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Mehmood Ali Noor*  

Abstract: Modern agriculture has posed a great impact upon natural resources in terms of exploitation and their luxurious use. Ever increasing population pressure has exhausted the sustainable agricultural production system by exploiting land and water resources. High external inputs, required for high yield targets, are nowadays heavily dependent upon artificial sources and ways, amongst which nitrogen (N) is of global concern. Nitrogen is required by crops for active growth and photosynthetic machinery throughout the crop cycle. Among cereals, maize has the highest yield potential, hence it removes huge amounts of N and water from soil. To achieve high yields, farmers mostly use high dose of N fertilizers, causing soil, water and environmental pollution in the form of soil degradation, eutrophication and volatilization, thus reducing nitrogen use efficiency (NUE). This review critically focuses on N fertilizer management and regulation in maize farming systems with high population density. Achievements have been summarized in regard of NUE improvement through certain advanced molecular breeding and transgenic approaches, as well as agronomic options to optimize N-uptake and its utilization, especially after silking in maize. Rational use of fertilizers with right doses at right time, as well as integrated agronomic management options are proposed. More research is required to modify assimilatory pathways during source-sink translocation through proteomic and
transcriptomic studies, to improve NUE in high density maize. Wider scope exists for molecular breeding to exploit natural variation for efficient root architecture, lodging resistance, anthesis-to-silking interval, photosynthetic efficiency, stay green and large sink capacity for optimum NUE.

**Subjects:** Crop Science; Soil Conservation Technology; Agronomy; Nutrition

**Keywords:** nitrogen; maize; nitrogen use efficiency; molecular approaches; recovery

1. **Introduction**

Nitrogen (N) fertilizer is an important measure to ensure maize yields, but excessive N inputs make it a difficult problem for optimum N utilization. In modern agricultural production, N fertilizer application is an important measure or necessary condition to ensure the high and stable crop yields. N is the key element for grain formation in maize (Evenson & Gollin, 2003; Hirel et al., 2001). However, excessive fertilization also brings a range of problems and challenges to agricultural soils and environments specifically (Guo et al., 2010).

Maize being important cereal crop feeds about 60% of the world dietary needs, along with wheat and rice (FAO, 2011). Therefore, improvement in maize yields is an important guarantee for food security. Among other agronomic managements for improved nitrogen use efficiency (NUE), rational use of N fertilizer is the key to increase maize production while reducing environmental pollution. Improving NUE is the only key to enhance utilization rates of chemical fertilizers (mainly N). In result of numerous researches, N uptake, transport and distribution patterns in maize plants are fully understood. For systematic discovery of maize potential for maximum N utilization, biological means are vital to identify genetic, physiological and molecular basis for maximizing NUE.

At the same time, a large number of studies have shown that increasing population density is also an effective way to increase maize yields as well as for increasing NUE (Tollenaar & Lee, 2002). Agronomic and physiological studies regarding higher grain yields in maize crop in recent era had focused on the key objective of increase in kernel weight, but high fertilizer dose and high plant density is also associated with occurrence of lodging, premature senescence, abortion and other related issues, thus a direct impact on high density maize cultivation breakthrough. In this perspective, to achieve high yields through high density maize cultivation the problems of lodging, senescence and grain development must be addressed in a systematic way. This mini review overviews possible breeding strategies, molecular techniques and agronomic managements in a critical way to enhance NUE, overcome N losses, better uptake and efficient source-sink distribution under high density maize cultivation.

2. **Nitrogen use efficiency and N uptake**

Being a major macronutrient, N often limits the plant growth and development. Significant increase in N fertilization was adopted after “green revolution”, which at that time resulted in bumper yields, had significantly attributed improvement in harvest index. To reduce the N losses and crop N requirement, it is suggested to increase the utilization efficiency of applied fertilizer, by selection of traits for higher yields with less inputs (Hawkesford, 2014). NUE is referred to as the grain yields per unit of available N (Moll, Kamprath, & Jackson, 1982), which can further be divided into N uptake and N utilization efficiency.

