Gene Expression Biomarkers for Evaluating Nitrogen Nutritional Status in Rice

A. Ferri, L. Fontanili, S. Morgutti, C. Lancilli, M. Corti, D. Cavalli, G. Cocetta, P. Marino Gallina, A. Ferrante, G.A. Sacchi and F.F. Nocito

Dipartimento di Scienze Agrarie e Ambientali – Produzione, Territorio, Agroenergia. Università degli Studi di Milano, Italy. alessandro.ferri@unimi.it, laura.fontanili85@gmail.com, silvia.morgutti@unimi.it, clarissa.lancilli@iis-codogno.com, martina.corti@unimi.it, daniele.cavalli@unimi.it, giacomo.cocetta@unimi.it, pietro.marino@unimi.it, antonio.ferrante@unimi.it, gianattilio.sacchi@unimi.it, fabio.nocito@unimi.it

Type of presentation: poster presentation

ABSTRACT

Over the last five decades the increase in rice yield has been associated with a dramatic increment in the use of nitrogen (N) fertilizers. Understanding of plant molecular responses to N is critical for our ability to improve the agricultural sustainability of rice cropping systems by developing a comprehensive approach that allows the selection of varieties with enhanced efficiency in their ability to use N and the development of new strategies to better manage N fertilization practices. In order to develop novel tools for real-time assessing of rice N nutritional status, we analyzed the expression profiles of seven putative gene expression markers in the shoots of rice plants grown under different N availability and environmental conditions. Our results suggest that five out of the seven genes analyzed have the potential to be used as agronomic tools to monitor and optimize the N nutritional status of rice.

Key words: nitrogen use efficiency, sentinel plants, biomonitoring

1. Introduction

Nitrogen plays an important role in plant growth and development. Its availability in the soil and its efficient use by plants represent the major limiting factors for rice production in many of the world's agricultural areas. During the last 50 years the increase in rice yield has been associated with a 20-fold increase in the global N fertilizer applications and this is expected to increase by at least three-fold by 2050. However, it is important to notice that the recovery of N fertilizers by rice plants is relatively low, since the current average of nitrogen use efficiency [NUE = (N removed by crop - N coming from soil + N deposited in rain)/N applied to crop] in the field may be as low as 33% (Glass, 2003; Vijayalakshmi et al., 2013). Thus, in this scenario, there is an urgent

need for developing a comprehensive approach to optimize N utilization, by managing the N fertilizers and improving the NUE of rice plants through different strategies, not only for increasing crop growth and yield but also for reducing production costs and environmental impact.

The NUE of a crop production system can be increased by growing varieties with enhanced efficiency and/or by modifying the environment where crops are grown. The selection of varieties with improved NUE is a generic approach which necessarily requires some knowledge of the genetic variation and inheritance of this trait; in such a context, understanding the mechanisms for N uptake, assimilation, and remobilization during the plant life cycle is crucial for increasing NUE. Differently, the improvement of the agricultural practices aimed at sustaining the N nutritional needs of a crop system may provide more immediate advantages in terms of cost and environmental quality. Although much progress has been made in the development of fertilizer management strategies able to reduce N losses, there remain considerable uncertainties about the timing and rate of N application for better synchronization between the supply and the demand of N by the rice plants. In fact, the physiological demand for N in a rice cropping system may vary depending on both genotype and environmental conditions (availability of other nutrients, biotic and/or abiotic stresses). Thus, quick and inexpensive bioassays to determine N bioavailability and/or N nutritional status are desirable, in order to monitor the soil N dynamics in different environments and to better manage the N fertilization practices for different rice varieties. In other words, developing specific bioassays based on the use of "sentinel plants", or bioindicators, may represent a reliable and efficient strategy to obtain valuable, timely and low-cost information about changes in N availability and/or nutritional requirement in a specific rice cropping system.

Generally, plants respond to nutrient supply or shortage through a complex of physiological, morphological, and developmental responses, which are under the control of several gene pathways. Genome-wide transcriptome analyses showed extensive changes in the expression of several genes involved in primary and secondary metabolism, nutrient transport, protein synthesis, regulation of gene expression and cellular growth processes (Yang et al., 2015). Such studies not only improved our general understanding of plant responses to N availability, but also provided reliable data from which to develop new molecular strategies for real-time monitoring of plant N nutritional status. Recently, Yang et al. (2011) used multiple whole genome microarray experiments to identify gene expression biomarkers capable of assessing plant responses under limiting and sufficient nitrogen conditions. By using logistic regression statistical approaches, they identified in maize a common set of genes whose expression profiles quantitatively assessed the extent of plant stress under different N conditions. Interestingly, such a biomarker gene set is independent of genotype, tissue type, developmental stage, and environment, being applicable to plants grown both under controlled conditions and in the field, and thus has the potential to be used as an agronomic tool for real-time monitoring of plant N nutritional status and, thus, to optimize NUE.

