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A Review of genetic improvement mechanisms of maize (*Zea mays* L.) in breeding for nitrogen use efficiency

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ABSTRACT

Maize is the third most prominent crop grown next to wheat and rice globally while extensively affected by stresses; critically abiotic factors like drought and soil acidity are the robust effect. Nitrogen use efficiency (NUE) is an imperative trait in maize to enhance yield with marginal contribution of nitrogen fertilizer. In this review, it has been tried to extant the current progress and the coming projections enlightening the NUE in maize by various mechanisms, especially through conventional breeding and molecular genetics. Of any kind, N fertilizer is amplified the understanding of the appliances prevailing maize N economy is indispensible for refining NUE and plummeting extreme use of fertilizer, whereas retaining an adequate yield and satisfactory income for agrarians. Consuming crops cultivated under managed environments, with various cultivation practices, independently or supplementary principles at minimum and great N inorganic mineral fertilizer expenditure or other organic fertilizer, it is currently conceivable to grow advance the entire maize agronomic and biological revisions. These can be shared with inheritable factor, protein and metabolic sketching to construct a widespread image showing the diverse paces of N acceptance, absorption and recovering to produce either in vegetative biomass or seed protein storage structures. Therefore providing a serious impression in what manner our empathetic of the agro-eco physiological, biological and molecular mechanisms of N adjustment in maize, at different ecological circumstances, has been enhanced. At the movement enduring sustainability is required at maize growing world to limit the application of synthetic N input and avoiding excessive environmental pollution. The knowledge and prospects of the future development and submission for breeding in maize improved at the deprived mineral fertilizer input.

1. Introduction

Maize (*Zea mays* L.) is originated in the Mexican highlands and diversified in a wide range of agro ecologies in the world. The USA, China, and Brazil being the top three maize producing countries globally while famous in Africa started about 500 years ago and in the late 16th and 17th in Ethiopia (Tsedeke *et al*., 2015).

Food security is a problem and international distress even today which has a direct effect to the people worldwide, especially in Sub Saharan Africa (FOA, 2017). Maize is globally serves as a source of diet, silage and industrial raw material in the manufacturing (Tahiru *et al*., 2015). In Ethiopia, it is the second largest food security crop after *tef* (*Eragrostis tef*) in area coverage and the first in its productivity (Abera *et al*., 2013). Maize provides proteins, carbohydrates, and minerals in the diet. However, now the production of this crop is insufficient globally due to its extensive demand for food, feed and bio fuel production in the industry (Banziger and Diallo, 2004; Ranum *et al*., 2014). The other studies also revealed that, its supply is also deficient as a result of most maize producing countries like conducted the production under low N availability, due to low natural soil fertility, low N fertilizers asset and production instability (Monneveux *et al*., 2005).

Currently the biotic and abiotic challenges are faced worldwide at maize production. Among the abiotic factors drought and soil acidity play a detrimental role in the deliberation of nutrients in maize seed significantly affects the ability of a seedling to tolerate various stresses (Chen *et al*., 2016). The rapid residents increase in synergy with factors of poor soil fertility and climate variability brings enormous compression on nutrient deficiencies like, P, boron (B), zinc (Zn), molybdenum (Mo) and also poor microbiological activity leading low nitrogen (N) and sulphur (S) availability (Rathod *et al*, 2017). Since maize is highly alimentary dependence stated by Cancellier (2011), critically it is more dependent on N nutrient and highly affected the yields when the N amount below the optimum level (Demari *et al*., 2016 and Noor, 2017). The other findings also conveyed that, it also contributed 1 to 4 % of dry matter and used for lateral root elongation in crops, but inhibits the lateral root growth if the N supply is extremely low (Sawyer and Mallarino, 2007). Eivazi and Habibi (2013) also reported that N increases the leaf area, cells and protoplasm content which facilitate the photo synthesis efficiency. Maize also takes half of its N supply at the growth period between 8th leaf collars to silking stage (Prester *et al*., 2003). Different agronomic studies showed that N emission is high in maize at the 4th and 6th leaves, and at the end of leaf differentiation stage (Pereira *et al*., 2013). The negative effect of urea application only at a time of eight leaves stage for maize was studied by Schiavinatti *et al*. (2011); hence split application was recommended to increase the production.