Therefore, it is vital to enhance both the uptake and utilization efficiency of N applied. In that regard, root structure and its functioning are greatly associated with N uptake, whilst sink capacity may limit the uptake (Hawkesford, 2014). Whereas, N utilization efficiency, referred to as grain yield at the expense of taken up N, is predominately associated with photosynthetic machinery of the plants and further better assimilation of photosynthates in grains, having sufficient storage proteins for maximum translocation (Shewry, 2007). More studies are required to explore the possible allocation of N in the grains, which is taken up after flowering and during grain filling process. Studying
physiological pathways regulating the translocation of N stored in crop canopy and also direct uptake of N from soil or foliar applied N fertilizers is the need of hour. High planting density of maize crop is a way to maximize uptake of applied nutrients in the form of fertilizers and also available N from other organic sources.

3. N management in high density maize

Mineral fertilizer N is the primary way nowadays to enrich soil with nutrients required for optimum crop growth and yields. Consumption of synthetic N fertilizers has eliminated the practice of enriching soils with organic C and N by adding organic manures, green manuring and fallowing (Hirel, Tétu, Lea, & Dubois, 2011). Estimated loss of applied N in intensive agricultural production systems is reported in range of 50–75%, mainly due to inefficient uptake by plants and possible soil leaching losses (Asghari & Cavagnaro, 2011; Hodge, Robinson, & Fitter, 2000; Ruan, Xue, & Tylkowska, 2002).

High plant density in summer maize is an important way to increase grain yields. In view of NUE, root length density and absorption area increases under plant high density, but it also creates competition for nutrients, water, light and space, forcing roots to grow deep into soil layers (Yang, Tao, & Wang, 2012; Zhang et al., 2012). In a recent report by Liu et al. (2017), they have observed a significant increase in root dry weight, absorption area, root/shoot ratio and with improved root structure for water absorption in summer maize in China. In their experiments, N application rate of 184.5 kg ha$^{-1}$ was found optimum among the range of 0–300 kg ha$^{-1}$, in improving root length density, root dry weight, unit leaf area and grain yield of summer maize sown under integrated agronomic practices management (Liu et al., 2017). Excessive dose of N is reported to inhibit root growth and development, thus reducing the ability of roots to absorb nutrients and water efficiently (Liu et al., 2017; Wang et al., 2000). This implies that excessive dose at initial stage would lead to N losses as well as makes it impossible to be taken up by continuously growing maize roots. Dathe, Postma, Postma-Blaauw, and Lynch (2016) suggested that older roots are continuously in decline stage and new roots tends to proliferate deep into soils. A number of studies had suggested that N applications in optimum quantities during mid and late growth stages significantly improves the N uptake, root characters, assimilation, source-sink translocation and final grain development process, thus leading to higher grain yields, harvest index and improved NUEs as compared to initial application of whole N (Liu et al., 2017; Sun et al., 2017; Zhou, Sun, Ding, Ma, & Zhao, 2017). Because strong root architecture per unit leaf area is reported to maintain high photosynthetic rates and duration as well as prolonged stay green leaf character, thus providing sufficient photosynthates to be deposited in grains to achieve high yields (Kang, Liang, Hu, & Zhang, 1998; Liedgens & Richner, 2001).

Maize yield potential per unit area has been seen increased as function of high plant population densities and due to hybrids introduction (Carlone & Russell, 1987; Duvick, 2005; Russell, 1991). Crowding stress is another important constraint due to high density cultivation of maize crop, in which inter-plant competition starts for the nutrients, water and light (Tollenaar, Deen, Echarte, & Liu, 2006; Tollenaar & Wu, 1999). Recently, Yan et al. (2017) have reported a study conducted with hypothesis of, high plant densities causes crowding stress reducing the plants nutrient uptake ability, thus impairing yields and NUE. They found that planting density of 7.5 plants m$^{-2}$ had resulted in maximum grain yield and agronomic use efficiency in maize crop. Further they described that planting density of 9 plants m$^{-2}$ tended to decrease the post-silking N accumulation, leaf N and net photosynthesis rate (Yan et al., 2017). Similarly, accelerated leaf senescence, impaired N uptake and inter-plant competition are amongst the major hurdles of high density maize cultivation (Tollenaar, Aguilera, & Nissanka, 1997; Tollenaar & Wu, 1999; Vas, Van Der Putten, & Birch, 2005), which can be overcome by agronomic N management strategies and through breeding approaches (Tokatlidis et al., 2011).

