Assesment of sorghum response to nitrogen availability

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Assessment of sorghum response to nitrogen availability

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Title : **Assessment of Sorghum response to Nitrogen availability**

Keywords: Sorghum, Nitrogen, Nitrate, Glutamine synthetase (GS), Metabolites, Intraspecific variability.

Abstract: Seven accessions of *Sorghum bicolor* were grown with low (N-) and optimal (N+) nitrate supply. Growth parameters (plant height and leaf numbers), physiological parameters (nitrate, protein, total N and total C contents) and the activity of glutamine synthetase (GS) were studied in leaves and roots of sorghum plants at three time points of early vegetative growth (2, 4 and, 6 weeks post emergence). Plant height and leaf number were higher with nitrate supply. Except for carbon, all studied parameters were sensitive to N availability and values were typically lower when nitrate supply was low. However, different genotypes displayed considerable variation in their response to N regimes. Variation among genotypes during early vegetative development was observed for plant height, but not for leaf number. Likewise, physiological parameters varied among accessions. A significant and strong correlation, N- and accession-dependent, was detected between plant height and nitrate content. Moreover, nitrate content and GS activity at early growth stages appeared to be good markers to discriminate between nitrate uptake and assimilation capacities of different accessions under both N conditions. In some sorghum accessions, protein and total N content were indicative of high nitrate reduction and assimilation even under N limitation. Chlorophyll content was also sensitive to N availability. Furthermore, expression studies of *SbNRT1.1*gene copies in leaves and roots of two accessions reflected variability in expression dependent on nitrogen condition, plant organ, plant age, and gene of interest. This study is helpful to characterize different aspects of the N metabolism in sorghum and may aid in the identification of sorghum genotypes with enhanced nitrogen use efficiency, a trait that is of key interest in one of the most important crop plants in arid and semi-arid regions.

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1 Introduction

1.1 Sorghum bicolor: Introduction

Sorghum (*Sorghum bicolor* (L.) Moench; 2n=20) is an important cereal crop. The Food and Agricultural Organization of the United Nations (FAO) ranks it as the 5th most important cereal crop worldwide, after wheat, rice, maize, and barley (De Wet, 1978; Wiersema and Dahlberg, 2007; Paterson, 2008; Dahlberg *et al*., 2011). Sorghum is cultivated in a broad range of environments. Its capacity to adapt to drought and salinity makes it one of the most important crops in dry regions (Paterson, 2008 ; 2009 ; Morris *et al*., 2013). Commercially, cultivated sorghums represent different agronomic types, including grain sorghum, forage sorghum, sweet sorghum and broomcorns (Berenji and Dahlberg, 2004 ; Singh and Lohithaswa, 2006 ; Berenji *et al*., 2011).

Sorghum is used for a variety of purposes (Dahlberg *et al*, 2011 ; Kimber *et al*., 2013). It is a staple food for millions of people in the semi-arid tropics. From a dietary perspective, it can substitute other cereals because it does not contain gluten but has otherwise the same nutritional value as maize grains (Table 1) (Sheorain *et al*., 2000 ; Singh and Lohithaswa, 2006 ; Dahlberg *et al*., 2011; Dial, 2012 ; Jacob *et al*., 2013 ; Morris *et al*., 2013). In India and Africa, sorghum is used for brewing beer, and in the United States for syrup production (Dahlberg *et al*., 2011). In addition, sorghum can be used as fodder and animal feed, as building material, or for the production of brooms (Dogget, 1988 ; Dahlberg *et al*., 2011 ; Hariprasanna and Patil, 2015). It is also of interest for plant biofuel production. After maize, it is the 2nd most important source of grain-based ethanol in the United States due to its high biomass yield and sugar content (Dahlberg *et al*., 2011; Jacob *et al*., 2013).

Table 1. Comparison of sorghum and maize grain contents (Sheorain *et al*., 2000 ; Jacob *et al*., 2013)

Component	Content %	
	Sorghum	Maize
Starch	63-68	60-64
Moisture	9-13	8-11
Proteins	9-11	9-11
Fats and oils	1-1.5	3-5
Crude fiber	1.5-2	1.5-2
Ash	1-2	1-2
Other organics	8-12	7-9

1.1.1 Morphology and development

Sorghum is an annual grass similar in appearance to maize in its vegetative stage. Mature *Sorghum bicolor* usually has a height between 1.5 - 2.1m but can sometimes reach 5 m. Its stem is erect and solid. It produces one or several tillers, depending on genotype and growth conditions. Tillers emerge initially from the base of the main stem, and later from stem nodes (Singh and Lohithaswa, 2006). The roots are adventitious with primary and secondary roots. Waxy leaves make the plant more tolerant to drought than most of other cereals (Kimber *et al*., 2013; Hariprasanna and Patil, 2015).

The first leaf (Figure1a) that appears at emergence is, as in all cereals, the coleoptile. This leaf differs from all true leaves in that it has a rounded leaf tip. The last leaf to emerge is the flag leaf which is considerably smaller than the other true leaves. The number of leaves can vary from 7 to 24 depending on the genotype (Singh and Lohithaswa, 2006). The inflorescence, called head or panicle, emerges from the flag leaf sheath and is supported by the portion of the stalk that is called the peduncle (Besancon *et al*., 2005; Hariprasanna and Patil, 2015). The panicle can be open or compact or between the two depending on the cultivar (De Wet., 1978).

The panicle has a central axis called the rachis where the primary branches develop. The primary branches give rise to secondary branches which, sometimes, give rise to tertiary branches. Primary, secondary and tertiary branches each can bear a group of spikelets (Figure 2a). The spikelets occur in pairs at each node (Burrow *et al*., 2014). One spikelet is always sessile, *i.e.*, directly attached to the branch, whereas the other is pedicelate, *i.e.*, attached to the branch by a pedicel. However, the terminal sessile spikelet at the end of each branch is accompanied by two pedicelate spikelets.

The sessile spikelet is fertile and contains one floret protected by two glumes. The florets consists of a central gynoecium with one ovary from which two stigmas protrude. Three anthers are attached to the base of the gynoecium by long threads like filaments (Hariprasanna and Patil, 2015). Gynoecium and anthers are enclosed in fibrous sheaths called lemmas (the upper may be awned) and a small palea. Two lodicules are found at each side of the ovary base. Each floret contains one ovule which will give rise to a seed enclosed in the two glumes after fertilization. The seeds can vary in shape, size and colour among sorghum cultivars. The seed is rounded, from 4-8mm in diameter, with white, yellow, dark brown, or reddish brown colour (Singh and Lohithaswa, 2006; Burrow *et al*., 2014; Hariprasanna and Patil, 2015).

The pedicellate spikelet never gives rise to a seed but can sometimes posses anthers (Singh and Lohithaswa, 2006; Burrow *et al*., 2014; Hariprasanna and Patil, 2015). The pedicellate spikelets can be persistent or deciduous depending on races or cultivars (Hariprasanna and Patil, 2015).

