Enhancing biological nitrogen use in crops: A translational research approach

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Abstract

Optimization of biological nitrogen (N) use by crops has assumed great significance in recent years due to ever increasing fertilizer costs and severe environmental pollution due to N leaching in the soil. This is particularly relevant for developing countries where farmers have limited resources and are confronted with the need to feed an ever increasing population amidst climate change. The present study is part of the Cambridge – India Network for Translational Research in Nitrogen (CINTRIN) which aims to translate our recent understanding of developmental N use in model plants such as *Arabidopsis* and *Brachypodium* to foxtail millet, wheat, sorghum and pearl millet by screening for nitrogen insensitive (NIS) ideotypes, analysing their relative N utilization, yield potential and subsequently establish candidate genes regulating N use in them. Among the all four studied crops, foxtail millet (*Setaria italica* L.) is a C_4 model cereal that requires very low growth inputs, and is perfectly adapted to tropical semi-arid climate. The crop has one of the highest nutritional indices for human consumption, making it one of the most suitable crops for arid and semi-arid environments. In the present study, we employ high-throughput phenomics platforms to examine agronomically important traits in 200 genetically and geographically diverse accessions of foxtail millet and use next generation comparative genomics and bioinformatic tools to identify candidate genes/QTLs regulating N use. We also propose to compare and analyse the variation in physiological N uptake/ use and allocation to grains by using high throughput $^{13}N/^{15}N$ partitioning experiments to identify parallels with nitrogen sensitive (NS) and nitrogen insensitive (NIS) genotypes. The overall objectives of the study are to couple molecular basis of plant development to the physiology of N uptake and partitioning thereby defining new NIS ideotypes and generate valuable information on cultivar-specific N fertiliser application and offering the same directly to farmers.

Introduction

Nitrogen (N) is one of the major limiting elemental nutrients that facilitate growth and development in most plants followed only by the importance of photosynthesis (Sulieman, 2011). Moreover, N is an expensive component of the fertilizer which requires significant investment from farmers especially those in the developing world including Asia and Africa. On the other hand, excessive utilization of N in agriculture leads to its leaching into the soil resulting in severe environmental pollution and climate change.

Wheat is an important crop in UK, and in India, wheat, sorghum, pearl millet and foxtail millet are widely cultivated. Millets are C_4 panicoid crops with significantly improved drought, heat and pathogen tolerance (Lata et al. 2013; Bergamini et al. 2013) in addition to being highly adaptive to low nutrient input conditions (Goron and Raizada, 2015) as prevalent in many parts of Asia, North America and sub-Saharan Africa. It is noteworthy that the prevalence of climate change transforms the arable regions into dry environments with increasingly severe weather patterns (Dai, 2011) while the legislations are

progressively professing reduced N use in order to slow climate change (Tester and Langridge, 2010). In this scenario, millets could serve as the staple food crop of choice for majority of population in arid and harsh climate regions of the world.

In view of the same, there is a greater need for crop specific understanding of optimal N use and identifying (and/or breeding) genotypes with higher nitrogen use efficiency (NUE) that translates into significantly reduced cost of agriculture without any penalty in quantity or quality of yield. In this regard, Cambridge – India Network for Translational Research in Nitrogen (CINTRIN) jointly funded by BBSRC, UK and Department of Biotechnology, India will provide innovative approaches to tackle crop biological N use. Firstly, it will promote a new understanding of the underpinning science associated with optimization of crop N use, built on an exciting new discovery of distinct life history strategies for N use in the model plants, *Arabidopsis thaliana* and *Brachypodium distachyon*. This work has identified N sensitive (NS) and N insensitive (NIS) types which vary fundamentally in their developmental response to N. This work indicates that the ability to protect seed yield under low N supply appears to come at the expense of the ability to exploit high N supply effectively. This model for developmental N use has the potential to revolutionise the way we think about the N requirements and uses of crops.

Within CINTRIN, a translational pipeline will couple the molecular basis of plant development to the physiology of N uptake and partitioning. Through advanced genomics and pre-breeding, new N ideotypes will be defined in crops important for the UK (wheat) and India (wheat, sorghum, pearl and foxtail millet millet). Foxtail millet (*Setaria italica* L.) is one the most important staple crop cultivated widely in semi-arid regions of Indian subcontinent and Africa and is the second most cultivated millet worldwide, apart from being a very popular poultry and bird feed (Baltensperger 2002, http://exploreit.icrisat.org/page/small_millets/875). Moreover, it is uniquely enriched with slowly digestible starch (SDS) and resistant starch (RS) and has higher concentrations of many mineral nutrients making it one of the most promising crop vis-à-vis human nutrition. In addition, the crop has recently been accentuated as a model for studying the traits related to C_4 photosynthesis, abiotic stress response and bioenergy (Lata et al. 2013; Muthamilarasan and Prasad, 2015).

