Indian Journal of Plant Sciences ISSN: 2319–3824
Online, International Journal, Available at http://www.cibtech.org/jps.htm
2024 Vol.13, pp.63-84/Basuchaudhuri
Review Article (Open Access)

GENETICS OF NITROGEN METABOLISM IN SORGHUM (*SORGHUM BICOLOR* L., MOENCH)

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ABSTRACT

Nitrogen nutrition is the most important aspect in cereal cultivation. Low and high levels of nitrogen in soil affect the growth, development and metabolism in cereal sorghum. In this review mainly genetic aspects of nitrogen uptake and metabolism are elaborated with special emphasis on nitrogen utilization efficiency. Genes associated with nitrogen uptake and metabolism were depicted under high and low nitrogen status so that low nitrogen tolerant varieties can be available through breeding and biotechnology programs. As nitrogen utilization is related to abiotic stresses with special reference to water, this is also being considered, though sorghum is inhabitant of semi-arid tropical region.

Key words: sorghum, nitrogen uptake, nitrogen utilization efficiency, nitrogen metabolism, low nitrogen tolerance, genes regulated these phenomena.

INTRODUCTION

Sorghum (*Sorghum bicolor* L., Moench) is the fifth most important cereal crop in the world and represents an important source of food, feed and energy in several countries. Recently, there has been an increasing interest in sorghum cultivation worldwide, since it is relatively more drought- and heat-tolerant than other cereal crops, and it is better suited for the predicted consequences of global warming. In Africa and Asia, sorghum is primarily used as food for more than 500 million people, while in the Americas and Australia, it is used mainly as a maize-substitute in livestock feed. In the United States, sorghum is also being used in the production of ethanol. In view of its diverse utility, sorghum offers a large number of target traits that could be modified to meet the required applications.

For pioneer U.S. grain sorghum hybrids, grain yield increased about 27 kg ha^{-1} yr^{-1} during the last decades (Demarco *et al.*, 2022). Overall, yield gain was strongly associated with the increase in grain number, harvest index (in minor proportion), and carbon remobilization during the reproductive period (Demarco *et al.*, 2022; Pfeiffer *et al.*, 2019). Nitrogen (N) represents the most important nutrient for crop yield improvement in crops and enhancing its use efficiency is desirable to reduce fertilizer costs (Cassman *et al.*, 2002; Traore and Maranville, 1999). Understanding the N internal efficiency (NIE, yield to N uptake ratio) of grain sorghum hybrids is a useful tool for genetic improvement programs, field crop management and efficient use of resources, with no clear trends on NIE over time. However, a more detailed retrospective characterization study is vital to better understand potential changes in NIE over time and to dissect the main physiological factors or mechanisms underlying the changes in this trait for U.S. grain sorghum hybrids.

Nitrogen Requirement

Despite greater drought tolerance, soil water stress and low soil nitrogen (N) are major factors that affect sorghum (*Sorghum bicolor*) productivity and profitability in dryland environments. A study was undertaken to determine the effects of available soil water at planting and N fertilizer on grain sorghum yield and net return. A study was conducted near Hays, KS, from Summer 1971 to Fall 2002. Treatments were four N rates (0, 22, 45 and 67 kg N ha^{-1}) and depth of moist soil determined prior to sorghum planting each year. Results showed a −0.96, 14.4, 29.3, and 36.5 kg ha^{-1} yield change per kg N applied for very low yielding (yield < 1,269 kg ha^{-1}), low yielding (1,269< yield <2,611), high yielding (2,611< yield <3,986), and very high yielding (yield > 3,986 kg ha^{-1}) environments, respectively. Sorghum yield increased by rates of 9.6

and 20.4 kg ha^{-1} per mm increase in available soil water (ASW) at planting for 0 and 67 kg N ha^{-1}, respectively. A multilinear function showed that grain yield increased by 20 kg ha^{-1} for each kg N ha^{-1} applied, by 15 kg ha^{-1} for each mm increase in ASW, and by 0.67 kg ha^{-1} for each mm increase of in-season precipitation (ISP). Net return was negative for all environments when depth of moist soil at planting was ≤76 cm. It was concluded that ASW prior to planting is critical for sorghum production and can be used to adjust sorghum N fertilizer rates early in the season. Sorghum yield response to N varied from −0.96 to 36.5 kg (kg N)$^{-1}$ based on environment. Yield increased by 9.6 kg ha^{-1} per mm increase in available soil water at planting for the control. Yield increased by 20.4 kg ha^{-1} per mm increase in soil water at planting for 67 kg N ha^{-1} treatment. Multilinear function best described the relationship between N, soil water, and precipitation with yield (Obour *et al.*, 2022).

Sorghum production in semi-arid lands is constrained by inadequate soil moisture and low nitrogen. Research was carried out in semi-arid Machakos between 2018-2020 to determine the effect of nitrogen fertilizer on nitrogen use efficiency, yield on selected sorghum genotypes and genotypes suitable for low soil fertility in semi-arid lands. The experiment was an RCBD in a split plot arrangement with 11 sorghum genotypes in the main plot and nitrogen (0, 6.5 and 32.5 kgha^{-1}) as the sub-plots in three replicates. Nitrogen use efficiency (NUE) and its indices agronomic efficiency (AE), nitrogen internal utilization (IE), physiological efficiency (PE), nitrogen recovery efficiency, nitrogen harvest index (NHI) and partial factor productivity (PFP) were obtained from sorghum yield data and nitrogen uptake. The results showed that nitrogen application at 6.5 kgha^{-1} and 32.5 kgha^{-1} significantly increased grain yield, stover weight and total dry matter (TDM) by 43% and 116%, 39% and 85% and 42% and 57% respectively. Sorghum genotypes had significantly higher grain yield and low N uptake implying they are N-efficient genotypes. NUE of sorghum decreased with increasing N application. Nitrogen internal utilization efficiency (IE) was significantly higher at zero N application rate implying better N assimilation by sorghum genotypes at low N. AE, PE, RE and PFP were all significantly higher at 6.5 kg N ha^{-1}. All tested genotypes had significantly high NUE (90 to 1148 kgkg^{-1}, RE (27 to 94 kgkg^{-1}), AE (41 to 139 kgkg^{-1}), PE (27 to 84 kgkg^{-1}) and IE (41 to 139 kgkg^{-1}) than the check (Gadam). It was concluded that sorghum genotypes yield parameters were increased by nitrogen application, NUE was highest at low N levels and its indices were significantly higher at 6.5 kg Nha^{-1}. Four genotypes were found to be highly N-efficient and are recommended for sorghum improvement (Ngulun *et al.,* 2024) (Table 1).

Table 1. Grain yield, stover weight, total dry matter (TDM), N uptake, NUE, and NUE indices of sorghum genotypes grown in Katumani during 2020 short rains season (after Ngulun *et al.*, 2024).

Treatment	Grain (kgha^{-1}	Stover (kgha^{-1}	TDM (kgha^{-1}	N (kgha^{-1}	NHI	NUE (moll)
KgNha^{-1}	2018 2020	2018 2020	2018 2020	2018 2020	2018 2020	2018 2020
0	803 3155	1697 4929	2503 8086	25 36	0.49 0.152	61 972
6.5	1436 3688	2887 5350	4323 9036	49 52	0.49 0.217	60 805
32.5	2396 4256	4291 5832	466910088	77 64	0.52 0.220	58 672

Treatment	NUE(Hi)	IE	AE	PE	RE	PFP
KgNha^{-1}	2018 2020	2018 2020	2018 2020	2018 2020	2018 2020	2018 2020
0	98 237	32 93	-	-	-	-
6.5	87 191	29 77	97 77	31 82	3.6 45	221 2.42
32.5	86 171	31 72	48 72	30 34	1.6 47	74 0.83

Nitrogen Utilization Efficiency

Nitrogen is the most important nutrient influencing the growth and development of sorghum plants; therefore, it is strongly associated with crop yields (Ciampitti and Prasad, 2016; van Oosterom *et al.,* 2010). Physiologically, in plants, N plays a key role in protein synthesis, enzyme activities, C fixation, biomass accumulation, and grain yield (Masclaux-Daubresse *et al.,* 2010). Nitrogen is absorbed by the plant during the vegetative and reproductive period. During the vegetative period N is allocated to the leaf to be utilized in photosynthesis and to the stems acting as reservoir organs for the plant growth (Ciampitti and Prasad, 2016; Ciampitti *et al.*, 2013). During the grain filling period, reserves of the plants are remobilized to fill the grains (van Oosterom *et al.,* 2010), and C and N are utilized for cell division at the lag phase of seed filling and cell growth during the exponential growth phase (Lemcoff and Loomis, 1994; Singletary & Below, 1989).Increase N remobilization has been described for sorghum and maize (*Zea mays* L.) as one of the mechanisms related to yield gain over time (Bender *et al.,* 2013; Ciampitti and Prasad, 2016;Ciampitti and Vyn, 2012; Mueller *et al.,* 2019)(Fig.1).