4. Molecular approaches

Transcriptome studies are gaining popularity now a days for identifying specific genes related to complex traits of NUE. Among those, significant advancements have been made to discover genes controlling specified steps such as senescence (Gregersen & Holm, 2007; Howarth et al., 2008) or
grain filling (Hansen, Friis, Bowra, Holm, & Vincze, 2009; Wan et al., 2008) in cereals, and also for nitrate supply in roots and shoots in model plants (Wang, Garvin, & Kochian, 2001; Wang, Okamoto, Xing, & Crawford, 2003). Studies on target genes and specific pathways for the improvement of NUE is still in progress but the expression of these genes is very complex under different environments and also due to the associated gene networks (Shrawat, Carroll, DePauw, Taylor, & Good, 2008).

Similarly, many other breeding and genomic approaches (Hirel, Le Gouis, Ney, & Gallais, 2007; Hirel et al., 2011) have been carried out to enhance the NUE in cereals, including wheat and maize (Reynolds et al., 2009; Shaibu, Adnan, & Jibrin, 2016). Root function needs to be optimum throughout the crop cycle, from establishment through to maturity with an important role of post-anthesis N uptake quality (Kichey, Hirel, Heumez, Dubois, & Le Gouis, 2007). Breeding strategies for improved capture can be a vital sign in broader terms of root architecture and proliferation. Research on roots system activity and studying variations in architecture is difficult under field situations and therefore most of the studies have been conducted in laboratory conditions, with different artificial systems. Studies reported that hydroponic, rhizotron and soil column methods may result in contrasting data outcome (Wojciechowski, Gooding, Ramsay, & Gregory, 2009).

Breeding strategies to improve the NUE should be focused on genetic modification of target genes, pathways or metabolic processes involved in assimilation process, or to breed for new varieties compatible with prevailing environments that can uptake efficiently maximum amount of available N from soil N bank, and utilize the taken up N very precisely into the economic part (grains) of the crop plant (Good, Shrawat, & Muench, 2004; Hirel et al., 2007). Whilst, manipulations done transgenically for many of assimilatory pathway steps showed satisfactory results under controlled conditions in pots but few have been employed under field conditions (Good et al., 2004; McAllister, Beatty, & Good, 2012). But still improved photosynthetic efficiency has the ability to cut down input requirements and to increase the final yields, thus influencing NUE. This implies less N uptake by efficient plant canopy with same C assimilation will result into improved NUE. Such an improved photosynthetic machinery can be evolved through natural variation identification among the processes involved, or manipulating assimilatory pathways by targeting for example Rubisco activity or RuBP regeneration (Parry, Madgwick, Carvalho, & Andralojc, 2007; Reynolds et al., 2009). Besides this, scientific society is also trying to incorporate C₄ metabolism into C₃ grasses, which will be vital achievement in terms of photosynthetic efficiency (Parry & Hawkesford, 2010). Breeding or biotechnology approaches are focusing on the traits of delayed maturity or functional stay green characteristics responsible for widening the grain filling duration and ultimately yield improvement (Figure 1). Among the uncontrollable factors, environmental risks are important in N uptake and its’ remobilization, such as disease attack and terminal drought conditions which enhances senescence and reduces NUE (Barbottin, Lecomte, Bouchard, & Jeuffroy, 2005). Exploitation of genetic variation for flowering, maturation and senescence time is amongst the controllable factors to improve NUE in maize crop.