Sorghum is a short day and photosensitive plant (Kimber *et al*., 2013). It is considered mostly self-pollinating, but outcrossing can be high in certain races (see below) and environments. In particular, panicle type, wind direction and velocity play important roles for outcrossing (Kimber *et al*., 2013; Hariprasanna and Patil, 2015).

Most sorghum races mature within 60-180 days (Kimber *et al*., 2013) but some varieties may require up to 300 days (Clerget *et al*., 2007). Cultivars that flower within 50 to 60 days after sowing are considered early flowering, while those that flower after 60 days are considered late flowering. Some late flowering cultivars may flower after 180 days. The duration between flag leaf emergence and heading (panicle emergence) does not vary between early and late flowering cultivars. Lateness and photoperiod sensitivity are strongly and positively correlated: All late-maturing sorghums are photoperiod-sensitive, with the degree of lateness increasing with photoperiod sensitivity (Clerget *et al*., 2007).

1.1.2 Life cycle of sorghum

The life cycle of sorghum is divided in three growth stages, the vegetative stage, the panicle initiation, and the grain filling stage (Gerik *et al*., 2003; Kelley, 2003).

1 The vegetative stage: GS1

This stage comprises germination, seedling development, emergence of leaves that will support the growth and grain (kernel) formation at late stages. The duration of this stage usually depends on genotype and temperature. Typically, late flowering plants tend to form more leaves at this stage (Clerget *et al*., 2007).

2 Panicle intiaition: GS2

It is characterized by the reproductive structures development. Maximum numbers of seeds per plant are set at this stage and accounts for 70% of sorghum final grain yield. The flag leaf has already emerged and enclosed the panicle which is nearly completely developed. This phase is called booting. Then, the panicle peduncle elongates rapidly leading to its exertion from the flag leaf sheath and it became visible. This is called heading.

3 Grain filling stage: GS3

At this stage, plant development centers on grain formation. This stage starts when mature anthers (yellow) appear at the tip of the head, usually five to seven days after head exertion. Dry matter accumulates in the grains and the plant reaches maturity. Seed development progresses in different phases, including milk phase, soft dough phase, hard dough phase, and, finally, physiological maturity. The period during which these processes take place can vary from 25 to 45 days after flowering, depending on genotype and growth conditions.

In the milk phase, the kernel reaches its maximum size; it is very soft and can be easily squeezed. The soft dough phase occurs approximately 15 to 25 days after flowering when 50 % of the grain weight has been accumulated; the kernel can still be squeezed between the fingers but little to no fluid appears. In the hard dough phase, when approximately 75 % of the final grain weight has accumulated, the grain can no longer be squeezed between the fingers. The phase of physiological maturity begins when a black layer appears near the base of the kernel, which still contains 30 to 35 % water. The kernels can be harvested when they contain less than 20 % moisture. However, for safe storage this value should be less than 14 % when no equipment for grain drying is available.

The three growth stages described above can be further subdivided into 10 sub-stages, numbered from zero (0) to nine (9) as shown below (Figure 3; Table 2), to more finely describe sorghum growth from plant emergence until physiological maturity (Vanderlip, 1993; Besancon *et al*., 2005).

Table 2. Characteristics of sorghum growth, subdivided into 10 sub-stages (Vanderlip, 1993; Besancon *et al*., 2005).

Growth Stage		Characteristics that identify the stage	
GS1	0	**Emergence**	The first leaf is visible at soil surface, 3-10 days after sowing
	1	**3-leaf stage**	The collar of 3 leaves can be seen, approximately 10 days after emergence
	2	**5-leaf stage**	The collar of 5 leaves can be seen; about 3 weeks after emergence
GS2	3	**Growing point differentiation**	The growth of sorghum changed from vegetative to reproductive: Panicle initiation
	4	**Flag leaf visible**	Flag leaf emerges
	5	**Boot stage**	All leaves are fully expanded. The head has developed to nearly full size and is enclosed in the flag-leaf sheath.
GS3	6	**Half bloom**	When half of the plants in a field have started to bloom. It usually represents two-thirds of the time from planting to physiological maturity
	7	**Soft dough**	Half of grain dry weight is accumulated during this period
	8	**Hard dough**	About 75% of the grain dry weight has accumulated
	9	**Physiological maturity**	Maximum total dry weight of the plant has been reached. Physiological maturity can be determined by the dark spot on the opposite side of the kernel from the embryo

1.1.3 Classification and Taxonomy

Sorghum belongs to the genus *Sorghum* in the *Poaceae* family (Clayton and Renvoize, 1986). Snowden (1936) used morphological characters, mainly of the spikelets, for classification. He divided the genus into the sections *Eu-sorghum* and *Para-sorghum* (Figure 5). He further sub-divided *Eu-sorghum* into two sub-sections: *Arundinacea* and *Halapensia. Arundinacea* contained the *Spontanea* series, which included the wild species or races, and the *Sativa* series which comprised the cultivated species (Snowden, 1936; Snowden, 1955). Altogether Snowden (1936) distinguished 31 cultivated species and 17 related wild species in the *Arundinacea*.

This classificaton later was refined based on additional morphological characters, considering in particular primary branch, node and spikelet morphologies (Garber, 1950; Celarier, 1959), resulting in a division of the genus *Sorghum* into *Chaetosorghum*, *Heterosorghum*, *Parasorghum*, *Stiposorghum* and *Sorghum* sections or sub-genera (De Wet and Harlan, 1970; De Wet, 1978). In the sub-genus *Sorghum* three species were recognized: two rhizomatous taxa, *S. halpense* and *S. propinquum*, and a third, *S. bicolor*, which included all annual cultivated, wild and weedy sorghums (De Wet, 1978; Dahlberg *et al*., 2011; Singh and Lohithaswa, 2006; Hariprasanna and Patil, 2015).

Subsequently, *S. bicolor* ssp. *bicolor*, *S. bicolor* ssp. *drummondii*, and *S. bicolor* ssp. *arundinaceum* were described as three subspecies of *Sorghum bicolor* (De Wet and Huckabay, 1967; De Wet, 1978). *S. bicolor* ssp. *arundinaceum* was later renamed *S. bicolor* ssp. *verticilliforum* (De Wet, 1978; Wiersema and Dahlberg, 2007; Dahlberg *et al*., 2011; Kimber *et al*., 2013).

Nowadays, all cultivated sorghum varieties belongs to *S.bicolor* ssp *bicolor*. Based on spikelet and panicle morphology, these varieties are divided into five basic races: *bicolor*, *caudatum*, *durra*, *guinae* and *kafir* (Figure 1), and ten hybrid races that exhibit the characteristics of at least two of these races (De Wet, 1978; Kimber *et al*., 2013; Dillon *et al*., 2007; Hariprasanna and Patil, 2015).

1 Bicolor

The race *bicolor* has an open and medium sized panicle. It has a long clasping and thick glumes. The glumes enclose totally the elliptic grain at maturity. The pedicellate spikelets are persistant and the pedicels are short. *Bicolor* has elongated and generally small sized seeds and is generally low yielding (De Wet, 1978; Mann *et al*., 1983; Hariprasanna and Patil, 2015).