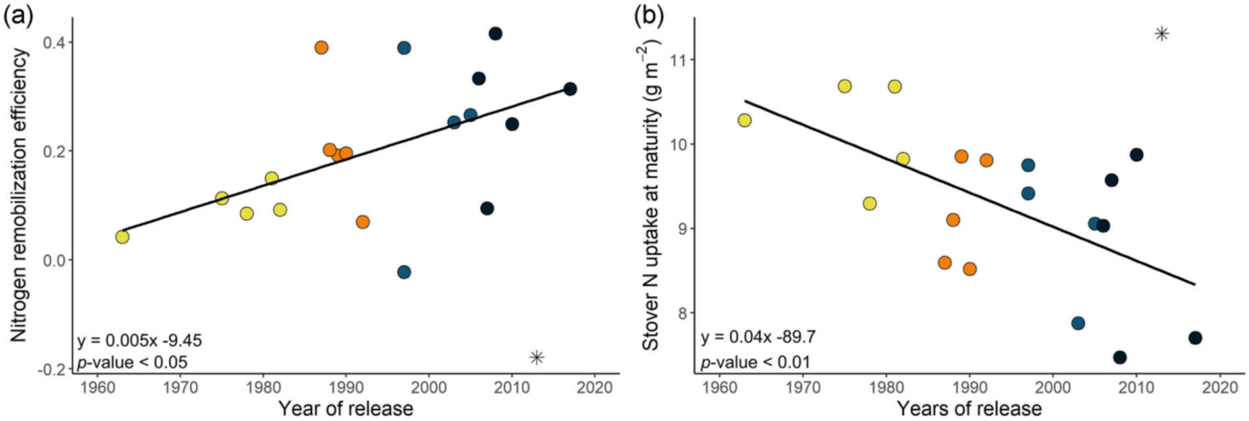


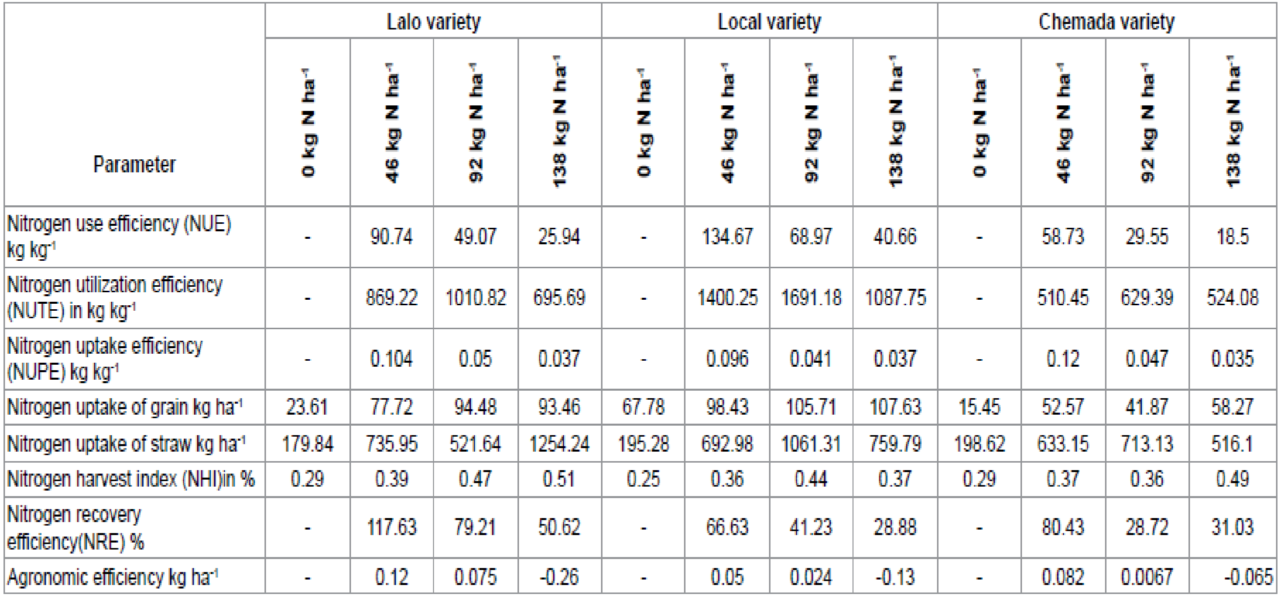
Fig.1.Changes in nitrogen remobilization efficiency and stover N uptake with time. Lines represent linear regression and colors represent the group of hybrids according to years of release: ●1963–1983, ●1984–1994, ●1995–2005, and ●2006–2017 **(after Demarco *et al.,* 2022).**

The efficiency of utilization of N by maize and sorghum (defined as grain yield per unit N uptake) varies under different climatic, soil and management conditions. To maximize N-use efficiency, the minimum N requirement for a given yield level must be established. The maximum N-use efficiency (NUE) of sorghum was smaller than for maize (48 vs. 61 g grain g^{-1} N absorbed) and was associated with a grain N concentration in sorghum. Cultivar differences in NUE in sorghum were related to grain N concentration. In both crops, NUE declined when N was supplied at high levels or when growth was limited by moisture supply. These conditions result in increased stem and grain N concentration. Crop models can integrate the effects of abiotic factors on grain yield and allow the development of strategies for improving the utilization of N to maximize grain yield and to minimize N losses from the production system (Muchow, 1998).

Sorghum is an important cereal crop, which requires a high dose of nitrogen for optimum growth and productivity, especially under rainfed farming situations in tropical regions. Field experiment was conducted at Haro Sabu Agricultural Research Center during main cropping season of 2014to investigate Nitrogen Use Efficiency (NUE) of two improved and a local sorghum cultivar in relation to graded rates of N levels and to investigate their effect on yield, N uptake and economics. The treatments comprised factorial combination of four nitrogen rates (0, 46, 92 and138 kg N ha^{-1}) and three sorghum genotypes (Lalo, Chemada and Local varieties) tested in a Factorial Randomized Block Design with three replications. The results revealed that there was significant effect of N rates on days to 50% flowering, days to 50% physiological maturity, lodging percentage, leaf area at 90 and 120 DAS, leaf area index, number of green leaves plant^{-1}, biological yield, grain yield, harvest index and nitrogen use efficiency. There was a significant interaction effect of N rates and sorghum genotypes on most of parameters studied. Significantly higher grain productivity was obtained in response to the application of 92 kg N ha^{-1} with Lalo variety in comparison with the rest of the genotype × N-rate combinations. Genotypic variations in N uptake, partitioning and NUE in plant parts like leaves, stems and grain were noted. Increase in the rate of applied N enhanced N uptake, nitrogen utilization efficiency, and N harvest index; while higher rates decreased N use efficiency, N uptake efficiency, N recovery efficiency, and agronomic efficiency. Economic analysis indicated higher net return with the application of 92 kg N ha^{-1} and Lalo genotype accrued the highest net return and benefit: cost ratio than Local variety (Shamme *et al.*, 2016).

Nitrogen Use Efficiency (NUE)is a broad term incorporating various traits that can be targeted for improvement, such as N uptake or utilization. In cereals, NUE ultimately refers to maximizing grain N yield per unit of soil N (Good *et al.*, 2004). It is often categorized into two subclasses: N Recovery Efficiencies (NRE)referring to the crops' ability to recover N from soil and N Internal Efficiencies (NIE)which refers to the crops' ability to efficiently assimilate and remobilize N. Many phenotypic traits can be manipulated, such as root morphology, total biomass and tiller number to affect NUE (Garnett *et al.*, 2009; Van Oosterom *et al.*, 2010a, b; Olson *et al.*, 2013), however the efficiency of cellular pathways involved in N uptake and utilization also play an important role. For example, elite maize varieties often display enhanced utilization of N; but none the less, N uptake has remained constant throughout domestication, suggesting conventional breeding programs have reached NRE capacity (Moose and Below, 2009) (Table 2).

Table 2. Nitrogen Utilization Parameters in sorghum (after Shamme *et al.*, 2016).



Parameter	Lalo variety				Local variety				Chemada variety			
	0 kg N ha^{-1}	46 kg N ha^{-1}	92 kg N ha^{-1}	138 kg N ha^{-1}	0 kg N ha^{-1}	46 kg N ha^{-1}	92 kg N ha^{-1}	138 kg N ha^{-1}	0 kg N ha^{-1}	46 kg N ha^{-1}	92 kg N ha^{-1}	138 kg N ha^{-1}
Nitrogen use efficiency (NUE) kg kg^{-1}	-	90.74	49.07	25.94	-	134.67	68.97	40.66	-	58.73	29.55	18.5
Nitrogen utilization efficiency (NUTE) in kg kg^{-1}	-	869.22	1010.82	695.69	-	1400.25	1691.18	1087.75	-	510.45	629.39	524.08
Nitrogen uptake efficiency (NUPE) kg kg^{-1}	-	0.104	0.05	0.037	-	0.096	0.041	0.037	-	0.12	0.047	0.035
Nitrogen uptake of grain kg ha^{-1}	23.61	77.72	94.48	93.46	67.78	98.43	105.71	107.63	15.45	52.57	41.87	58.27
Nitrogen uptake of straw kg ha^{-1}	179.84	735.95	521.64	1254.24	195.28	692.98	1061.31	759.79	198.62	633.15	713.13	516.1
Nitrogen harvest index (NHI)in %	0.29	0.39	0.47	0.51	0.25	0.36	0.44	0.37	0.29	0.37	0.36	0.49
Nitrogen recovery efficiency(NRE) %	-	117.63	79.21	50.62	-	66.63	41.23	28.88	-	80.43	28.72	31.03
Agronomic efficiency kg ha^{-1}	-	0.12	0.075	-0.26	-	0.05	0.024	-0.13	-	0.082	0.0067	-0.065

Remobilization of Nitrogen to grains

The effect of nitrogen (N) supply on the relative contributions of pre- and post-anthesis net above-ground biomass accumulation and N uptake to grain-yield and grain N concentration was examined in four contrasting environments in semi-arid tropical Australia. The four environments had different radiation and temperature regimes, and varying levels of water deficit. The grain-yield achieved under high N supply ranged from 156 to 621 g m^{-2} (on an oven-dry basis).