As several investigations indicated that in order to meet the increasing desires of the world population, food security in the starvation areas and improving the use of non-renewable resources could be proficient through the development and use of productive varieties (Tahiru *et al*., 2015). The agronomic solution as well provides to increase the productivity such as urea [CO $(NH_2)_2$] and ammonium sulfate [$(NH_4)_2 SO_4$] are the two main N bases exploited for maize (Alva *et al*. 2006). Sawyer and Mallarino, 2007 also reported that the adoption of high yielding maize varieties increase with the demand of additional fertilizer for enhancing the yield. Continuous N-endorsement from soil during the dough stage reduces the remobilization of N from vegetative to procreative tissues. Thus the crop does not have to cannibalize the leaves to provide N for grain expansion allows the plant to retain more green leaf and increases the duration of photosynthesis, carbohydrate production and grain yield (Muhammad *et al*., 2017).

2. The concept of nitrogen use efficiency (NUE) and its role for the crop physiology

Critically the NUE in maize has been studied seriously by various scientists. The N-use efficiency is defined as the ability of a genotype to produce higher grain yields under low soil N conditions in comparison with other genotypes (Kamprath *et al*., 1982; Prester *et al*., 2003). Nitrogen uptake efficiency (NUpE) obtained by calculating the total amount of N in the developed plant, shared by the amount of N applied to soil whereas N utilization efficiency (NUtE) is the ratio between grain weight and the total amount of N in the full-fledged plant (Moose and Below, 2009). The NUtE is influenced by different physiological factors like N acceptance, metabolism, distribution and remobilization (Gironde *et al*., 2015). According to Hawkesford (2014), to minimize the N losses and the crop N option, it is better growing the exploitation efficiency of applied nutrient by selecting traits contributing for high yield with less input. The amount of NUpE, NUtE, and NUE were quantified based on the formula developed by Moll *et al*. (1982).

$$NUpE = \frac{total\ shoot\ N}{N\ supplied},\ NUtE = \frac{Grain\ Yield}{total\ shoot\ N}$$

and

$$NUE = [\frac{Grain\ Yield}{total\ shoot\ N}]\ x\ [\frac{total\ shoot\ N}{N\ supplied}].$$

The existence of N from 95 to 99 % in a field is not readily available to crops because found unavoidable form as soil organic matter (Rangel and Silva, 2007). The organic matter and nutrients also currently decreased due to the land use intensification and fast population increments (Kamara*, et al*., 2014). Soil N is available to monocot crops in the form of nitrate (NO_3) and ammonium (NH_4) ions (Araujo *et al*. 2012), but as Sharma and Bali, 2017, reported that ammonia is the cheapest form of nitrogen fertilizer comprehensively used with simply submission and complete accessibility. Mineral N in the soil combined over the season and the root layer, governs the potential of N availability to the crop critically in maize (Mastrodomenico *et al*., 2018). Hence N is a vital nutrient for maize growth and development. This element is not only provides N source for amino acids, nucleic acids, chlorophyll and adenosine tri phosphate (ATP), but also arbitrates the intake of phosphorus, potassium and other nutrients in maize (Lv *et al*., 2016). According to Nunes *et al*. (2013), nitrogen also enhanced the grain quality by increasing the protein content and significantly related with the number of ears per plant and biomass for maize.

3. Breeding mechanisms to improve NUE of maize

The environmental heterogeneity that exists for nutrients in the soil is feasibly not remarkable that there is important extent of genetic variation and phenotypic flexibility for NUE. The strategy of improving the NUE is the genetic modification make to improve the take up more organ N or mineral N from the soil and utilize the engaged N proficiently. The research findings revealed that obviously crop genetic improvement has been responsible for 50 % to 60 % of increasing the crop yields (Han *et al*., 2015). Different studies also showed that breeding technique highly contributed to enhance the NUE in maize (Hirel *et al*., 2011; Shaibu *et al.*, 2016). The study on the physiology and genetics of N uptake and utilization is crucial to the development of N**-**efficient cultivar (Perchlik and Tegeder, 2017). One operative strategy is reducing fertilizer necessities by improving maize genotypes with high N use efficiency and yield potential (Haegele *et al*. 2013). Genotypes with high yield potential are also needed to support the rapidly growing population and provide incentives to farmers who are annoying to make modest increase in N application in their maize fields (Noelle *et al*., 2017). Generally there are two key meanings improving the NUE in the crop, one is the economic gain of the yield and the second advantage is the reduction of N pollution to the environment (Lv *et al*., 2017). The primary bio chemical reactions in the crop occur due to the involvement of N. Since N has several physiological role having different organic compositions in the cell including amino acids, proteins, enzymes, and chlorophyll. Nitrogen is mainly plentiful in leaves, chiefly in photosynthetic enzymes. Hence, biomass production, and grain yield are strongly correlated with N in maize (Kappes *et al.* 2013; Otie *et al*., 2016).