5. Agronomic options
It is widely accepted that NUE varies greatly worldwide (Raun & Johnson, 1999). Locally adapted application practices for N fertilizers by farming communities are the main reason for this variation in NUE, and this factor sometimes can be considered as controllable and it becomes unavoidable under certain prevailing socioeconomic conditions and local customs, under which farmers are forced to do common practice (Hawkesford, 2014). Therefore, for wider options to improve NUE in a certain locality, timely application of fertilizer with proper irrigations according to plant nutrient requirements at critical stage of plant growth is the primary solution. Furthermore, balanced plant fertilization according to essential and non-essential nutrients ensures good yields and crop quality. Integrated agronomic management options for optimum plant yields with higher NUE is nowadays focus of many researchers (Liu et al., 2017; Yan et al., 2017), which can be a hand-guide for maize growers to adopt certain agronomic practices (tillage, N rates, irrigation scheduling, population density, cropping patterns/system, crop rotations and sowing times) for specific localities or maize growing regions under prescribed climatic conditions (Figure 1).
As N sources vary greatly over spatial spectrum ranging from organic manures to inorganic urea, anhydrous ammonia, ammonium and nitrate salts, therefore, careful management regarding fertilizer applications at right time and right dose thus ensures minimum N loss in the form of leaching and volatilization (Matson, Naylor, & Ortiz-Monasterio, 1998). In that scenario, government bodies and agricultural research institutes should ensure and disseminate the site-specific production technologies among the crop growers to enhance yields with higher harvest index and NUE by applying N fertilizers in right amounts during the maximum N uptake vegetative stage of crop plants. Similarly, split applications of N also help photosynthetic apparatus with continuous N supply through root N uptakes at silking and after silking in maize plants, which enhances the grain N deposition with improved grain quality (Zhou et al., 2017).

The detrimental effects of nitrate loss from soil impose some toxicological impacts on humans, animals and especially on environment causing freshwater eutrophication and ecosystems contamination (Beman, Arrigo, & Matson, 2005; Camargo & Alonso, 2006; Giles, 2005). Therefore, farmers can enhance the sustainability of agricultural lands by practicing more legumes in their crop rotations as well as in cover crops to restore the soil fertility status naturally. Other agronomic approaches to enhance the NUE in field crops especially maize, include rational use of fertilizers, crop rotations, establishing ground cover and by incorporating crop residues in fields after harvesting. Amongst these approaches, rational use of fertilizers can be performed by applying organic and inorganic nutrient sources under optimum conditions to minimize the runoff or volatilization at appropriate plant growth stages with precise dosage according to crop requirement (Hirel et al., 2007). Similarly, different cropping systems can allow the better uptake of nutrients from different depths of soil profile and restoration of nutrient balance by N fixation process in case of legumes. One other option to improve NUE is the conservation tillage practices or no-till, which can further be fortified by continuous cover cropping, which is known to enhance significantly the plant colonization by N fixing bacteria (Kabir, O’Halloran, Fyles, & Hamel, 1997; Kabir, O’Halloran, & Hamel, 1997; Neelam, Gaur, Bhalla, & Gupta, 2010).

6. Conclusion
Yield increments in maize crop without increasing N input sources can be a way to uplift NUE. This milestone can be achieved by employing integrated agronomic management approaches in
addition to molecular applications in enhancing photosynthetic apparatus efficiency with maximum C fixation and minimum N requirements (Figure 1). Certain physiological studies are required to optimize the performance of two components of NUE (uptake and utilization) by independent selection of target traits, having potential for significant NUE improvement. Although, transgenic studies have made significant achievements in maximizing N uptake and remobilization pathways through transgenic germplasm with satisfied performance under controlled conditions. But a very few studies have been reported for successful performance of these transgenic crops under field conditions, one reason is technically difficult and social dislikes.

Significant progress has been made recently on regulation of inorganic N metabolism at cellular and organ level. Particularly, the efforts to integrate transcriptomic and physiological data sets at whole-plant level has improved the understanding about N assimilation regulation under controlled and also in field conditions up to some extent. Similarly, sufficient research has been carried out in recent decades to locate the rate limiting steps of NUE by agronomic, physiological and molecular means. However, in perspective of physiological and genetic studies, the key steps for inorganic N metabolism as well as NUE regulatory mechanisms are still unclear, from gene expression to metabolic activity. The reason, NUE is administered by a complex array of physiological, developmental and environmental interactions which are specie-dependent. This implies the identification of broad range of genetic diversity amongst a wider germplasm with high N-uptake efficiency and its utilization (Figure 1).

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