In all but the lowest-yielding environment, there was substantial biomass accumulation during grain filling, and it increased with N application. Only in the lowest-yielding environment, there was substantial mobilization of pre-anthesis biomass to grain.

Sorghum is one of the most drought-tolerant cereals. However, strong water stress, especially during the reproductive phase, causes significant grain yield reduction. Drought events are common in second crops planted after soybean harvest in Brazil. The study was to assess grain sorghum hybrids grown under pre- and post-flowering drought stress. Twenty-five hybrids were subjected to two water stress environments (pre- and post-flowering) and one environment with non-water stress. The experimental design was a split plot based on a randomized complete block with three replicates. Water stress at pre-flowering reduced plant height, accelerated flowering, and increased panicle index compared with water stress at post-flowering. Both pre- and post-flowering stress reduced yield by 45% and 48%, respectively. Grain mass was greatly affected by stress occurring post-flowering but not by that occurring pre-flowering. Therefore, the reduction in yield caused by pre-flowering stress affected more to the reduction of grain number in the panicle, whereas the reduction when stress occurs during post-flowering affected more to reduction in grain size. The plant chlorophyll index decreased when stress occurred pre-flowering and reverted after irrigation had resumed, demonstrating recovery in the photosynthetic capacity of the sorghum plant. With post-flowering stress, the chlorophyll index decreased significantly 30 days after the stress, affecting grain filling, with consequent yield loss (de Souza *et al.*, 2021).

Grain Yield

Results highlight that grain yield in sorghum, unlike many morpho-physiological traits, exhibits substantial variability of genotype specific responses to long term low severity nitrogen deficit stress. Metabolic response to long term nitrogen stress showed higher proportion of variability explained by genotype specific responses than did morpho-pysiological traits and several metabolites were correlated with yield. This suggests that it might be possible to build predictive models using metabolite abundance to estimate which sorghum genotypes will exhibit greater or lesser decreases in yield in response to nitrogen deficit, however further research needs to be done to evaluate such model (Grzybowski *et al.*, 2022) (Fig.2).

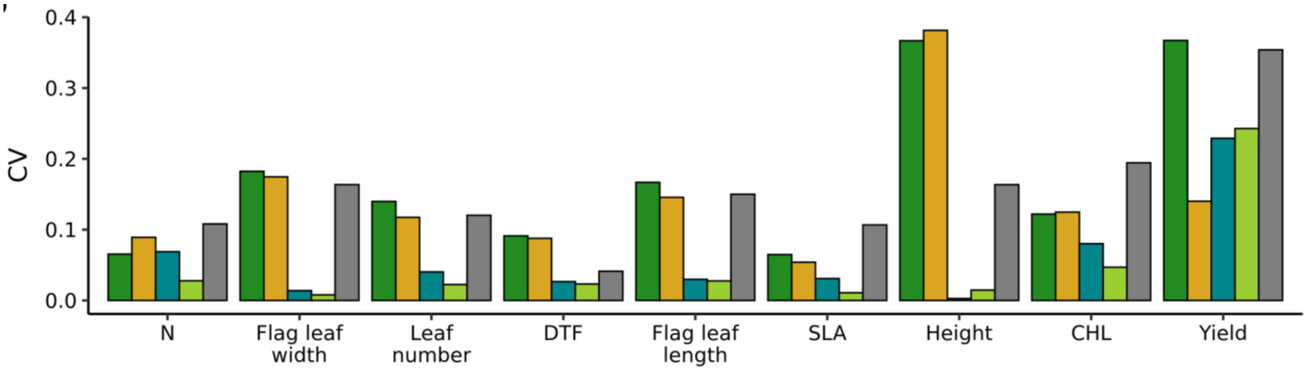


Fig.2.Variance of different traits and yield of sorghum under spell and severity of nitrogen deficit

Sorghum [*Sorghum bicolor* (L.) Moench] has been gaining attention as a feedstock for biomass energy production. While it is obvious that nitrogen (N) supply significantly affects sorghum growth and biomass accumulation, our knowledge is still limited regarding the effect of N on the biomass quality of sorghum, such as the contents and structures of lignin and other cell wall components. Therefore, in a study, researchers investigated the effects of N supply on the structure and composition of sorghum cell walls. The cell walls of hydroponically cultured sorghum seedlings grown under sufficient or deficient N conditions were analyzed using chemical, two-dimensional nuclear magnetic resonance, gene expression, and immunohistochemical methods. It was found that the level of N supply considerably affected the cell wall structure and composition of sorghum seedlings. Limitation of N led to a decrease in the syringyl/guaiacyl lignin unit ratio and an increase in the amount and alteration of tissue distribution of several hemicelluloses, including mixed linkage (1 → 3), (1 → 4)-β-d-glucan, and arabinoxylan. At least some of these cell wall alterations could be associated with changes in gene expression. Nitrogen status is thus one of the factors affecting the cell wall properties of sorghum seedlings (Rivai *et al.*, 2021).

Root Architecture

Root architecture shows high developmental plasticity to combined N and W availability. At least part of these adaptations may be the result of co-ordination between transcriptional programs in response to N and W signals. Known signaling components of N and ABA signaling pathways may be involved in this coordination. The NLP7 TF and NRT1.1 nitrate transceptor mediate the expression of N-responsive genes, and—as proposed herein through genome-wide analysis—the expression of drought-responsive genes. Similarly, analysis showed that TFs in the ABA pathway mediate drought responses as well as the expression of N-responsive genes. The effect of perturbing signaling components of one pathway and evaluating the impact on the other at a genome-scale level—in a single experimental design—remains to be determined. Such an approach would provide further insight into how plants coordinate transcriptional programs to adapt organ responses to changes in both N and W availability. It was speculated that as our understanding of signaling pathways in different crops growth, species will differ in how they integrate N and W signals at the molecular level. These differences may in turn explain why some crop varieties vary in their phenotypic responses to N and W combinations (Swift *et al.*, 2019).

Exploring the natural genetic variability and its exploitation for improved Nitrogen Use Efficiency (NUE) in sorghum is one of the primary goals in the modern crop improvement programs. The integrated strategies include high-throughput phenotyping, next generation sequencing (NGS)-based genotyping technologies, and a priority selected candidate gene studies that help understand the detailed physiological and molecular mechanisms underpinning this complex trait. A set of sixty diverse sorghum genotypes was evaluated for different vegetative, reproductive, and yield traits related to NUE in the field (under three N regimes) for two seasons. Significant variations for different and related traits under 0 and 50% N confirmed the availability of native genetic variability in sorghum under low N regimes. Sorghum genotypes with distinct genetic background had interestingly similar NUE associated traits. The Genotyping-By-Sequencing based SNPs (>89 K) were used to study the population structure, and phylogenetic groupings identified three distinct groups. The information of grain N and stalk N content of the individuals covered on the phylogenetic groups indicated randomness in the distribution for adaptation under variable N regimes. This study identified promising sorghum genotypes with consistent performance under varying environments, with buffer capacity for yield under low N conditions. These also report better performing genotypes for varied production use—grain, stover, and dual-purpose sorghum having differential adaptation response to NUE traits. Expression profiling of NUE associated genes in shoot and root tissues of contrasting lines (PVK801 and HDW703) grown in varying N conditions revealed interesting outcomes. Root tissues of contrasting lines exhibited differential expression profiles for transporter genes [ammonium transporter (SbAMT), nitrate transporters (SbNRT)]; primary assimilatory (glutamine synthetase (SbGS), glutamate synthase (SbGOGAT[NADH], SbGOGAT[Fd]), assimilatory genes [nitrite reductase (SbNiR[NADH]3)]; and amino acid biosynthesis associated gene [glutamate dehydrogenase (SbGDH)](Bollam *et al.*, 2021) (Fig.3).

