen use efficiency in maize, was then to see whether there was a genetic variation in ^{15}N discrimination and whether this variation could be related to certain agronomic or physiological traits. We also examine whether ^{15}N is distributed homogeneously within the maize plant or if there is compartmentation according to N-input. Furthermore, as we have used a population of recombinant inbred lines, using molecular markers, the detection of involved quantitative trait loci (QTLs) will be possible. Study of coincidences of detected QTLs with those already detected with the same material for agronomic traits (Bertin and Gallais, 2000, 2001) and physiological traits (Hirel et al., 2001; Limami et al., 2002) could be useful for finding the physiological meaning of QTLs for ^{15}N fractionation.

2. Material and methods

To develop the study, we have used the set of 99 recombinant inbred lines (RILs) already used by Bertin and