As highlighted earlier, studies in model plants, *A. thaliana* and *B. distachyon* have demonstrated that apart from other morphological and temporal variations, NIS lines bear more seeds under low N supply than their N sensitive counterparts (NS). It is in this backdrop that we at the CINTRIN envisage to translate this knowledge to foxtail millet, wheat, sorghum and pearl millet by examining the natural variation in N partitioning and physiological N for growth (and subsequently yield). We intend to identify NS and NIS ideotypes and characterize them through a set of high throughput genomic, phenomic and transcriptomic approaches and establish genes/QTLs related to NUE of foxtail millet. The overall objective is therefore to deliver a pipeline to propose new ideotypes for efficient N use and suggest breeders and farmers for application in commercial farming practices.

Phenomics: Identifying N dependent traits and screening for NIS ideotypes

Nitrogen substantially influences plant development and growth. We are using high-throughput phenomic platforms to characterize major agronomically important growth and physiological parameters across a diverse set of genetically and geographically diverse foxtail millet accessions (~200 accessions). Optimal growth conditions under soil-like nutritionally inert media are being standardized for N deficient and sufficient treatments in order to screen for NS and NIS genotypes. Furthermore, apart from estimating total N content, we will estimate $[NO_3^-]$ amounts in the conducting tissue (Prasad and Spiers, 1984,1985; Olsen and Lyons, 1994; Fontes and Ronchi, 2002; Farneselli et al, 2014) in order to have a better understanding of N status in individual accessions. In addition, major phenotypic traits under consideration are shoot and root biomass, height, chlorophyll content, tiller number and photosynthetic rate. Similar parameters have been considered in other crops to identify effects of N on plant growth (Hoffman et al. 2012; Gelli et al. 2016; Liu et al. 2016).

The successful uptake of belowground resources by plants depends mainly on root length or root surface area than total root biomass (Sattelmacher et al., 1990). Recently, higher N availability to *Setaria viridis* has been found to induce increased lateral root numbers (Pankievicz et al. 2015) implicating the important role of root depth and surface area as an important determinant of efficient N uptake and therefore better adaptability to N deficiency. In view of the same, we intend to explore dynamics of root morphology (root length, diameter, surface area, lateral root density and root hair density) of foxtail millet under contrasting N nutrient provisioning using conventional and state-of-the-art imaging techniques.

Radioisotope tracing: Mechanistic understanding of the physiological basis to N/C partitioning in foxtail millet

Conventional methods of tracking the balance of resource allocation in different parts of the plant are primarily destructive and are not suitable for high throughput assays. In association with our collaborators at Sainsbury Laboratory Cambridge University (SLCU), we will develop methodologies to evaluate genetic variation in N use using stable radioisotopes. Preliminary research at NIAB, CU has indicated that leaf N (%), specific leaf area and stable natural abundance ^{13}C composition are interrelated.

Comparative genomics and bioinformatics: To establish candidate genes regulating N use from models leading to pre-breeding

A comprehensive and useful understanding of N use requires elaboration of underpinning genetic protagonists and processes regulating its uptake, distribution and homeostasis. We intend to use the natural genetic variation and leads from phenotypic characterizations of 200 foxtail millet accessions to mine for important genes and QTLs related to N use by using high throughput comparative genomics and bioinformatics platforms. To this effect, a genome wide association study (GWAS) of the accessions will be performed based on a high density SNP map generated through genotyping-by-sequencing/ re-sequencing approach. The GWAS loci will be validated and further investigated for their genic locations/functions. The approach will help gene discovery and facilitate identification of germplasms for pre-breeding, breeding and production.

Field level evaluation of optimal N use and generating resources for breeders and farmers

Germplasms with better NUE will be trialled with contrasting N levels in the field and responses (with yield) generated will provide the basis for advice to farmers and breeders in the wider CINTRIN network.

Conclusion

CINTRIN will actively engage in training, collaboration and development. This will be achieved via frequent exchanges between partners, and focussed CINTRIN workshops. CINTRIN will also develop a map of the N knowledge base to identify targets for new participants to join the wider network. It will also identify new areas of N research to develop, as well as new sources of funding to expand the network. Further, the information generated will provide to the farmers and breeders in the wider CINTRIN network.

Acknowledgements

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