

Urgency to Understand Nitrogen Metabolism in Organic Agriculture

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Abstract

N fertilizers together with the development of high yielding varieties were major drivers of the enormous increase in crop productivity during the past 50 years. Despite increasing food production, application of higher nitrogen fertilizers in intensive agriculture also contributed in global warming. In context to environment there is an increasing interest to breed crop varieties for organic agriculture. However, we know much about regulation of nitrogen metabolism in intensive agriculture as compared to organic agriculture. The current need and gaps in understanding of nitrogen metabolism in organic environment is discussed.

Keywords: Nitrogen metabolism; Nitrogen use efficiency; Organic agriculture

Need to Understand Nitrogen Use Efficiency in Organic Agriculture

Nitrogen (N) is quantitatively the most important nutrient for plant development. Limited N availability has severe consequences for plant metabolism and growth [1]. Inadequate N supply, for example leads to reduced leaf area, chlorophyll content and photosynthetic rate, resulting in lower biomass and yield of storage compounds. Indeed high application of N fertilizers together with the development of high yielding varieties were major drivers of the enormous increase in crop productivity during the past 50 years, allowing for significant decrease in world hunger despite a doubling of the population [2] Approximately 85 to 90 Million Metric tons (MMt) of nitrogenous fertilizers are added to soil worldwide annually up from 1.3 MMt in 1930 and 10.2 MMt in 1960 [3] and this is predicted to increase up to 240 MMt by the year 2050 [4] which dramatically affect the N cycle and associated processes [5,6]. Over 40 years the amount of mineral N fertilizer applied to agricultural crops increased by 7.4 fold whereas the overall yield increase was only 2.4 fold [7]. This means that Nitrogen Use Efficiency (NUE) which may be defined as the yield obtained per unit of available N in the soil has declined sharply. In most intensive agricultural production system the nitrogen use efficiency is approximately 33% and a substantial proportion of the remaining 67% is lost into the environment [8].

N introduced into the environment largely through N fertilization has resulted in significantly negative environment consequences [9-11]. Nitrogen lost from agricultural system will entered to groundwater, lakes, estuaries and coastal water where the reactive nitrogen can participate and induces in a wide range of biotic and abiotic process [12]. The link between agriculture and nitrate pollution is well established with impact on drinking water. For example, in the United State, 89% of total N inputs into Mississippi river come from agricultural runoff and drainage [13]. The production and excessive uses of N fertilizer also play a large role in ozone depletion and global warming [14]. Nitrous oxide (N₂O) is the third most abundant greenhouse gas (GHG) with only carbon dioxide (CO₂) and Methane (CH₄) being most prevalent [15] and is a 300 times more potent GHG than CO₂ [16]. In India, N fertilizer application contributed most of N₂O emission, a 49% share in 2005 (Out of 267 Gg, where Gg=1000000 kg) compared to 40% in 1985 (144 Gg) [17]. Agriculture sector activities (mainly nitrogen fertilizer use) are the main contributor of global anthropogenic N₂O emission (ca. 58%) [18]. In addition to these negative environmental effects, synthetic nitrogen fertilizer is typically the single highest input cost

for many crops and since commercial fertilizer production (via Haber Bosch method) is energy intensive process which require approximately 1% of the world's annual energy supply. This cost is dependent on the price of energy [19] and adding to food production cost [20].

Increasing consciousness of conservation of environment and mitigation of climate change brought a major shift in cultivation practices of major crops towards organic agriculture. Organic agriculture has been shown to improve many different environmental and human components of the agro ecosystem [21-23]. It is based on minimizing the use of external inputs through use of on-farm resources efficiently compared to intensive agriculture and thus the use of synthetic fertilizers is avoided. The demand for organic food is steadily increasing both in developed and developing countries with annual average growth rate of 20-25% [24]. Worldwide over 130 countries produce certified organic products in commercial quantities [25]. An important issue to the acceptance of organic agriculture is found in the question of its productivity. Existing analysis have indicate the carrying capacity of organic agriculture at 3-4 billion, well below the present world population (\approx 7 billion) and that projected up to 9 billion for 2050 [26]. Several yield trial comparisons between organic and conventional (intensive) farming system have shown significantly lower yield for organic system [27-29]. In organic agriculture crop productivity is mainly limited due to nitrogen availability which is not easily controllable [30]. The N availability dependent on mineralization of crop residues and farm yard manures applied on the farm. In early crop growth stages when demand is low, N is lost while in later stages the demand from the plant is often much greater than the supply from mineralization. Matching N need and mineralization is indeed one of the major limiting factors in organic agriculture system [31]. One of the basic principles of soil fertility management in organic agriculture is that crop nutrition depends on biologically derived nutrient. Organic residues added to the soil surface or incorporated into soil undergo decomposition by soil microbes. In addition to the readily available ammonium and nitrate ions, soil of organic agriculture can contain a

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wide range of organic nitrogen compounds such as peptides, proteins, free amino acids, amino sugars and nitrogen heterocyclic compounds [30,32-34]. The organic nitrogen fraction typically comprises 0.1 to 0.5% of total soil N [35]. Soil micro-organisms secrete proteases into the soil which facilitate the breakdown of proteins and peptides into their constituent amino acid units [36].