2 Caudatum

The panicle range from compact to open. The glumes are shorter than the grains. It has obovate to elleptical sessile spikelets while pedicellate spikelets are deciduous. The grains are large, turtle-like, asymmetrical, flat on one side and curved on the oppsite side, chalky white or pigmented. This race is generally high yielding (De Wet, 1978; Mann *et al*., 1983; Hariprasanna and Patil, 2015).

3 Durra

The panicle is dense and compact. The sessile spikelet are flattened and ovate and the pedicellate spikelets are large and persistent. The texture of the tip of glumes is different from that of their base. It is with transverse crease until the middle and coriaceous. Durra sorghums have rounded white seeds. This race is generally high yielding (De Wet, 1978; Mann *et al*., 1983; Hariprasanna and Patil, 2015).

4 Guinea

The race *guinea* has a large and open panicle. The glumes are long. The grains have small to medium size, are shorter than the glumes, and are whitish. The pedicellate spikelets are both persistent and deciduous. The sessile spikelet opens at maturity and exposes the seed. The *guinea* race tends to be low yielding (De Wet, 1978; Mann *et al*., 1983; Hariprasanna and Patil, 2015).

5 Kafir

Kafir sorghums have a more or less compact panicle that is often cylindrical and elongated. Glumes are much shorter than the grains. The sessile spikelets tend to be hairy. The grains are symmetrical, and more or less spherical. This race is generally high yielding (De Wet, 1978; Kimber *et al*., 2013; Hariprasanna and Patil, 2015).

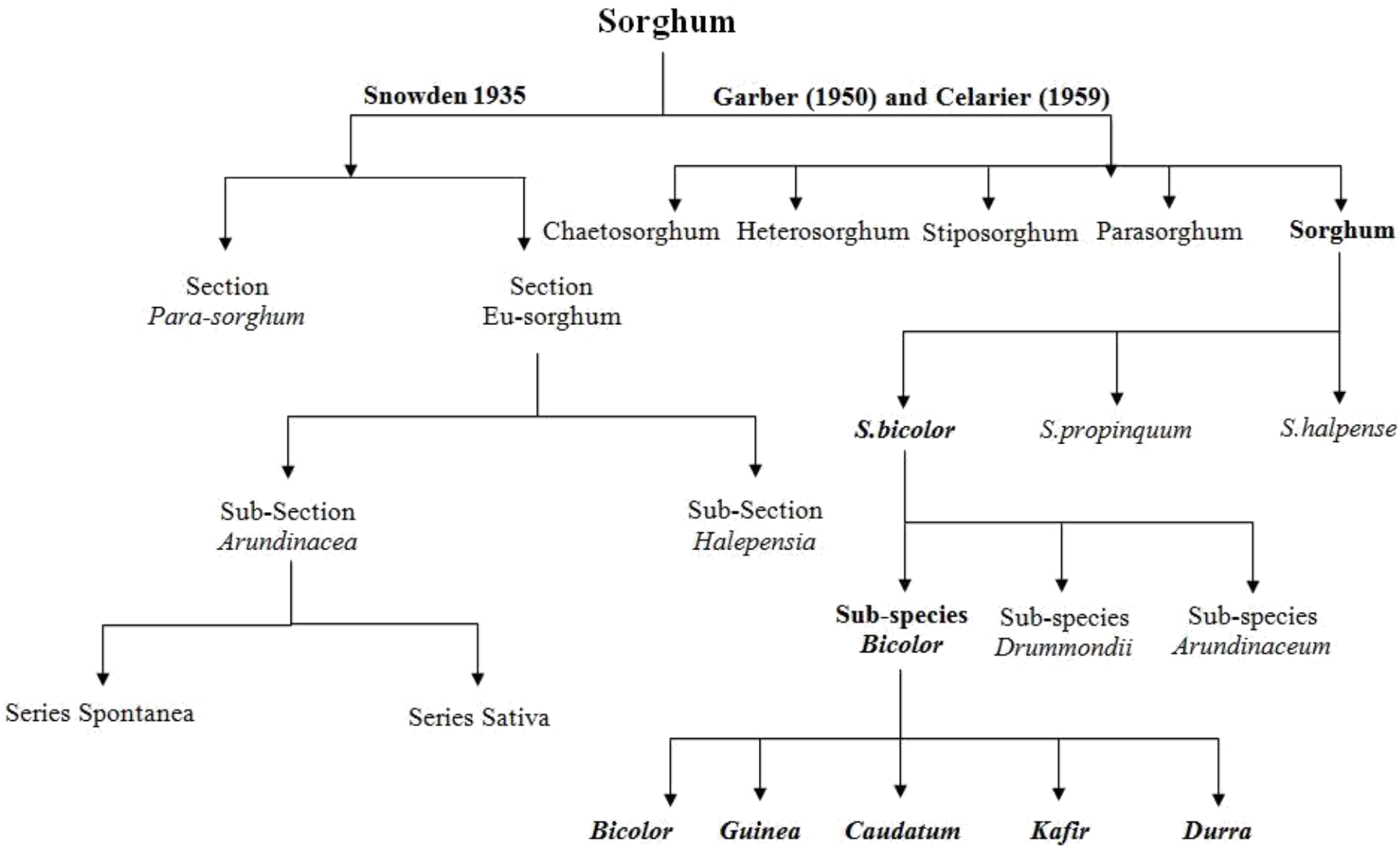

Figure 1. Classification of sorghum (Inspired from Hariprasanna and Patil, 2015)

1.1.4 Origin and domestication of sorghum

Sorghum is an ancient crop originating from Africa where actually the largest diversity of wild and cultivated sorghum is found (Kimber *et al*., 2013). The early domestication of sorghum has taken place about 5000-8000 years ago in the north east of Africa, in an area that corresponds to the border region between Egypt and Sudan (Mann *et al*., 1983; Wendorf *et al*.,1992). Carbonized grains of sorghum have been excavated at an early Holocene archeological site at Nabta Playa near the Egyptian-Sudanese border (Wendorf *et al*., 1992; Dahlberg and Wasylikowa, 1996; Jacob *et al*., 2013; Hariprasanna and Patil, 2015). Sorghum probably dispersed with the migration of people across the Sahel-Sudan grasslands and southward from the Nile Valley region along the Great Rift. This hypothesis is supported by the distribution pattern of wild and cultivated sorghums in Africa (Murdock, 1959; De Wet and Harlan, 1970; Kimber *et al*., 2013).

Afterwards, more than 5000 years ago, sorghum spread from east Africa to India by semitic speakers who carried their crops with them (Figure 2). Sorghum reached Indonesia and China from India around 4000 years ago (Singh and Lohithaswa, 2006; Kimber *et al*., 2013). Around 3000 years ago sorghum arrived in the Middle East, and around1500 years ago in the Far East, as a consequence of migrating people and trade (Figure 5) (Dillon *et al*., 2007). Finally, in the mid 19th century, African slaves carrying sorghum seeds with them introduced this crop in the US, which is nowadays the largest sorghum-growing country (Singh and Lohithaswa, 2006; Hariprasanna and Patil, 2015).