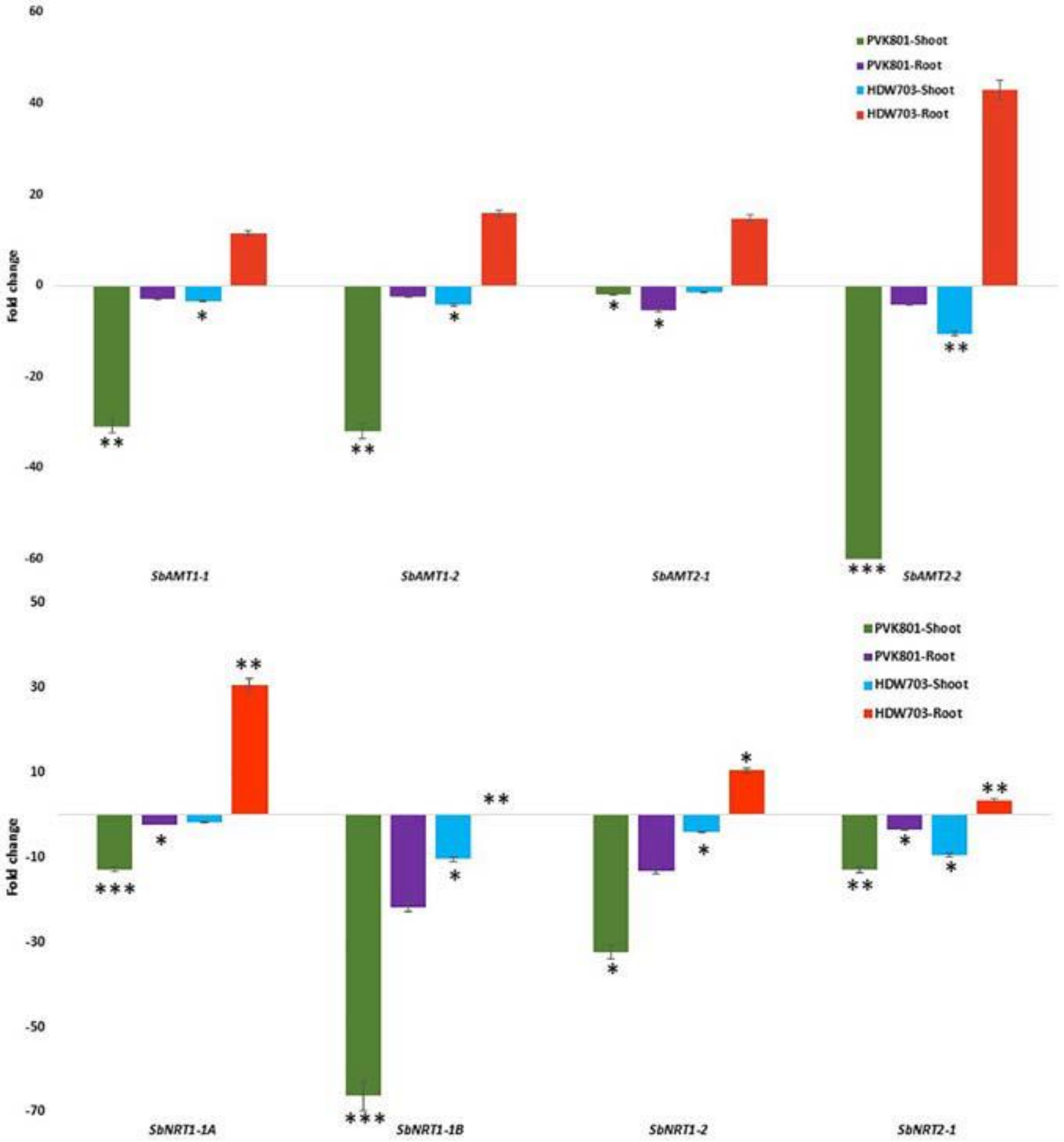


Fig.3. Changes in transporter and assimilatory genes of sorghum shoot and root grown under varying nitrogen conditions (after Bollam *et al.*, 2021).

High NUE

Profiling of contrasting sorghum genotypes in varying N dosages will provide new information to understand the response of NUE genes toward adaptation to the differential N regimes in sorghum. High NUE genotypes identified from this study could be potential candidates for in-depth molecular analysis and contribute toward the development of N efficient sorghum cultivars.

A field experiment was conducted on improving nitrogen use efficiency through split application of nitrogen in rainfed kharif sorghum (*Sorghum bicolor* (L.) Moench) at AICRP on Sorghum, Main Agricultural Research Station, Dharwad-Karnataka-India during kharif 2014. The experiment was laid out in a split plot design with fourteen treatment combinations and three replications. The treatment consisted of two genotypes as main plots and seven methods of split application of nitrogen as sub plot. Among the genotypes DSV-6 recorded significantly higher plant height (225.2 cm), leaf area index (3.14), leaf area duration (114.77 days), total dry matter production (237.28 g plant^{-1}), grain weight ear^{-1}(100.59 g), number of grains ear^{-1}(4376), nitrogen use efficiency (59.36 kg kg^{-1}), grain yield (5937 kg ha^{-1}), fodder yield (9.09 t ha^{-1}), net returns (33241 ha^{-1}) and B:C ratio (1.86). Split application of nitrogen @ 50 % N at sowing + 25 % N at 30 DAS + 25 % N at boot leaf stage recorded significantly higher plant height (196.4 cm), leaf area index (3.51), leaf area duration (95.50 days), total dry matter production (220.48 g plant^{-1}), grain weight ear^{-1}(99.56 g)(Khidrapure and Kubsad, 2017).

A split-split plot experiment, replicated three times, was conducted in Mt Zonwe smallholder farming area from 2016/17 to 2018/19. The results showed that water content under tied contour (TC) and infiltration pits (IP) had significantly higher water content compared to standard contour (SC) and moisture content significantly decreased with an increase in distance from RWH practices. TC and IP had higher agronomic efficiency than SC across all nitrogen applications, distance from RWH practice, and seasons. Sorghum variety Macia had higher nitrogen use efficiency indices than Sc Sila at nitrogen application of 50 and 70 kg N/ha while nitrogen application >100 kg N/ha had no difference in nitrogen use in both varieties. A decreasing trend in nitrogen productivity with an increase in nitrogen application was shown in both varieties (Kubiku *et al.,* 2023).

Quantification of crop yield and eight morpho-physiological traits from a large and diverse sorghum population (*Sorghum bicolor* L.) grown to maturity in field conditions under both nitrogen limiting and nonnitrogen limiting conditions. For a subset of 24 replicated genotypes, large scale metabolic profiling was conducted from leaf tissue collected at the flowering stage. Significant plasticity and genotype x environment interactions were observed for both yield and a subset of metabolic traits, while substantially less genotype x environment interaction was observed for morpho-physiological traits. The abundance of several metabolites at flowering exhibited significant correlations with plant performance (e.g. yield) at maturity (Grzybowski *et al*., 2022).

Known high nitrogen utilization efficiency (NUE j, biomass per unit plant N) China lines of sorghum, China 17 and San Chi San, were compared with relatively low NUEj U.S. lines, CK60 and Tx623, for both their physiological and biochemical adaptations to tolerate an imposed N stress in the greenhouse. Assimilation efficiency indices (ACi) were significantly greater for the China lines than the U.S. lines at both low and high soil nitrogen levels by about two-fold. Chlorophyll levels in leaves of high NUEI lines were lower at both soil N treatments. Immunoblots of leaf extracts of sorghum subjected to N stress indicated reduced levels of both phosphoenolpyruvate carboxylase (PEPcase) and ribulose 1,5-bisphosphate carboxylase (Rubisco) while NADP-malic enzyme levels, in general, appear not to be affected. However, NUEI China line, China 17, retained a significantly greater PEPcase activity than the less-NUEI U.S. lines, also the NUEI China line San Chi San, when grown under N stress conditions.

This suggests that PEPcase and enzymes associated with phosphoenolpyruvate synthesis, perhaps, are significant factors in maintaining relatively high photosynthesis under N stress. Carbon isotope ratios of leaves from sorghum genotypes, as indicated by 8 ^{13}C values, became less negative when sorghum plants were grown under N stress, but a genotypic variation either at a low or high N was not observed (Maranville and Madhavan, 2002).

Maranville and Settimi (1989) proposed that observed differences of C4 crops for NUEI at low leaf N levels may lie in the comparative ability to mobilize N from carboxylating enzymes with minimal loss of catalytic function, thus maintaining photosynthesis at levels which sustain growth as N supply is reduced.