N is most often taken up by plants as water soluble nitrate (NO_3^-), ammonium (NH_4^+) and to a lesser extent as proteins, peptides or amino acids [37-40]. Comparison of rates of root uptake of amino acids, NH_4^+ and NO_3^- have been made for several species and it seems that NH_4^+ is absorbed at the highest rate followed by amino acids while the lowest rate of uptake are usually displayed for NO_3^- [41-45]. The actual pool size of organic forms of N can be large in agricultural soils [46,47]. Therefore an ability of crops to take up organic N seems advantageous for crop productivity under organic system. In fact some agricultural crop species have been shown to absorb organic N [48], for example, in rice, the N uptake rate increases with organic N supply rather than on nitrate application [49]. Studies on plant grown in solution culture or upon excised roots have also demonstrated that uptake of organic N can occur at a rate comparable to or in excess of N uptakes from inorganic N sources [50-52]. Several studies has led to the hypothesis that plants have two distinct transporter system, one for neutral/acidic amino acids and one for basic amino acids [53-57] and a range of such amino acid transporters have been identified in the roots of some plants [58]. An uptake of free amino acids through active transport system has also been demonstrated in maize [59]. However, from the literature it is clear that dissolved organic N constitute a major soluble N pool in soil and that plant root have potential to access some of this pool, yet its importance in plant nutrition is unclear and debatable [60].

Interestingly, in studies where organic crop productivity potential has been estimated actually used a variety of a crop species which have been selected and breed under conventional high fertilizer input agriculture, that may not accurately represent the soil condition present in organic system. Similar inference can also be drawn from the occurrence of interaction of genotype and N level, which indicate that the best performing varieties at high N fertilization are not necessarily the best ones where the supply of N is lower [61]. Thus breeding crops varieties suited for organic system is gaining attention as farmers and researchers realize that beneficial traits for the system should be very different from those which produce high yield under intensive agriculture [62], for example, genotypic differences were reported in wheat cultivars for the capacity to take up amino acids and this may certainly affect their performance in organic farming system [63].

Developing crop varieties with high nitrogen use and uptake efficiency is of particular importance in organic agriculture [64]. Indeed, it is also one of the top objectives in breeding for conventional agriculture too. Traditional breeding strategies to improve NUE in crop plants have experienced a plateau i.e., increase in N applied do not result in yield improvements. FAO data indicate that cereal crops including wheat, soya and maize have slowed to a growth rate of 1% annually and that in some cases specifically in developed countries, growth of crop yield is close to zero [65].

However, with advancement of molecular biology and biotechnology, the search to identifying genes that regulate NUE of crop plant made progress and successfully transgenic traits have developed [66]. But, very less is known about genes regulation of NUE under organic environment. Moreover, the transgenic approach is associated with problem of expression and NUE phenotype development and these approaches also conflicted with the core concept of organic

agriculture where integrity of plant must be maintained [67]. However, DNA based diagnostic techniques seems to fit with this concept. As NUE be a multigenic controlled trait, identifying quantitative trait loci (QTLs) linked to NUE is a promising way for genetic improvement of NUE in organic agriculture [68]. NUE as being the yield of grain per unit of available N in the soil [69] depends on uptake efficiency and utilization efficiency of genotype. NUE is under strict quantitative genetic control and a NUE phenotype is conditioned by several genes having trait enhancing (positive) and trait depressing (negative) alleles. The efficient cultivar accumulates more positive alleles though negative alleles harbor in the genetic pool [70]. The advent of molecular markers has opened new vistas of looking into segregation pattern of quantitative traits, popularly known as QTL mapping. The basic idea of QTL mapping is contemplated by making recombination between positive and negative alleles, within a controlled population. Quantifying individual phenotype in this population under targeted environment will facilitate in determining the association between the segregation of markers and the phenotype. It is established that the marker loci linked to the positive or negative alleles show co-segregation pattern with the phenotype and are mapped as putative QTL [71]. In practice, QTL mapping is done by selection and validation of quantitative loci linked with the target trait by associating the quantitative trait data on segregation population with the marker segregation data through multivariate statistical computation [72-74].

Indeed, despite the growing interest of organic agriculture, nothing is known at the molecular level about differences in molecular regulation of NUE of a crop grown solely with organic fertilizers compared to crop grown under conventional inorganic conditions. For such type of investigation, maize provides an ideal system because of its higher genetic diversity between any two genotypes [75,76]. Maize is the third most important cereal crop after wheat and rice and it is predicted to become the crop with the greatest production globally and in the developing world by 2025 [77]. Nitrogen fertilization is one of the most crucial inputs for maize production in many regions of Asia and North and South America and represents a significant production cost for the farmers [78]. It is estimated that a 1% increase in NUE could save \approx \$1.1 billion annually [79]. Hybrid corn have a strong demand for N over a short time period and it is believed that this may not be easily compatible with organic fertilizer [80] and higher NUE including use and uptake efficiency is required for increase organic maize productivity [81,82]. Previous studies on the genetic basis of NUE in maize using different population of recombinant inbred lines have found that a few yield and yield component associated quantitative trait loci (QTL) detected under low nitrogen conditions were co-located with genes encoding key enzymes involved in nitrogen metabolism such as glutamine synthetase, nitrate reductase, sucrose phosphate synthase, sucrose synthase and invertase and these were considered QTLs for NUE [83-87]. Nitrogen Reductase and Glutamine Synthetase are also identified as key regulator for nitrogen assimilation in young plants [88].

However, these QTLs identified for low nitrogen condition i.e., no synthetic nitrogenous fertilizer added, may not accurately represent soil and fertility status of organic farms. Organic and low input systems have very different seasonal N cycling and availability. As QTL with a significant effect in low nitrogen environment may often show no effect in organic fertilization as the underlying gene responses only to specific factors [89]. For example, significant differences in gene expression were noticed in wheat grown with organic and inorganic fertilization [90]. Low N condition is characterized by low N mineralization pool [91,92] which is higher in organic farm with less mineral N [93]. NUE

may also be influenced by soil bacterial processes and organic and inorganic N sources [94,95].

It is high time to understand the basic molecular differences if any, in NUE of crop plants under organic and inorganic environment will lead to discover the underlying molecular regulator(s) of NUE and provide key guidelines for crop breeding meant for organic agriculture.

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