As in all cereals, human selected voluntary or involuntary specific morphological and physiological traits during sorghum domestication. These traits include a nonshattering panicle, large inflorescences and seeds, high yield, easy threshing, suitable height [for easier harvesting] and a short period of seed dormancy (Mann *et al*., 1983; Singh and Lohithaswa, 2006). These characteristics were better suitable in cultivation for human needs. After initial domestication, in the course of sorghum dispersion to different regions, additional selection led to adaptation to new environments and brought about new characteristics. As a result, a huge phenotypic and physiological diversity arose which was the basis for the generation of numerous landraces. These landraces are nowadays a valuable genetic resource for sorghum improvement through modern selection (Singh and Lohithaswa, 2006; Dahlberg *et al*., 2011; Kimber *et al*., 2013; Morris *et al*., 2013).

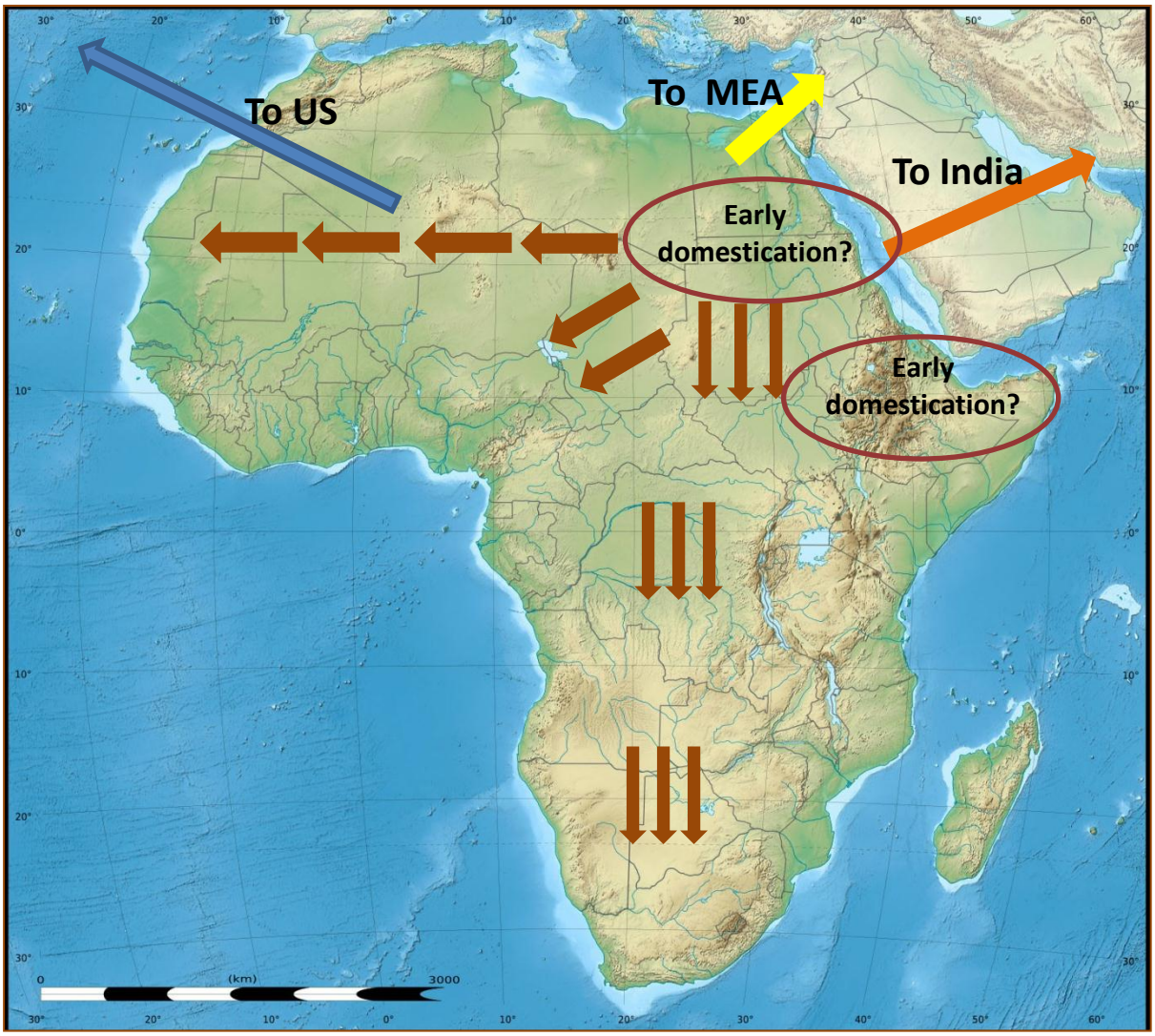

Figure 2. Origin and suggested movements of the domesticated races of *Sorghum bicolor* (Inspired from Kimber *et al*., 2013)

1.1.5 Current distribution of *Sorghum bicolor*

Sorghum bicolor ssp. *bicolor* is widely distributed around the world. Cultivated races of sorghum are dominant in Africa. Likewise, wild sorghum sub-species are also common on the African continent. *S. bicolor* ssp. *verticilliforum*, formerly known as *S. bicolor* ssp. *arundinaceum*, includes the varieties *arundinaceum*, *virgatum*, *aethiopicum*, and *verticilliforum*. The variety *arundinaceum* is present in tropical forests of central and West Africa in environments which are not suitable for cultivated sorghum races. The race *virgatum* occurs in the parts of the Nile valley that have seasonal flooding, as well as in some parts of Sudan. *Aethiopicum* is a desert grass, it occurs in the Kassala region of the Sudan and in Ethiopia. The widest distributed race is *verticilliforum;* it is present in the savannas of east and south Africa (De Wet and Harlan, 1970; De Wet, 1978; Kimber *et al*., 2013).

The current distribution pattern of cultivated sorghum races is partly explained by their biological traits and partly by their historical dispersion across the continents (Figure 2)(Kimber *et al*., 2013; Hariprasanna and Patil, 2015). The race *bicolor* is grown across the whole range of sorghum cultivation areas in Africa and Asia but is rarely an important crop. Instead, *bicolor* sorghums are mainly grown for their sweet stem and bitter grains that are used to flavor sorghum beer. The distribution of the race *caudatum* in Africa is closely associated with the settlement areas of people speaking the Chari-Nile language. It is adapted to arid regions which receive between 250 to 1300 mm of rainfall per year. The race *durra* is present in the mid altitude highlands of Ethiopia, the Nile valley of Sudan and Egypt, Pakistan and parts of India (De Wet, 1978; Mann *et al*., 1983; Kimber *et al*., 2013).