Nitrogen Metabolism

Nitrogen is a primary macronutrient in plants, and nitrogen fertilizers play a critical role in crop production and yield. In this study, it revealed that the effects of overexpressing a glutamine synthetase (GS) gene on nitrogen metabolism, and plant growth and development in sorghum (S*orghum bicolor* L., Moench). GS catalyzes the ATP dependent reaction between ammonia and glutamate to produce glutamine. A 1,071 bp long coding sequence of a sorghum cytosolic GS gene (Gln1) under the control of the maize ubiquitin (Ubq) promoter was introduced into sorghum immature embryos by *Agrobacterium*-mediated transformation. Progeny of the transformants exhibited higher accumulation of the Gln1 transcripts and up to 2.2-fold higher GS activity compared to the non-transgenic controls. When grown under optimal nitrogen conditions, these Gln1 transgenic lines showed greater tillering and up to 2.1-fold increase in shoot vegetative biomass. Interestingly, even under greenhouse conditions, a seasonal component to both these parameters and the grain yield were observed. Results, showing that the growth and development of sorghum Gln1 transformants are also affected by N availability and other environmental factors, suggest complexity of the relationship between GS activity and plant growth and development. A better understanding of other control points and the ability to manipulate these will be needed to utilize transgenic technology to improve nitrogen use efficiency of crop plants (Urriola and Rathore, 2014).

Native Genotypes

Sorghum bicolor (sorghum) is an important cereal crop, particularly in resource-poor semi-arid regions, and is known to have a high NUE in comparison to other major cereals under limited N conditions. This study provides the first assessment of genetic diversity and signatures of selection across 230 fully sequenced genes putatively involved in the uptake and utilization of N from a diverse panel of sorghum lines. This comprehensive analysis reveals an overall reduction in diversity as a result of domestication and a total of 128 genes displaying signatures of purifying selection, thereby revealing possible gene targets to improve NUE in sorghum and cereals alike. A number of key genes appear to have been involved in selective sweeps, reducing their sequence diversity. The ammonium transporter (AMT) genes generally had low allelic diversity, whereas a substantial number of nitrate/peptidetransporter1 (NRT1/PTR) genes had higher nucleotide diversity in domesticated germplasm. Interestingly, members of the distinct race *Guinea margaritiferum* contained a number of unique alleles, and along with the wild sorghum species, represent a rich resource of new variation for plant improvement of NUE in sorghum.

Different genetic engineering approaches to address two important issues in sorghum are: seed quality and nitrogen use efficiency. First, it examined the temporal and spatial activity of a rice glutelin gene (GluA-2) promoter, in transgenic sorghum. Results from quantitative and histochemical GUS assays, as well as from transcript analyses, showed that this promoter is highly active during the middle stages of sorghum seed development and that it controls transgene expression specifically in the seed endosperm. This means that the GluA-2 promoter can serve as a useful tool in introducing novel traits into sorghum seed to improve the quality of this important cereal. Furthermore, when investigated the effects of cytosolic glutamine synthetase (GS1) and alanine aminotransferase (AlaAT) gene overexpression on nitrogen metabolism and plant growth in sorghum. T (2) generation plants transformed with a sorghum GS1 gene (Gln1) driven by the maize ubiquitin promoter exhibited enhanced grain yield and biomass accumulation under optimal nitrogen levels.

Identification of the genes responsible for low-N tolerance in sorghum will facilitate understanding of the molecular mechanisms of low-N tolerance and facilitate the genetic improvement of sorghum through marker-assisted selection or gene transformation. In this study the transcriptomes of root tissues from seven sorghum genotypes having differential response to low-N stress (Table 2) (Massel *et al.,* 2016).

Table 2. Performance of five sorghum inbreds, five worst (1-5) and five best performing (6-10) CK60 × San Chi San RILs grown under normal-N and low-N field conditions

Genotype	Plant height (cm)		Biomass yield (t ha^{-1})		Grain yield (t ha^{-1})	
	NN	LN	NN	LN	NN	LN
RIL-1	113	95	4.1	3.0	1.3	0.9
RIL-2	132	83	9.0	3.1	3.1	1.4
RIL-3	149	77	7.5	3.2	1.6	1.1
RIL-4	147	98	7.6	3.5	3.2	1.7
RIL-5	122	109	8.1	3.7	2.5	0.9
CK60	115	91	6.6	3.1	2.9	1.1
BTx623	140	126	8.2	4.0	2.8	1.2
KS78	132	76	10.1	5.9	4.1	2.2
San Chi San	157	137	16.5	7.6	6.4	5.0
China-17	170	157	13.8	7.3	5.5	3.9
RIL-6	124	93	13.0	11.4	6.2	4.6
RIL-7	152	122	13.2	9.6	4.0	1.0
RIL-8	161	125	13.4	9.4	3.4	2.6
RIL-9	185	168	17.7	15.1	6.2	5.2
RIL-10	163	137	18.4	16.5	7.7	6.7

NN- normal N field (100 kg ha^{-1}); LN- low N field (0 kg ha^{-1}).

Low Nitrogen Tolerance

Illumina RNA-sequencing detected several common differentially expressed genes (DEGs) between four low-N tolerant sorghum genotypes (San Chi San, China17, KS78 and high-NUE bulk) and three sensitive genotypes (CK60, BTx623 and low-NUE bulk). In sensitive genotypes, N-stress increased the abundance of DEG transcripts associated with stress responses including oxidative stress and stimuli were abundant. The tolerant genotypes adapt to N deficiency by producing greater root mass for efficient uptake of nutrients. In tolerant genotypes, higher abundance of transcripts related to high affinity nitrate transporters (NRT2.2, NRT2.3, NRT2.5, and NRT2.6) and lysine histidine transporter 1 (LHT1), may suggest an improved uptake efficiency of inorganic and organic forms of nitrogen. Higher abundance of SEC14 cytosolic factor family protein transcript in tolerant genotypes could lead to increased membrane stability and tolerance to N-stress. In order to analyze the mechanisms of different sorghum genotypes to low-N tolerance and provide the theoretical basis for the breeding of low nitrogen tolerant sorghum varieties, two materials (KZ15 and SZ7) with different tolerance to low-N were treated with nitrogen by hydroponics, the phenotypes were observed, physiological indexes were measured and transcripts were analyzed in this study. Compared to SZ7, KZ15 showed significantly higher capacity of low-N tolerance. The results showed that the change of KZ15 chlorophyll content was lower than that of SZ7 under low-N stress. The activity of nitrate reductase (NR) and glutamine synthetase (GS) had a smaller reduction in KZ15 than SZ7 under low-N stress. High-throughput sequencing revealed several common differentially expressed genes (DEGs) between KZ15 and SZ7, including nitrogen transport and nitrate assimilation genes. In the tolerant genotype KZ15, there were more transcripts related to high affinity nitrate transporters (NRT2.4, NRT3.1 and NRT4.5), suggesting an improved uptake efficiency of inorganic and organic forms of nitrogen. In addition, an abundance of certain transcription factors in the tolerant genotype KZ15 suggests that they may play roles in regulating the response to N-stress. This study will enable us to discover candidate genes potentially useful in improving the resistance to low nitrogen stress by genetic engineering technology and provide theoretical basis for the breeding of low nitrogen tolerant sorghum varieties.

Comparison of transcriptomes between N-stress tolerant and sensitive genotypes revealed several common DEG transcripts. Some of these DEGs were evaluated further by comparing the transcriptomes of genotypes grown under full N. The DEG transcripts showed higher expression in tolerant genotypes could be used for transgenic over-expression in sensitive genotypes of sorghum and related crops for increased tolerance to N-stress, which results in increased nitrogen use efficiency for sustainable agriculture (Gelli *et al.*, 2014).

Variation in the metabolic, physiological, and morphological responses of a sorghum association panel assembled to represent global genetic diversity to long term, nitrogen deficit stress and the relationship of these responses to grain yield under both conditions. Grain yield exhibits substantial genotype by environment interaction while many other morphological and physiological traits exhibited consistent responses to nitrogen stress across the population. Large scale nontargeted metabolic profiling for a subset of lines in both conditions identified a range of metabolic responses to long term nitrogen deficit stress. Several metabolites were associated with yield under high and low nitrogen conditions (Fig.4) (Grzybowski *et al.*, 2022).

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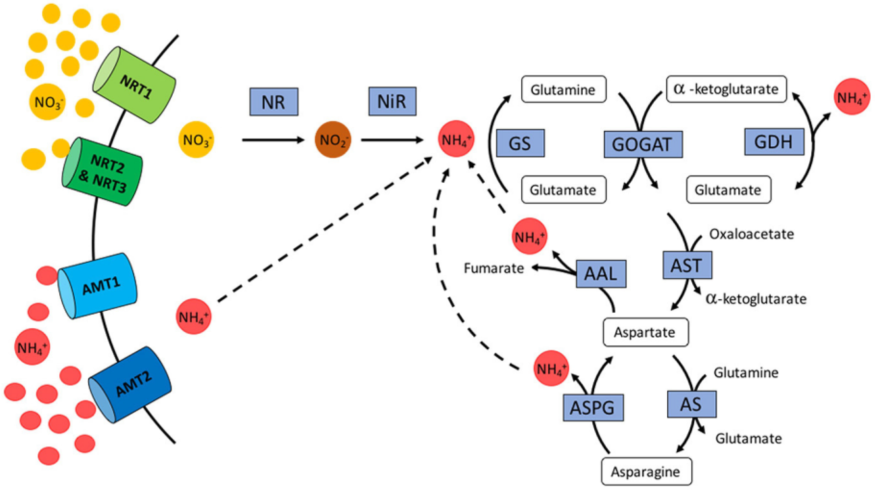

Gene Families	Number of genes
High-Affinity Ammonium Transporter (AMT1)	2
Low-Affinity Ammonium Transporter (AMT2)	7
Low-Affinity Nitrate Transporter (NRT1/PTR)	87
High-Affinity Nitrate Transporter (NRT2)	5
High-Affinity Nitrate Transporter (NRT3)	3
Nitrate Reductase (NR)	5
Nitrite Reductase (NiR)	30
Glutamine Synthetase (GS)	49
Glutamate Dehydrogenase (GDH)	3
Glutamine Oxoglutarate Aminotransferase (GOGAT)	15
Aspartate Aminotrasnferase (AST)	13
Asparagine Synthetase (AS)	4
Asparaginase (ASPG)	5
Aspartate Ammonia-Lyase (AAL)	2

Fig.4. Nitrogen transporters and metabolic genes in sorghum (after Bollam *et al.*, 2021).