Seven orthologous genes have been identified in rice and their expression profiles under different N availability has been studied, in order to evaluate their possible exploitation in developing gene expression-based markers or sentinel plants useful to assess rice N nutritional status. Preliminary results are presented and discussed in this paper.

2. Methods

All the analyses were carried out on shoots of rice (*Oryza. sativa* L. cv. Gladio) plants grown under different N availability, growth media (hydroponic solutions or natural soil) and environmental conditions (growth chamber or greenhouse). Plants were grown for different time periods in order to obtain shoot samples in various phenological phases (from the III to the VIII leaf stage). At the end of the growing period, shoots were collected, frozen in liquid N_2 and then stored at -80°C. Each sample was analyzed for total N content, using an elemental analyzer, and for relative amounts of the transcripts of seven marker genes previously identified and cloned. Gene expression analysis was performed by quantitative reverse transcription-PCR, using different genes as housekeeping.

3. Results

3.1. Identification of seven expression biomarkers in rice

Seven out of the eight genes proposed by Yang et al. (2011) as expression biomarkers for assessing N nutritional status in maize have been identified as putative orthologous genes in rice, and cloned. The marker gene sequences and the IDs of the relative loci were obtained from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/index.shtml).

Searching for molecular function revealed that only two of these loci, *LOC_Os03g62200* and *LOC_Os03g07570*, encoded proteins directly associable to N transport or N metabolism, respectively. The remaining five loci encoded proteins of unknown function or, where the function was deducible, with activities not directly associable to N metabolism (Table 1).

Table 1. Annotation of the seven rice genes tested as potential expression biomarkers for N nutritional status.

Probeset (from Yang et al. 2011)	Rice locus ID	Gene product name
A1ZM006239_at	*LOC_Os01g54340*	Plant-specific domain TIGR01615 family protein, expressed
A1ZM001292_s_at	*LOC_Os02g31030*	Glycerophosphoryl diester phosphodiesterase family protein, putative, expressed
A1ZM019982_at	*LOC_Os03g62200*	Ammonium transporter protein, putative, expressed
A1ZM058664_at	*LOC_Os09g37710*	NIN, putative, expressed
A1ZM011316_s_at	*LOC_Os08g38700*	C3-BTB2 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain with

|		C3 subfamily conserved sequence, expressed
A1ZM004474_at	*LOC_Os03g07570*	Alanine-glyoxylate aminotransferase 2, putative, expressed
A1ZM016678_at	*LOC_Os05g49240*	Homeodomain-related, putative, expressed (MYB)

3.2. Expression analysis

In order to validate the possible use of the seven genes as expression biomarkers for N nutritional status in rice, we performed a gene expression analysis using cDNAs synthesized from total RNAs extracted from the shoots of plants grown under different N availability and environmental conditions (hydroponic solution in growth chamber; pots with natural soil in greenhouse) for different time periods. Such experiments were specifically designed with the final aim of obtaining plants in different phenological phases (from the III to the VIII leaf stage) with an as wide as possible concentration of total N in the shoot.

The complete data set obtained for each marker was analyzed as a function of the total N concentration measured in the shoots, in order to obtain characteristic curves describing the absolute relationship between marker expression and N status of the plants independently of their particular "story".

Data analysis revealed that each marker gene significantly changed in its relative expression level together with the total N concentration in the shoot (Figure 1). The relative transcript levels of *LOC_Os03g62200*, *LOC_Os09g37710*, *LOC_Os08g38700*, *LOC_Os03g07570,* and *LOC_Os05g49240* decreased as the concentration of total N in the shoot increased. Least squares fitting revealed that data point distributions can be properly described by a linear function, for *LOC_Os03g62200,* or by exponential decay functions [$y = y_0 + ae^{(-bx)}$] for *LOC_Os09g37710*, *LOC_Os08g38700*, *LOC_Os03g07570*, and *LOC_Os05g49240*.

Concerning *LOC_Os01g54340* and *LOC_Os02g31030*, no apparent trend was deducible from data point distributions.