Guinea is basically known as a west African race, but it is also present in the mountains of eastern Africa, in areas with high rainfall. This race is preferred in humid regions because it is not strongly affected by grain mold and insect damage, which are brought about by long rainy seasons in west Africa (Stemler *et al*., 1977; De Wet, 1978; Mann *et al*., 1983; Morris *et al*., 2013; Kimber *et al*., 2013).

Kafir sorghums are important staple crops. This race is associated with Bantu-speaking people who live in the eastern and southern savannas from Tanzania to South Africa. In these regions, which are characterized by short rainy periods, the dense panicle of *kafir* and that of *durra* are preferred by farmers because of their high grain yield under these conditions (De Wet, 1978; Mann *et al*., 1983; Kimber *et al*., 2013; Morris *et al*., 2013).

1.1.6 Genomics of Sorghum

Genomic analysis of crops can play an important role in supporting sustainable agriculture all over the world, in particular in Africa, Asia, and in semi-arid regions (Morris *et al*., 2013). *Sorghum bicolor* is a diploid species (2n=20) (Singh and Lohithaswa, 2006). It is a C4 plant, *i.e.*, it possesses specific biochemical and morphological characteristics that allow high carbon assimilation at high temperatures (Edwards et al., 2004). It is the first African crop for which the entire genome sequence is available. The genome of *Sorghum bicolor* is one of the smallest among the grasses; it encompasses ca. ~730 Mb. This size corresponds to about 25% of the maize or sugarcane genomes (Paterson *et al*., 2009). As a consequence, sorghum has become an attractive species for genetic research and for comparative studies among cereals. For example, to facilitate the identification of genes of agronomic interest for sorghum improvement, Morris *et al*. (2013) have characterized ~265,487 single nucleotide

polymorphisms (SNPs) in a panel of 971 sorghum accessions chosen from throughout the world and adapted to diverse agro-climatic conditions. The authors performed a genome-wide association study (GWAS) to identify genes underlying natural variation in agro-climatic traits, with specific focus on plant height and inflorescence architecture. The results obtained by this study by scanning sorghum genome using GWAS identified three major QTLs for plant height (Morris et al., 2013). These QTLs have distinct allelic distributions among sorghum accessions in Asia and Africa thus revealing plant height differences between accessions.

1.2 Nitrogen Metabolism

Nitrogen (N) is quantitatively the most important nutrient for plants. It is a major limiting factor in plant growth, crop productivity and yield (Hirel *et al*., 2007; Lea and Azvedo, 2007). N is taken up by plants from the soil in differrent forms, either directly by the plant roots or by mycorrhizae associated with the roots (Andrews *et al*., 2013). Nitrate (NO_3^-) is the major N source available to plants in the soil (Cawford, 1995), but ammonium (NH_4+) can also be important, in particular in undisturbed and unfertilized soils. Some plants such as legumes can take their N by making symbiosis with microorganisms (as *rhizobia*) that fix atmospheric nitrogen. This provides an advantage when plants are growing in soil with low N content (Franche *et al*., 2009; Raven and Andrews, 2010; Andrews *et al*., 2013). The N cycle in plants involves many processes: uptake, assimilation, translocation and, in particular in aging plants, recycling and remobilization.

Two different nitrate uptake systems play key roles in N uptake and transport throughout the plants. A high affinity transport system (HATS), is responsible for nitrate uptake when the external nitrate concentration is low (below ≤ 1mM). A low affinity transport system (LATS) is more important at nitrate concentrations > 1mM (Forde, 2000). Two HATS have been described. In addition to a constitutive system, cHATS, there is an inducible system, iHATS, which becomes functional only after exposure to nitrate (Aslam *et al*., 1993; Forde, 2000; Glass, 2003).

Two classes of genes, *NRT1* and *NRT2*, have been found to be involved in LATS and HATS respectively. In *Arabidopsis thaliana*, 53 *NRT1* and seven *NRT2* members have been identified (Orsel *et al*., 2002; Tsay *et al*., 2007). *AtNRT1.1* was the first gene to have been isolated and extensively studied for its dual affinity. Its gene product has a dual function; it serves as nitrate transporter and also as nitrate sensor to activate the expression of nitrate-

related genes (Forde, 2000; Masclaux-Daubresse *et al*., 2010; Bai *et al*., 2013). Grasses have multiple closely related co-orthologous to *AtNRT1.1*. Four copies were identified in maize (*ZmNRT1.1A*, *ZmNRT1.1B*, *ZmNRT1.1C*, *ZmNRT1.1D*) (Plett *et al*., 2010) three in rice (*OsNRT1.1A, OsNRT1.1B and OsNRT1.1C*), and in sorghum (*SbNRT1.1A, SbNRT1.1B* and *SbNRT1.1C*) (Plett *et al*., 2010). Moreover, the results done on *NRT2* gene family showed the presence of two *NRT2* genes in rice (*OsNRT2.1* and *OsNRT2.2),* three in maize (*ZmNRT2.1*, *ZmNRT2.2*, and *ZmNRT2.3),* and sorghum (*SbNRT2.1*, *SbNRT2.2, and SbNRT2.3*) (Plett *et al*., 2010).

Following uptake by the roots, nitrate can be stored or reduced to nitrite by nitrate reductase (NR) (Figure 3). The resulting nitrite is then reduced to ammonium under the action of nitrite reductase (NiR) (Figure 3). NR is the first enzyme in the nitrate assimilation pathway. Thus, the reduction of nitrate by NR can be a limiting step (Srivastava, 1980). Nitrate and nitrite reduction can take place in roots or in shoots depending on the genotype and on environmental conditions, especially nitrate concentrations in soils (Figure 3) (Lea and Azevdo, 2007; Masclaux-Daubresse *et al*., 2010; Andrews *et al*., 2013).

Ammonium, the second N source for plants, can be taken up from soil, produced by reduction of nitrite, or from photorespiration of amino acid recycling (Figure 3) (Andrews *et al*., 2013). It is assimilated into amino acids via the glutamine synthetase (GS)/ glutamate synthase (GOGAT) pathway (Andrews *et al*., 2013). GS catalyses an ATP-dependent reaction that fixes ammonia (NH_3) to glutamate, forming glutamine (Figure 6). Plants possess two major isoforms of GS: GS1 and GS2 (Cren and Hirel, 1999). GS1 is located in the cytosol and present in a variety of organs and tissues such as roots, leaves, and phloem cells. GS2 is located in the chloroplasts of photosynthetic tissues and the plastids of the roots. The two isoforms can occur in different ratios depending on organ and species (Martin *et al*., 2006; El-Omari *et al*., 2010; Hirel *et al*., 2011; Andrews *et al*., 2013). GS1 is involved in the assimilation of ammonium taken up from the soil and plays a key role in N recycling and remobilization, while GS2 is responsible for assimilation of ammonium derived from nitrate reduction and photrespiration (Tabuchi *et al*., 2007; El-Omari *et al*., 2010).