Nitrogen use efficiency is an important movement to be genetically improved for this trait through breeding for developing more productive maize varieties (Moose and Below, 2009). As the studies reported by Haegele *et al*. (2013) and Mastrodomenico *et al.* (2018), indicated that the existence of genetic variation for NUE in maize contributed to synthetic varieties conveying N uptake and utilization efficiency at broad**-**sense heritability. However, the molecular breeding has not yet further practiced to improve the NUE of maize before globally, the two breading strategies conventional and molecular approaches are important to develop stress tolerant varieties.

Various studies indicated that great variations have shown among the evaluated maize genotypes in NUE and directs traits genetically determined and improved through breeding. Genetic improvement mechanisms for agricultural crops in NUE have initially studied using conventional breeding systems (Cassman *et al*., 2002). Hereditary studies with small grains have been mainly concerned with enlightening NUE as a means of enhancing the grain protein and yield response in the application of N fertilizer (Dawson *et al.*, 2008).

The science of genetics was used in the art of breeding to improve the NUE and determine the level of genetic variation existing in different landraces and hybrids of maize. Nitrogen uptake efficiency has been widely studied about its trait components in maize and assessed by growing inbreed and hybrids at a series of high and low N conditions (Bubert, 2014). Most maize breeders used selection at the screening phase to identify high yield potential, resistance/tolerance to stresses based on the desirable grain traits and plant type through successive selection and back crossing recurrently until the desired trait acquired (Brusamarello Santos *et al*., 2017). Varieties which exhibit stress tolerance or stress avoidance through acclimation and adaptation mechanisms evolved through natural selection (Mickelbart *et al.,* 2015). Various studies showed that direct selection for yield improvement under stressed conditions has been successful in breeding (Sadras and Lawson, 2013). Selection for prospective yield in combination of genotypes and environments favor stress variation, strategy to be effective improving stress adaptation (Sadras and Richards, 2014).

Biotechnology applied to enhance the detection and validation of genes controlling NUE and its constituent traits, to develop molecular markers introducing transgenes that modify key physiological processes contributing NUE **(**Moose and Below, 2009). The studies reported before indicated that, NUE is a multifarious trait for which substantial heritable dissimilarity happens in maize germplasm and identified QTL controlling the NUE (Agrama *et al*., 1999).The modern detailed molecular characterization of QTLs and associated genes aid stress survival has enabled the expedient transfer of genes into the crop by molecular markers related with the key genes in breeding (Mickelbart *et al*., 2015). The studies used in molecular breeding indicated that root traits are controlled by multi genes. Some quantitative trait loci (QTLs) governing maize root growths to nutrient uptake have been identified, the extent of genetic variation of root traits affecting nutrient acquisition in maize is also unclear (Mu *et al.,* 2015). Near isogenic inbred lines (NILs) are a powerful tool for reviewing the function of plant traits. For instance different researchers identified a number of genes controlling N utilization using the hybrid mapping population of Inter mated B73 x Mo17 RILs (IBM) by Illinois high protein (Liu 2014). Crops with higher yields in good environmental conditions are more likely to have higher yields in stressed conditions. This approach also increased the yield in high crop environments. But it is increasingly apparent that specific selection strategies are needed to enhance yield in low-yield under stressed environments. In molecular breeding there are different selection techniques for the desired gene in maize for NUE improvement. Marker assisted selection (MAS) used a marker such as a specific phenotype, chromosomal banding, a particular DNA (RNA motif), or a chemical label that associates with the desired trait. For instance DNA marker closely linked to a stress resistance locus used to predict whether a crop is likely to be resistant to that stress or not**.** Hirel *et al*. (2005) highlighted those QTLs for the leaf enzyme activity showed to coincide with QTLs for yield and the putative role of glutamine synthetase (GS) enzyme in grain efficiency of maize. Similarly candidate genes were obtained as reported by Hirel *et al.* (2007) using quantitative trait mapping, RNA communication and metabolite describing and transgene testing. Li *et al. (*1993) also investigated that two cytosolic GS enzymes (GS1) in maize, Gln1-3 and Gln1-4 genes by studying the molecular and physiological properties of Mutator insertion mutants. The impact of knock out mutations on grain yield and its components was studied grown under suboptimal N feeding (Martin *et al*., 2006). Marker-assisted recurrent selection (MARS) also involves crossing in selected individuals at each cycle and selection. Genome-wide (GW) or genomic selection (GS)-relies on MAS for the feasibility of incorporating desirable alleles at many loci that have slight genetic outcome when used independently. This is applied to progeny in a breeding based on marker data only without the need of phenotypic evaluation (Tester and Langridge, 2010). For instance Ms44 is an example of specific gene which was done for NUE (Lv *et al*., 2016; Afsar *et al.,* 2017).