To survive under N-stress, some genes involved in alleviating the detrimental effect of stress are abundantly expressed, which could facilitate tolerance to the stress. In this study, cell wall invertase-2 (CWINV2) transcript was massively increased in San Chi San and China17, indicating that sucrose degradation was increased in tolerant genotypes. A similar observation was made in the leaves of a water stress resistant cultivar of wheat (Saeed and Moradi, 2011). It is believed that the enhanced invertase expression in the roots of tolerant genotypes may contribute to the rapid cycling of sucrose, thus promoting carbon partitioning in favor of sucrose accumulation for counteracting the stress condition (Crawford *et al.*, 2002). In addition, the transcript of SEC14 cytosolic factor family protein was abundantly expressed in tolerant genotypes compared to CK60 and BTx623. It is also known as phosphatidylinositol /phosphatidylcholine transfer protein and is located in the Golgi membrane. There, it acts as a signal precursor and activates stress responsive genes, phospholipids and galactolipids (Liu *et al.*, 2013), which increase the membrane stability and provides stress tolerance (Larsson *et al.*, 2006). Gene transcripts responsible for numerous cellular activities, including protein biosynthesis, modification, and degradation enzymes were abundantly expressed in tolerant genotypes. Transcripts encoding ribosomal genes involved in protein biosynthesis, including structural constituent of ribosome L16p/L10 and translation elongation factors (EF1A) were also abundant in tolerant genotypes (Table 3).

Table 3. List of DEG transcripts abundant in tolerant genotypes (Bollam *et al.*, 2021).
Log (FC) = log2 (sensitive/tolerant genotype)

Gene annotation	Gene id	1/3 1/4 1/5 1/6	2/3 2/4 2/5 2/6	7/3 7/4 7/5 7/6
Ankyrin repeat	Sb07g002190	-7.5 -8.4 -7.7-6.9	** ** ** **	** -3.0 -2.2 **
LHT1	Sb01g038720	-7.0 -7.7 ** -6.7	-7.0 -7.6 ** -6.7	** ** ** **
SEC1	Sb05g023680	-7.8 -6.4 -7.7 **	-3.7 ** -3.6 **	** ** ** **
Drought induced 19 protein	Sb04g013790	-3.8 -4.6 ** -3.2	** -2.3 -3.1 -1.7	** -2.3 ** **
Translation elf-Tu	Sb02g007166	-4.7-4.6 ** -4.5	-4.1 -4.0 ** -3.9	-2.4 -2.3 ** **
BTB-POZ and MATH domain 1	Sb07g026735	-5.1 -5.6 ** **	-7.3 -7.8 ** -6.5	** -2.3 ** **
Ribosomal protein (L16p/L10e)	Sb01g036330	-2.7 -3.4 -2.3-2.0	** -1.7 ** **	** -1.4 ** **
Glutathione s-transferase	Sb09g003700	-2.1 -2.6 ** **	-2.4 -3.0 ** -1.3	-1.9 -2.5 ** **
Phosphatases	Sb08g019110	-2.1 -2.9 -2.1-2.0	-2.9 -3.6 -2.9-2.8	** -1.6 ** **
Lucine-rich receptor-like kinase	Sb04g003800	-2.7 -2.6 -2.7 **	-1.9 -1.8 -1.9 **	** ** -1.5 **
Phosphoglycerate mutase	Sb06g000380	-3.4 -3.3 ** -3.6	** -1.5 ** -1.7	** ** ** -1.3
Rm1C-like cupins	Sb01g019830	-3.5 -5.0 -3.2-3.2	** -2.1 ** **	** -2.0 ** **
Homeobox associated lucine zipper	Sb07g029150	-3.6 -2.7 ** **	-4.3 -3.4 -3.1-2.8	** ** ** **
Expressed protein	Sb08g019270	-3.7 -3.7 ** -3.0	-3.9 -4.0 ** -3.3	** ** ** **
Transducin	Sb04g022100	-3.8 -3.6 ** -3.3	-3.2 -2.9 ** -2.6	** ** ** **
Expressed protein	Sb04g000700	-3.9 -3.9 ** **	-6.9 -6.9 ** **	-3.5 -3.5 ** **
Trypsin family protein with PDZ domain	Sb08g015916	-4.3 ** ** -4.9	-4.2 ** ** -4.9	-4.2 ** ** -4.8
3-oxo-5-alpha streroid 4-dehydrogenase	Sb02g003510	-4.9 -4.5 ** -3.2	-3.2 -2.8 ** -1.6	-2.8 -2.4 ** -1.1
F-box domain containing protein	Sb02g001640	-5.4 ** ** -4.4	-5.3 ** ** -4.3	-3.5 ** ** -2.4
DNA binding transposon protein	Sb05g020750	-7.0 -7.4 ** -7.4	-7.0 -7.3 ** -7.3	** -2.6 ** **
Expressed protein	Sb04g000690	-7.7 -7.9 ** -7.0	-7.6 -7.9 ** -7.0	-5.5 -5.8 ** -4.9
Lucin eriched repeat family protein	Sb06g001645	-7.9 -7.6 ** -7.2	-7.9 -7.5 ** -7.1	-** ** ** **
Expressed protein	Sb04g012640	-8.3 ** ** -8.2	-6.0 ** ** -5.9	-3.9 ** ** -3.8
Cell wall invertase2	Sb0067s002240	-9.1 -7.4 ** -7.1	-6.7 -5.1 ** -4.8	-3.5 ** ** **

Hypothetical protein	Sb04g012541	-9.1 ** ** -9.3	-9.0 ** ** -9.3	-4.2 ** ** -4.5
Cupin domain containing protein	Sb07g005307	** -2.9 ** **	-7.7 -9.2 -8.0-7.9	** -2.7 ** **
UDP-Glycosyltransferase	Sb04g027470	** ** ** **	-6.2 -4.5 -5.2-4.3	-2.3 ** -1.2 **

The transcriptional abundance of DEGs from 12 pair-wise comparisons (1/3, 1/4, 1/5, 1/6, 2/3, 2/4, 2/5, 2/6, 7/3, 7/4, 7/5, and 7/6) made between three sensitive genotypes [CK60 (1), BTx623 (2) and the low-NUE bulk (7)] with each of the four tolerant genotypes [San Chi San (3), China17 (4), KS78 (5) and the high-NUE bulk (6)] were summarized. **Not significant when FDR ≤ 0.001; Log (FC) is the log2 ratio of gene transcript between sensitive and tolerant genotypes; If Log (FC) <0, negative values indicate transcript is abundant in tolerant genotypes.

Exploring the natural genetic variability and its exploitation for improved Nitrogen Use Efficiency (NUE) in sorghum is one of the primary goals in the modern crop improvement programs. The integrated strategies include high-throughput phenotyping, next generation sequencing (NGS)-based genotyping technologies, and a priori selected candidate gene studies that help understand the detailed physiological and molecular mechanisms underpinning this complex trait. A set of sixty diverse sorghum genotypes was evaluated for different vegetative, reproductive, and yield traits related to NUE in the field (under three N regimes) for two seasons. Significant variations for different yield and related traits under 0 and 50% N confirmed the availability of native genetic variability in sorghum under low N regimes. Sorghum genotypes with distinct genetic background had interestingly similar NUE associated traits. The Genotyping-By-Sequencing based SNPs (>89 K) were used to study the population structure, and phylogenetic groupings identified three distinct groups. The information of grain N and stalk N content of the individuals covered on the phylogenetic groups indicated randomness in the distribution for adaptation under variable N regimes. This study identified promising sorghum genotypes with consistent performance under varying environments, with buffer capacity for yield under low N conditions. It was also reported better performing genotypes for varied production use—grain, stover, and dual-purpose sorghum having differential adaptation response to NUE traits (Bollam *et al.*, 2021).

Under low N conditions, the July sampling revealed many root metabolites that were correlated with reduced biomass including several proteinogenic amino acids: serine, threonine, asparagine, valine, and phenylalanine. Oleamide, an amide derivative of oleic acid, was associated with higher biomass under full N for both sampling dates and under high N in September. However, Oleamide was negatively correlated with biomass in September under low N conditions. Higher biomass in the low N field was associated with root lactic acid, an end product of anaerobic respiration (Rivoal and Hanson, 1994), but only for the September sampling. Roots with reduced shikimic acid, a precursor of auxin (Fig.5).