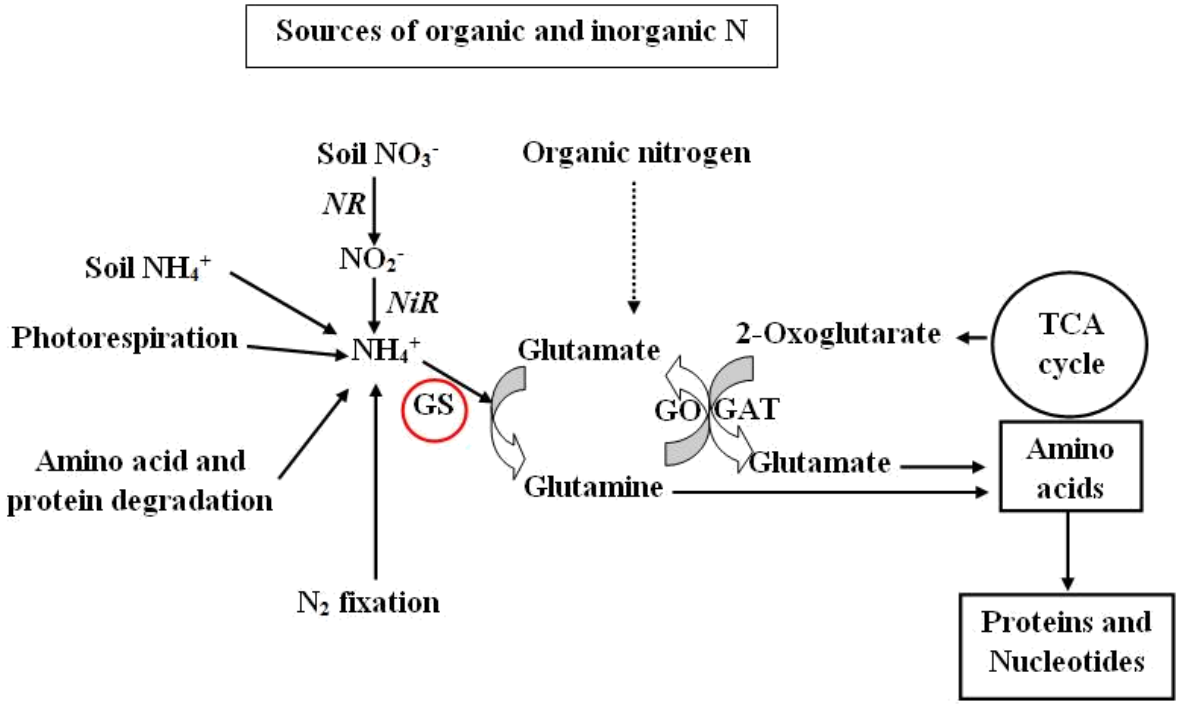

Figure 3. Main reactions of nitrogen assimilation in plants. Modified from Hirel *et al.*2011.

GOGAT (glutamine 2-oxoglutarate amino transferase) catalyses the conversion of glutamine and 2-oxoglutarate to two molecules of glutamate (Figure 3) (Cren and Hirel, 1999; Forde and Lea, 2007 ; Masclaux-Daubresse *et al*., 2010). GOGAT has also two isoforms: a ferredoxin-dependent form that is, in conjunction with GS2, involved in the assimilation of ammonium derived from nitrate reduction and photorespiration. The second isoform is pyridine nucleotide-dependent, and is involved in the synthesis of glutamate in both photosynthetic and non-photosynthetic tissues (Hirel and Lea, 2001; El-Omari *et al*., 2010; Hirel *et al*., 2011; El-Omari and Nihiri., 2015). Glutamine and glutamate are the main donors for amino groups to all N-containing molecules including other amino acids, proteins, and nucleotides for RNA and DNA synthesis (Hirel *et al*., 2011). Thus, GS/GOGAT is of key importance for N assimilation in plants. All the N in a plant, without regard of its source, is channeled through the reaction catalyzed by GS/GOGAT which is, hence, considered as a checkpoint for plant growth and productivity (Hirel *et al*., 2005b; Tabuchi *et al*., 2005; Martin *et al*., 2006).

During senescence of annual plants, leaf proteins and particularly photosynthetic proteins are degraded to serve as a source of carbon, nitrogen and other elements for nutrition of late growing organs, in particular the seeds. All organs that absorb and assimilate inorganic

nitrogen during vegetative growth start to operate as a N source at late developmental satges, a phenomenon called N remobilization (Hirel *et al*., 2001; 2007; Masclaux-Daubresse *et al*, 2010). This phenomenon is important to route organic N to seeds during grain filling in cereals because N uptake and assimilation are typically insufficient to meet the high demand for this element in developing seeds (Masclaux-Daubresse *et al*, 2010).

1.3 Nitrogen Use Effieciency

To ensure high crop yield synthetic fertilizers are frequently used. The main ingredient of these fertilizers is nitrate, since the availability of this nutrient is usually limited in soil (Andrews *et al*., 2013). However, excess input of mineral fertilizers has a negative impact on the environment, in particular soil and water pollution. As an anion, nitrate is easily washed out of the soil, into deeper layers of the soil and into the groundwater, or into streams, rivers and lakes. Leaching can cause a loss of 50% to 70% of N input (Raun and Johnson, 1999). Furthermore, the use of synthetic fertilizers can lead to the emission of nitrous oxide (N_2O) by soil bacteria that use nitrate as a substrate. N_2O is a more powerful green house gas than carbon dioxide (CO_2) (Galloway *et al*., 2003; Butterbach-Bahi *et al*., 2013).

From both an ecological and an economic point of view, and in light of a growing world population, the development of productive crop varieties that require low nitrogen input is of high priority. Highly productive crops are of great importance to meet the high demand of the growing world population. Thus, it is necessary to understand how nitrogen can be efficiently used in crops (Canas *et al*., 2009; Gupta *et al*., 2012).

In general Nitrogen Use Efficiency (NUE) is defined as grain production per unit of N available in the soil (Moll *et al*., 1982; Hirel *et al*., 2001; Gallais and Hirel, 2004). Two factors contribute to NUE : (i) Nitrogen uptake efficiency, which is the ability of a plant to take up N from the soil in form of nitrate and/or ammonium, and (ii) Nitrogen utilization efficiency, which is the ability to use acquired N for grain production (Good *et al*., 2004). The components of NUE are species-and genotype-specific, but can also be influenced by genotype-environment interactions (Hirel *et al*., 2007).

Different approaches have been used to investigate the basis of NUE in crops at an agronomic, metabolic, biochemical, molecular or genetic level. Most research has been carried out in maize (Hirel *et al*., 2001; 2005a; 2005b; Gallais and Hirel, 2004; Canas *et al*., 2009; 2010; Amiour *et al*., 2012; Gupta *et al*., 2012). Biochemical markers are frequently used as indicators for the plant nutritional status at different developmental stages. These

markers include GS activity, nitrate content, total protein content, chlorophyll content and total N content. These markers have been used to understand what limits N assimilation and remobilization in maize (Gallais and Hirel, 2004; Hirel *et al*., 2005a; 2005b; Canas *et al*., 2009; 2010; Amiour *et al*., 2012), but also in other crops such as wheat (Kichey *et al*., 2005; Ping *et al*., 2011).