4. Challenges improving the NUE in maize and constraints of N fertilizer in the environment

One of the challenges to improve a trait, since it is determined by multiple genes is the collection of high-quality phenotypic data. Economically important traits are frequently polygenic; therefore, it is unlikely that a single genotype or recombinant inbred line would contain the necessary contrast in the relevant genes behind the trait(s) and a resource intensive. The absence of genetic variability under N stressed condition, the requirement of long breeding cycle and selection intensity, Nature of the trait to be transferred (polygenic traits), investment (initial cost of research investment (for molecular breeding), lack of integration across professions and the skill and facility of stress management are the key factors. To achieve this principle requires understanding the nature of the crop and how to use stress management, a suite of useful secondary traits that relate to yield under stress, improved statistical designs for use during selection, and appropriate choice of germplasm and breeding schemes are crucial.

The use of natural variation for crop improvement has generally been without the knowledge of specific causal genes and associated biological mechanisms. Currently, genetic determinants can be identified through quantitative trait locus (QTL) mapping, association mapping and screening by recurrent selection.

Studies on target genes and specific path ways for the development of NUE is static in progress but the expression of these inheritable factors is very multifaceted under diverse settings and also due to the related genetic material (Shrawat *et al*., 2008). As different research findings indicated that operations can be done transgenically for various assimilatory pathway paces and revealed satisfactory results under precise in pots and field conditions (McAllister *et al*., 2012).

Maize hybrids are commonly evaluated under high N, but N deficiency has a big influence on canopy variables, such as green leaf number at the grain filling period, leaf area index (LAI) and specific leaf nitrogen (SLN), and cause reduction in radiation use efficiency and light interception ultimately lower kernel number (KN) and kernel weight (KW) due to yield loss under N deficient in Kernel number per plant affected by plant growth rate (PGR) during the silking period as indicated in figure one (Chen *et al*, 2016).

Reducing N fertilizer use efficiency or retrieval competence by crops is the most problem associated with the use of chemical fertilizers (Lv *et al*., 2016). Nitrogen fertilizers also account about 25 % of the total input costs (seeds and pesticides) in maize production (Afsar, *et al.*, 2017). The other studies also confirmed that N has a (Hirel *et al*., 2007), N fertilizer contribute annual energy supply and significant greenhouse gas emissions (Li *et al*., 2015), due to its volatile nature, it was estimated that 35% to 65% of applied N lost from soil (Koocheki *et al*., 2013). The application of extreme N fertilizer inhibits root growth and development influencing the ability of roots to absorb nutrients efficiency (Liu *et al*., 2017). In the contrast the use of excessive N does also bring environmental problems and agricultural soils that pollute the aquatic systems through runoff (Guo *et al*., 2010; Santos *et al*., 2010; Zhu *et al*., 2016). For instance nitrous oxide (N_2O) is a strong glasshouse gas; and nitric oxide (NO) also plays a role in tropospheric ozone depletion (Sharma and Bali, 2017). As so many investigators argued that the best solution to elucidate N pollution is the development and production of crop varieties which have the high NUE ability and produce high grain yield (McAllister *et al*., 2012; Garnett *et al*., 2015). Figure 1 specifies the influence of sensible N stress on every of these kernels digit apparatuses characters for a distinctive maize crosses. The ear size and the total amount of ovules are alike, but may reveal the variances due to N stress. As the figures showed that both ears also designed a trivial amount of ovules at their tip that did not be fertilized, because of dawdling development of silks comparative to pollen accessibility.