Expression profiling of NUE associated genes in shoot and root tissues of contrasting lines (PVK801 and HDW703) grown in varying N conditions revealed interesting outcomes. Root tissues of contrasting lines exhibited differential expression profiles for transporter genes [ammonium transporter (SbAMT), nitrate transporters (SbNRT)]; primary assimilatory (glutamine synthetase (SbGS), glutamate synthase (SbGOGAT[NADH], SbGOGAT[Fd]), genes [nitrite reductase (SbNiR[NADH]3)]; and amino acid biosynthesis associated gene [glutamate dehydrogenase (SbGDH)]. Identification and expression variability for NUE in sorghum profiling of contrasting sorghum genotypes in varying N dosages will

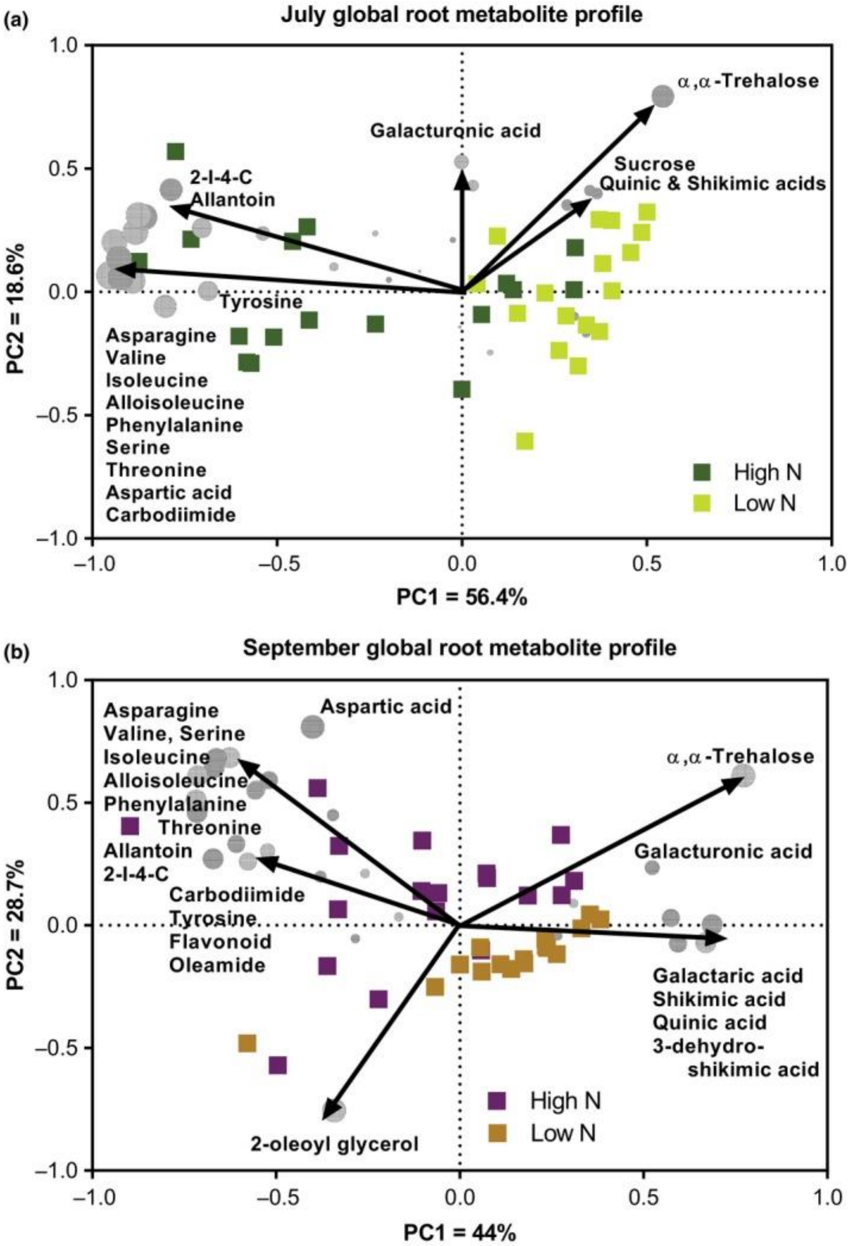

Fig.5. Principal component analyses of metabolites in sorghum under July(a) and September(b) samplings (after Rivoal and Hanson, 1994).

provide new information to understand the response of NUE genes toward adaptation to the differential N regimes in sorghum. High NUE genotypes identified from this study could be potential candidates for assimilatory in-depth molecular analysis and contribute toward the development of N efficient sorghum cultivars (Bollam *et al.*, 2021).

Abiotic Stresses

An open-top chamber experiment was conducted to investigate the growth and biochemical responses of six sorghum genotypes under two irrigation regimes (based on 40% and 75% soil-water depletion) and two N fertilizer levels (0 and 105 kg N ha^{-1}), at two atmospheric CO_2 conditions (390 ± 50 and 700 ± 50 μmol mol^{-1}). The results revealed that water limited stress decreased the plant dry weight by reducing the plant leaf area, SPAD value, Fv/Fm, leaf RWC and membrane stability index (MSI), while it increased the specific activity of APX , CAT and POX enzymes, DPPH, LPC, Phe, TSC, H2O2, MDA and EL. The lowest decrease of the plant dry weight due to limited water stress was observed in the GS5 genotype, which was followed by the lowest decrease in the leaf RWC and MSI; also, the highest increase was seen in APX, Phe and TSC, while the lowest one was recorded in EL. Elevated CO_2 improved DPPH and Phe under both control and water limited conditions, resulting in the decrease of APX, POX, H2O2 and EL, while a more pronounced effect was observed in the stress conditions. Furthermore, with the application of nitrogen, the reduction in DPPH, H2O2 and MDA values was greater under water limited stress rather than control conditions. It could be, therefore, concluded that the responses of sorghum genotypes to water deficit stress had interaction with atmospheric CO_2 concentrations and nitrogen levels; this could be considered in breeding programs as well as planting management of sorghum (Asadi and Eshghizadeh, 2021).

To evaluate and explicate the changes in the nitrogen compounds and enzyme activities in *Sorghum bicolor* plants (cv. BR-700) submitted to water deficit during vegetative, reproductive and maturation stages. The experimental design used was entirely randomized in factorial scheme, with two conditions (control and stress) combined with three stages (vegetative, reproductive and maturation). The nitrate level was increased at 180.6, 72.9 and 78.9% during the vegetative, reproductive and maturation stages, respectively. The total soluble amino acids and glycine betaine increased only during vegetative and reproductive stages, if compared with control plants. However, besides protein levels during all stages, significant reductions were reported in nitrate reductase and glutamine synthetase activities; free ammonium presented decreases at 37.3 and 77.6% in vegetative and reproductive stages, respectively, when compared with control plants (Fig.6) (Oliveira Neto *et al.*, 2009).

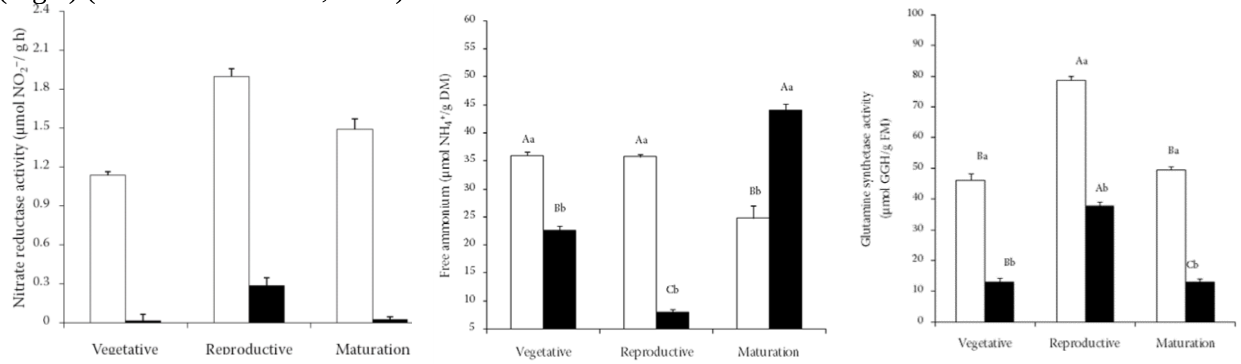

Fig.6. Variations of Glutamine synthetase, free ammonium and Nitrate reductase at different growth stages of sorghum under control and water stress (black) (Oliveira Neto *et al.*, 2009).