GS activity is one of the major factors that control growth in cereals, as mentioned before (Hirel *et al.*, 2005b; Tabuchi *et al.*, 2005; Martin *et al.*, 2006). It has been used as a marker for both inorganic N assimilation and N recycling (Hirel *et al*., 2001). A quantitative genetic study with a focus on maize kernel productivity showed strong coincidence between QTLs for leaf GS activity and grain yield (Hirel *et al*., 2001), and there is a positive correlation between GS activity and grain yield among maize genotypes (Gallais and Hirel, 2004). Similarly, in wheat, there was a strong positive correlation between GS activity and total plant N content (Kichey *et al*., 2005). Moreover, in sorghum, leaf GS activity observed under nitrate and ammonium and mainly nitrate nutrition is due to an accumulation of chloroplastic GS2 while cytosolic GS1 activity accumulated in roots icreased also under nitrate and ammonium but it showed to be higher in ammonium treated plants (El-Omari and Nhiri, 2015).

Nitrate content can be considered as a metabolic marker for N uptake ability during early stages of maize development because, after silking, nitrate assimilation slows down as shown in field-grown maize. Where a significant positive correlation was observed between the leaf nitrate content of maize young plants and grain yield regardless of the level of N supply (Hirel *et al*., 2001; Gallais and Hirel, 2004).

The N status of the plant was shown to be monitored using total protein and total N content during grain filling stages in maize and wheat (Hirel *et al*., 2005b; Kichey *et al*., 2005). Where, during late stages, leaf proteins and leaf total N decreases progressively due to degradation of leaf protein to provide N for the grains during grain filling (Waters et al., 1980).

Chlorophyll is one of the main markers that used during leaf senescence because its degradation is one of the major events that occurs during the shift from N assimilation to N remobilization (Hirel *et al*., 2005a). As N is an essential constituent of chlorophyll (Kafle and Sharma, 2015), it was shown by studies done on maize (Hirel *et al*., 2005b) and wheat (Kichey *et al*., 2005) a positive correlation between chlorophyll ant total N content in different leaf stages.

At molecular level, the basis of plant response to different N conditions was identified by different responsive genes. In Arabidopsis, microarray analysis showed a difference in the expression of some genes involved in N metabolism as *NR*, *NRT1*, and *GS* genes in plants grown under different nitrate concentrations. *NRT1* gene, for example showed a higher response at low nitrate supply with some decrease when exposed to high nitrate conditions (Wang *et al*., 2000). In maize, the transcript levels of *NRTs* genes changed under reduced nitrate levels in the soil. Where, *ZmNRT2.1* and *ZmNRT2.2* genes were found to be with higher transcript levels than those of *ZmNRT1.1A* and *ZmNRT1.1B* at N limiting-conditions (Garnett et al., 2013). In wheat, the expression of *NRT2.1*, *NRT2.2*, and *NRT2.3* decreased under high N supply (Ping *et al*., 2011).

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1.4 Sorghum bicolor: Introduction (En francais)

Sorgho (Sorghum bicolor (L.) Moench; 2n = 20) est une culture céréalière importante. L'Organisation pour l'alimentation et l'agriculture des Nations Unies (FAO) se classe comme la 5ème récolte céréalière la plus importante dans le monde, après le blé, le riz, le maïs et l'orge (De Wet, 1978; Wiersema et Dahlberg, 2007; Paterson, 2008; Dahlberg et al., 2011). Le sorgho est cultivé dans un large éventail d'environnements. Sa capacité d'adaptation à la sécheresse et à la salinité rend l'une des cultures les plus importantes dans les régions sèches (Paterson, 2008; 2009; Morris et al, 2013.). Commercialement, sorghos cultivés représentent différents types agronomiques, y compris le sorgho, sorgho fourrager, le sorgho sucré et broomcorns (Berenji et Dahlberg, 2004; Singh et Lohithaswa, 2006;. Berenji et al, 2011).

Sorgho est utilisé pour une variété de buts (Dahlberg et al, 2011; Kimber et al, 2013.). Il est un aliment de base pour des millions de personnes dans les zones tropicales semi-arides. Du point de vue alimentaire, il peut se substituer à d'autres céréales, car il ne contient pas de gluten, mais a autrement la même valeur nutritive que les grains de maïs (tableau 1) (Sheorain et al., 2000; Singh et Lohithaswa, 2006; Dahlberg et al., 2011 ; cadran, 2012; Jacob et al, 2013;.. Morris et al, 2013). En Inde et en Afrique, le sorgho est utilisé pour le brassage de la bière, et aux États-Unis pour la production de sirop (Dahlberg et al., 2011). En outre, le sorgho peut être utilisé comme fourrage et des aliments pour animaux, comme matériau de construction, ou pour la production de balais (Dogget, 1988; Dahlberg et al, 2011;. Hariprasanna et Patil, 2015). Il est également d'intérêt pour la production de biocarburants de l'usine. Après le maïs, il est la deuxième source la plus importante de l'éthanol à base de céréales aux États-Unis en raison de son rendement et la teneur en sucre élevée de biomasse (Dahlberg et al, 2011;. Jacob et al, 2013).

Tableau 1. Comparaison des sorgho et de maïs contenu de grains (Sheorain et al., 2000; Jacob et al., 2013)

Component	Content %	
	Sorghum	**Maize**
Starch	63-68	60-64
Moisture	9-13	8-11
Proteins	9-11	9-11
Fats and oils	1-1.5	3-5
Crude fiber	1.5-2	1.5-2
Ash	1-2	1-2
Other organics	8-12	7-9

1.1.1 Morphologie et développement

Sorgho est une graminée annuelle semblable en apparence au maïs dans son stade végétatif. Sorghum bicolor Mature a généralement une hauteur comprise entre 1,5 - 2,1 m, mais peut parfois atteindre 5 m. Sa tige est dressée et solide. Elle produit un ou plusieurs talles, selon le génotype et les conditions de croissance. Tillers émergent initialement à partir de la base de la tige principale, et plus tard à partir de nœuds souches (Singh et Lohithaswa, 2006). Les racines sont adventives avec des racines primaires et secondaires. feuilles Waxy rendent la plante plus tolérante à la sécheresse que la plupart des autres céréales (Kimber et al, 2013;. Hariprasanna et Patil, 2015).

La première feuille (Figure1a) qui apparaît à l'émergence est, comme dans toutes les céréales, coléoptile. Cette feuille est différente de toutes les vraies feuilles en ce qu'elle a une pointe de la feuille arrondie. La dernière feuille à émerger est la feuille de drapeau qui est considérablement plus petite que les autres vraies feuilles. Le nombre de feuilles peut varier 7-24 selon le génotype (Singh et Lohithaswa, 2006). L'inflorescence, appelée tête ou panicule, émerge de la gaine de la feuille et est supportée par la partie de la tige qui est appelé le pédoncule (Besancon et al., 2005; Hariprasanna et Patil, 2015). Le panicule peut être ouvert ou compact ou entre les deux en fonction du cultivar (De Wet., 1978).