The gap between pollen shed and silk appearance or anthesis silking interval (ASI) usually turn into more prominent under sever N stress.

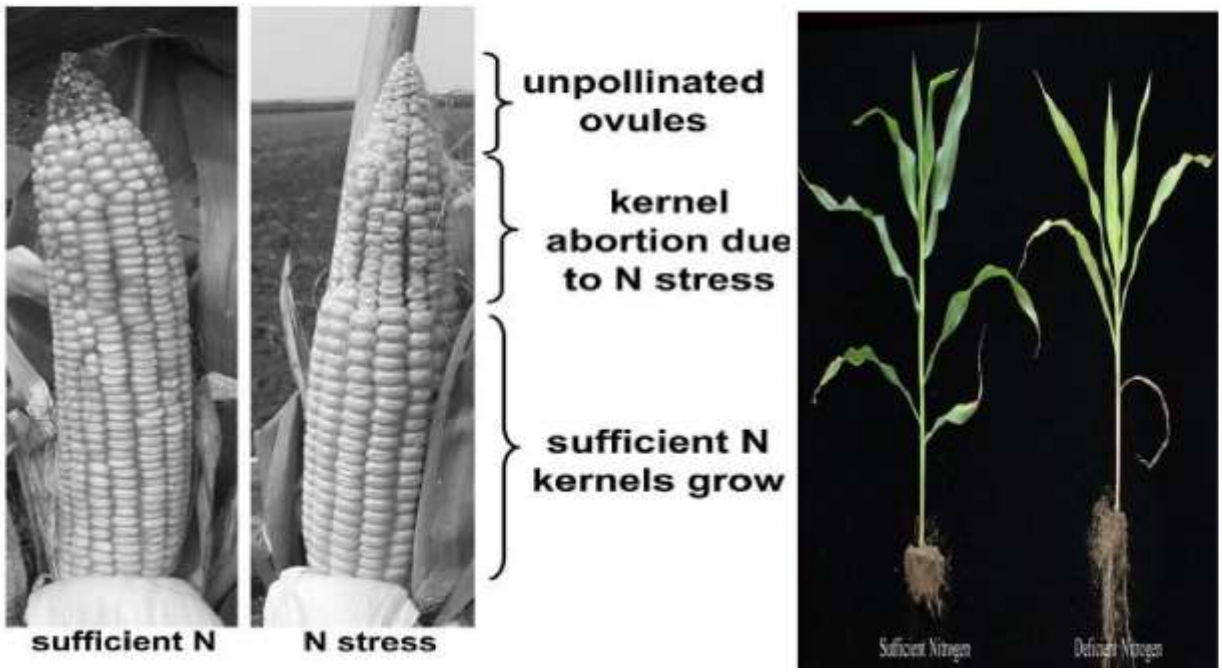

Figure 1 .Phenotypic and physiological changes in response to N stress.

5. Conclusions

Food security is an international concern today which has a direct effect on the human population, especially in Sub Saharan Africa. But the deliberation of nutrients in maize seed greatly affected by the ability of a seedling to tolerate various biotic and abiotic stresses. It is strong-minded by the examination that both conventional and molecular breeding techniques contributed for improvement of NUE, but difficult separately, especially using only conventional technique because of extended time obligation. In long term prospectus molecular breeding (genetic engineering) is the most important methods to improve the NUE of the crop combined with stepwise recurrent selection and back crossing. In general developing N stress tolerant variety is still in infant stage and it is better to strengthen the advanced breeding strategies like genetic engineering. Since the use of stress tolerant variety is cost effective, sustainable solution and environmental friendly.

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