The objective of the present research was to study nitrogen metabolism in sorghum plants subjected to salt stress and silicon concentration. The experiment was conducted at the Amazon Federal Rural University, Capitão Poço Decentralized Unit for 1 month, in 2013, using the cultivar BR 700 of forage sorghum plants (*Sorghum bicolor* [Moench.]). The experimental design was completely randomized, in a 5 × 3 factorial arrangement (0, 50, 100, 150 and 200 µM of silicon) and saline concentrations (0, 1.5 and 2.0 M), consisting of 4 replications. Analyses were conducted of amino acids, proteins, free ammonium, nitrate and nitrate reductase. Nitrate content increased in the leaves and root in the treatments 0 and 1.5 roots, the treatments 1.5 and 2.0 of SC caused reduction and increase, respectively, of ammonium levels. The silicon doses attenuated the negative effects of the treatments on the biochemical compounds caused by higher salt concentrations in sorghum plants (Table 4 & Fig.7) (Souza *et al.*, 2016).

Table 4. Analysis of variance for nitrate, amino acid, ammonia, protein and NRA of sorghum leaf and root under salinity and silicon doses.

Source of variation	Degree of freedom	Leaf-Nitrate	Root-Nitrate
Silicon doses (Si)	4	**	**
Salt conc. (SC)	2	**	**
Si X SC	8	**	**
CV%	-	7.24	15.30
	Degree of freedom	Leaf-Amino acid	Root-Amino acid
Silicon doses (Si)	4	**	**
Salt conc. (SC)	2	**	**
Si X SC	8	**	**
CV%	-	0.44	0.07
	Degree of freedom	Leaf-Ammonium	Root-Ammonium
Silicon doses (Si)	4	**	**
Salt conc. (SC)	2	**	**
Si X SC	8	**	**
CV%	-	3.56	0.51
	Degree of freedom	Leaf-Protein	Root-Protein
Silicon doses (Si)	4	**	**
Salt conc. (SC)	2	**	**
Si X SC	8	**	**
CV%	-	2.52	5.36
	Degree of freedom	Leaf-NRA	Root-NRA
Silicon doses (Si)	4	**	**
Salt conc. (SC)	2	**	**
Si X SC	8	**	**
CV%	-	3.48	10.17

*CV = Coefficient of variation NRA = Nitrate reductase activity; ** = significant ($p \leq 0.01$).*

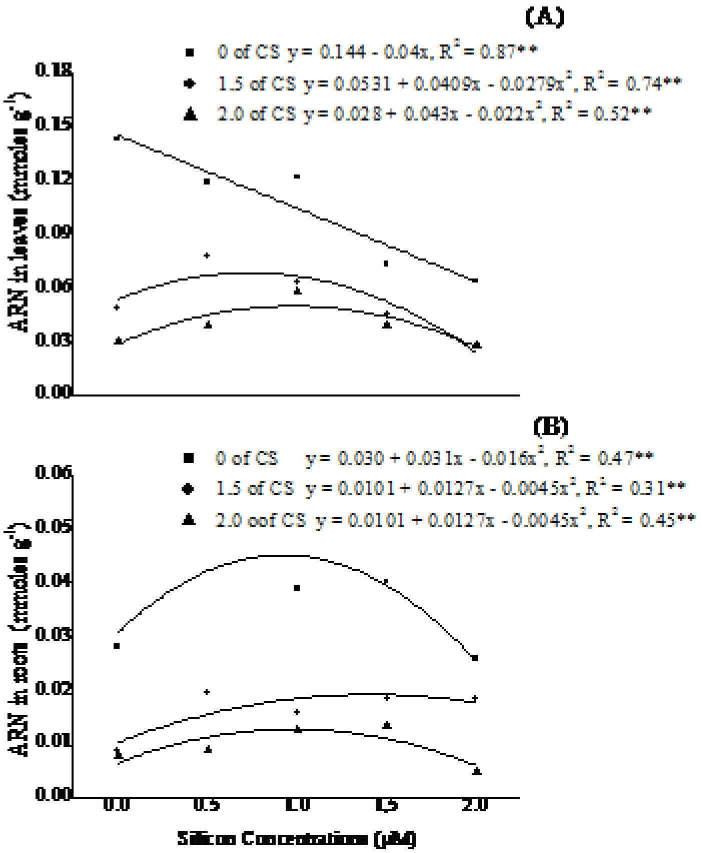

Fig.7. Changes in NRA in leaves(A) and roots(B) of sorghum under different concentrations of silicon at different growth phases.

Global gene expression and comparative analysis of genotypes contrasting for NUE allow the enrichment of candidate genes for NUE. Considerable literature is now available on transcriptomics, proteomics, as well as on quantitative trait loci (QTL) affecting N response and NUE (Sinha *et al.*, 2018). However, only limited studies explored the regulatory role of different classes of phosphatases in N responses and NUE (Waqas *et al.*, 2018; Sinha *et al.*, 2018; Xiong *et al.*, 2019).

Soil salinization is more aggravating than ever before, and techniques are needed to mitigate this problem; understanding salinity-fertilizer relationship is of considerable economic importance for promoting crop growth and productivity in saline soil. A pot experiment was done in a greenhouse to determine whether the N could alleviate the negative effect of different salts compositions on the growth, and physiological attributes of the two most used Sudan sorghum (*Sorghum bicolor* (L.) Moench) cultivars (Wadahmed and Tabat). The plant was subjected to the salt stress at 0, 0.3419 M NaCl, and 0.1408 M Na_2SO_4, and four levels of N application 0, 4, 8, and 12 g N kg^{-1} soil, designated as N0, N1, N2, and N3, respectively. Results showed that Na_2SO_4 was reduced plant height, leaf area index, number of leaves, dry weight (DW), chlorophyll a, total chlorophyll content, and increased soluble protein content, activities of peroxidase (POD) and catalase (CAT) activities by 16.4%, 29.7%, 61.5%, 28.7%, 39.6%, 12.7%, 99.7%, 34.3%, 51.8%, respectively, when compared with the non-saline treatment. The Na_2SO_4 salt had a more harmful effect than NaCl. This study revealed that N fertilizer was successful in alleviating the adverse impacts of both types of salt. In this study, 12 g N kg^{-1} soil was most effective on most of the measured parameters of two sorghum cultivars. These findings demonstrated that the N soil amendment application could alleviate the harmful impact of salinity. ‘Wadahmed’ was more tolerant to salinity stress than ‘Tabat’ during the boot stage of sorghum plant (Elsiddig *et al.,* 2023).

A set of 284 genes that were top predictors in the sorghum and maize-trained models, and also differentially expressed in both datasets. The majority of these genes showed increased expression during drought in sorghum dataset. Using gene ontology enrichment analysis, we found significant enrichment for well-characterized abiotic and biotic stress-responsive pathways as well as genes related to protein folding. It was found that these conserved drought-responsive genes were also significantly more likely to have shared differential expression (>50% of genotypes) as opposed to differentially expressed in only one sorghum genotype (Fisher’s exact P = 7.37e−29). Previous researchers have identified sets of shared differentially expressed genes related to drought responses in other species (Shaar-Moshe *et al.,* 2015). To test for overlap between conserved drought genes in maize and sorghum and across broader species, we used conserved Ortho groups to link gene identities between studies. It was identified orthologs for 282 of the 284 conserved drought-responsive genes reported in Shaar-Moshe *et al.,* and found 39 had shared drought responsiveness in maize and sorghum. This represents significant enrichment (Fisher’s exact test P = 2.49e−20); however, a substantial portion of the drought response genes between maize and sorghum are not shared with more distantly related species.

Genes with the highest predictive power in the sorghum and maize models are overwhelmingly involved in response to abiotic stresses. This include orthologs of the abscisic acid-mediated transcription factors drought-induced protein 19 (Di19-3; Sobic.003G443000) (Qin *et al.,* 2014) and abscisic acid-responsive element-binding factor 2 (AREB1; Sobic.004G309600) (Yoshida *et al*., 2015), which play central roles in the regulation of drought and high-salinity stress responses. Top predictors also include aquaporin orthologs to plasma membrane intrinsic proteins PIP 1 and 4 (Sobic.004G288700, Sobic.004G238100), which has been linked to various osmotic stresses in *Arabidopsis* (Jang *et al.,* 2007). The most abundant group of top predictors are reactive oxygen species scavengers such as L-ascorbate peroxidase (Sobic.001G410200, Sobic.006G084400, Sobic.006G204000), polyamine oxidase (Sobic.001G472000), thioredoxins (Sobic. 001G173500 Sobic.001G386200, Sobic.002G421600, Sobic. 008G117600), ferredoxin 3 (Sobic.001G022900), and oxidoreductase (Sobic.006G140700), as well as various heat shock proteins and a tandem array of orthologs to heat shock protein 22 (HSP22; Sobic.003G081900, Sobic.003G082000, Sobic.003G082100, Sobic.003G082200, Sobic.003G082300, Sobic.003G082500). HSP22s play essential roles in epigenetic memory to heat stress in *Arabidopsis* (Yamaguchi *et al.,* 2021) and may function in the regulation of osmotic stress. Late embryogenesis-abundant proteins (LEAs) protect cellular macromolecules during water deficit (Hand *et al.,* 2011), and orthologs of LEA2 (Sobic.001G017100) and dehydrins (Sobic.009G116700) are also included in this list of top predictors. These patterns suggest top predictors are involved in deeply conserved and central responses to water deficit.

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