Figure 1. Gene expression analysis as a function of total N concentration in the shoot.
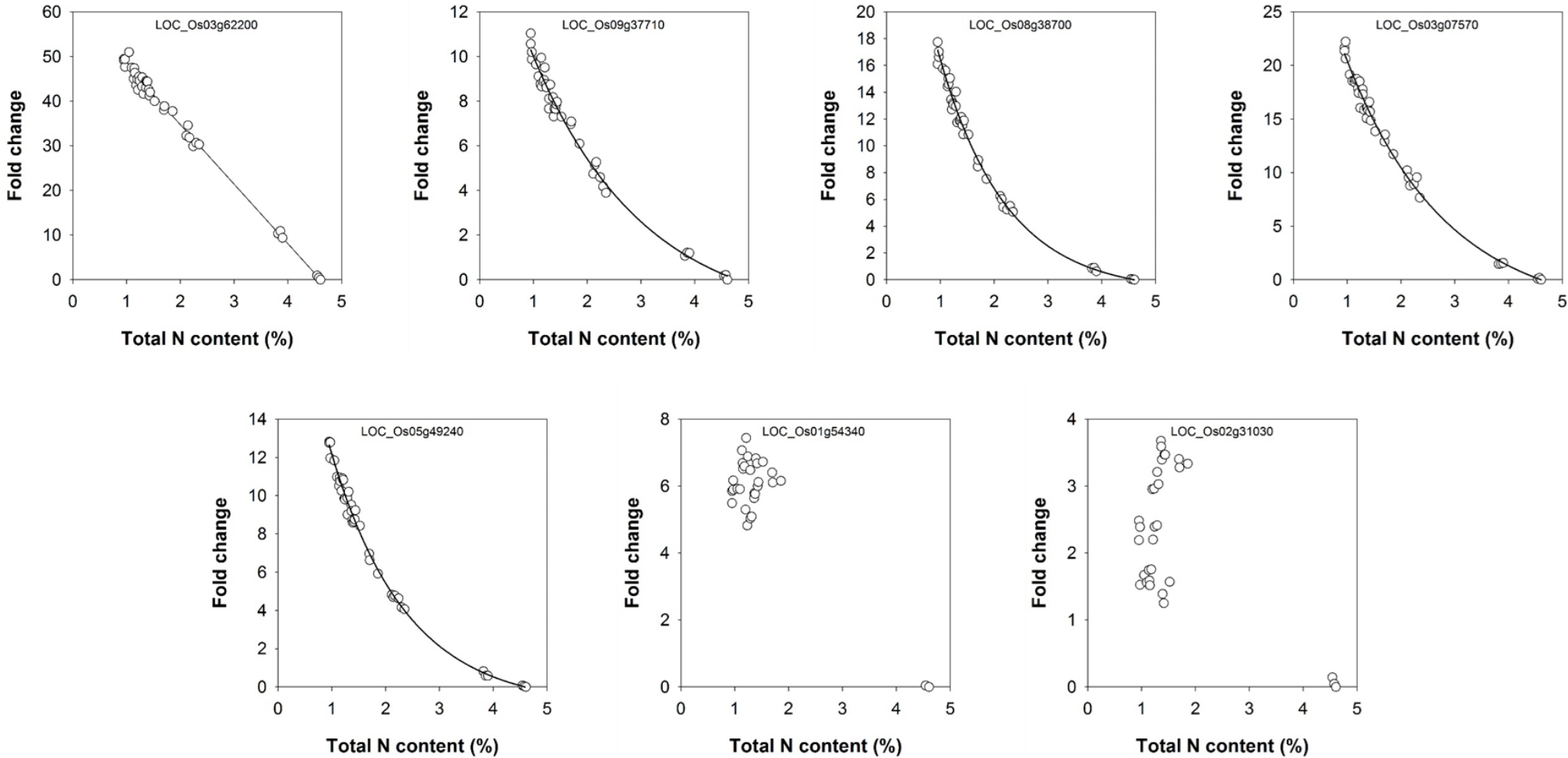
4. Discussion

For five out of the seven genes analyzed, it has been possible to deduce the existence of clear relationships between the relative amounts of their transcripts and the levels of total N measured in the shoots. Since such relationships seem to be independent of developmental stage and environment, we can reasonably suggest the potential use of these genes for developing agronomic tools to monitor and optimize the use of N fertilizer in rice. The demand for such tools is widespread, and the results reported here could provide a promising base for developing new molecular strategies for real-time monitoring of rice N nutritional status. In fact, the existence of genes, which specifically respond to the N nutritional status of the plant, potentially allows to develop a new class of bioindicators, or sentinel plants, based on the concept of gene fusion, in which the promoter of a marker gene controls the expression of a reporter gene able to provide accurate and low-cost information about N dynamics in a given rice cropping system.

Conclusions

The use of gene expression biomarkers for monitoring agronomic traits has until today been limited. In this study, we provide evidences that validate five rice genes as highly quantitative expression biomarkers able to provide information about the N nutritional status of the plant.

References

Glass A.D.M, 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Crit. Rev. Plant Sci. 22, 453-470.

Vijayalakshmi P., Vishnu Kiran T., Venkateswara Rao Y., Srikanth B., Subhakara Rao I., Sailaja B., Surekha K., Raghuveer Rao P., Subrahmanyam D., Neeraja C.N., Voleti S.R., 2013. Physiological approaches for increasing nitrogen use efficiency in rice. Ind. J. Plant Physiol. 18, 208-222.

Yang W., Yoon J., Choi H., Fan Y., Chen R., An G., 2015. Transcriptome analysis of nitrogen-starvation-responsive genes in rice. BMC Plant Biol. 15, 31.

Yang X.S., Wu J., Ziegler T.E., Yang X., Zayed A., Rajani M.S., Zhou D., Basra A.S., Schachtman D.P., Peng M., Armstrong P.M., Caldo R.A., Morrell J.A., Lacy M., Staub J.M., 2011. Gene expression biomarkers provide sensitive indicators of in planta nitrogen status in maize. Plant Physiol. 157, 1841-1852.