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La panicule a un axe central appelé le rachis où les branches primaires se développent. Les branches primaires donnent lieu à des branches secondaires qui, parfois, donnent lieu à des branches tertiaires. branches primaires, secondaires et tertiaires chacun peut porter un groupe d'épillets (Figure 2a). Les épillets se produisent par paires à chaque noeud (Burrow et al., 2014). Un épillet est toujours sessiles, à savoir, directement rattaché à la branche, alors que l'autre est pédicellé, à savoir, attaché à la branche par un pédoncule. Cependant, l'épillet sessile terminal à l'extrémité de chaque branche est accompagné de deux épillets pédicellé.

L'épillet sessile est fertile et contient un fleuron protégé par deux glumes. Les fleurettes se compose d'un gynécée central avec un ovaire dont deux stigmates dépassent. Trois anthères sont attachés à la base du gynécée par de longs fils comme filaments (Hariprasanna et Patil, 2015). Gynécée et anthères sont enfermés dans des gaines fibreuses appelées lemmes (la partie supérieure peut être aristées) et un petit palea. Deux lodicules se trouvent de chaque côté de la base de l'ovaire. Chaque floret contient un ovule qui donnera lieu à une graine enfermée dans les deux glumes après la fécondation. Les graines peuvent varier en forme, la taille et la couleur parmi les cultivars de sorgho. La graine est arrondie, de 4-8mm de diamètre, blanc, jaune, brun foncé, ou de couleur brun-rougeâtre (Singh et Lohithaswa, 2006; Burrow et al, 2014;. Hariprasanna et Patil, 2015).

L'épillet pédicellée ne donne jamais lieu à une graine, mais peut parfois posséder anthères (Singh et Lohithaswa, 2006;. Burrow et al, 2014; Hariprasanna et Patil, 2015). Les épillets pédicellées peuvent être persistantes ou caduques en fonction des races ou des cultivars (Hariprasanna et Patil, 2015).

Sorgho est une courte journée et végétale photosensible (Kimber et al., 2013). Il est considéré comme la plupart du temps d'auto-pollinisatrices, mais outcrossing peut être élevé dans certaines races (voir ci-dessous) et les environnements. En particulier, le type de panicule, la direction du vent et le jeu de vitesse des rôles importants pour outcrossing (Kimber et al, 2013;. Hariprasanna et Patil, 2015).

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La plupart des races de sorgho à échéance dans 60-180 jours (Kimber et al., 2013), mais certaines variétés peuvent nécessiter jusqu'à 300 jours (Clerget et al., 2007). Cultivars cette fleur dans les 50 à 60 jours après le semis sont considérés comme une floraison précoce, tandis que ceux qui fleurissent au bout de 60 jours sont considérés comme fin de la floraison. Certains cultivars à floraison tardive peut fleurir au bout de 180 jours. La durée entre l'émergence de la feuille étendard et le cap (panicule émergence) ne varie pas entre les cultivars précoces et tardives de floraison. Retards et sensibilité à la photopériode sont fortement et positivement corrélés: Tous les sorghos tardives sont sensibles à la photopériode, avec le degré de retard croissant avec sensibilité à la photopériode (Clerget et al., 2007).

1.1.2 Cycle de vie du sorgho

Le cycle de vie du sorgho est divisé en trois étapes de croissance, le stade végétatif, l'initiation de la panicule, et la phase de remplissage du grain (Gerik et al., 2003; Kelley, 2003).

1. Le stade végétatif: GS1

Cette étape comprend la germination, le développement des semis, l'émergence de feuilles qui soutiendront la croissance et le grain (noyau) la formation à des stades tardifs. La durée de cette étape dépend habituellement du génotype et de la température. En règle générale, les plantes à fleurs tardives ont tendance à former plus de feuilles à ce stade (Clerget et al., 2007).

2. Panicule intiaition: GS2

Elle est caractérisée par le développement des structures de reproduction. Nombre maximal de graines par plante sont fixés à ce stade et représente 70% de rendement en grain finale de sorgho. La feuille de drapeau a déjà vu le jour et fermé la panicule qui est presque complètement développé. Cette phase est appelée démarrage. Puis, le pédoncule de la panicule s'allonge conduisant rapidement à son effort de la gaine de la feuille et il est devenu visible. Ceci est appelé cap.

3. Phase de remplissage Grain: GS3

A ce stade, le développement des plantes se concentre sur la formation des grains. Cette étape commence lorsque anthères matures (jaunes) apparaissent à la pointe de la tête, généralement de cinq à sept jours après la tête effort. La matière sèche accumule dans les grains et la plante atteint sa maturité. le développement des semences progresse dans les différentes phases, y compris la phase de lait, phase de pâte molle, la phase de pâte dure, et, enfin, la maturité physiologique. La période pendant laquelle ces processus se déroulent peut varier de 25 à 45 jours après la floraison, selon le génotype et les conditions de croissance.

Dans la phase de lait, le noyau atteint sa taille maximale; il est très doux et peut être facilement pressé. La phase de pâte molle se produit environ 15 à 25 jours après la floraison lorsque 50% du poids du grain a été accumulée; le noyau peut encore être coincé entre les doigts, mais peu ou pas de liquide apparaît. Dans la phase de pâte dure, quand environ 75% du poids du grain finale a accumulé, le grain ne peut plus être coincé entre les doigts. La phase de maturité physiologique commence quand une couche noire apparaît près de la base du noyau, qui contient encore 30 à 35% d'eau. Les noyaux peuvent être récoltées quand ils contiennent moins de 20% d'humidité. Cependant, pour un stockage sûr, cette valeur doit être inférieure à 14% en l'absence de l'équipement pour le séchage du grain est disponible.

Les trois stades de croissance décrits ci-dessus peuvent être subdivisés en 10 sous-étapes, numérotées de zéro (0) à neuf (9) comme indiqué ci-dessous (Figure 3; tableau 2), pour décrire plus finement la croissance de sorgho de la levée de la plante jusqu'à la maturité physiologique (Vanderlip, 1993; Besancon et al, 2005).

Tableau 2. Caractéristiques de la croissance du sorgho, subdivisés en 10 sous-étapes (Vanderlip, 1993;. Besancon et al, 2005).

Growth Stage		Characteristics that identify the stage	
GS1	0	**Emergence**	The first leaf is visible at soil surface, 3-10 days after sowing
	1	**3-leaf stage**	The collar of 3 leaves can be seen, approximately 10 days after emergence
	2	**5-leaf stage**	The collar of 5 leaves can be seen; about 3 weeks after emergence
GS2	3	**Growing point differentiation**	The growth of sorghum changed from vegetative to reproductive: Panicle initiation
	4	**Flag leaf visible**	Flag leaf emerges
	5	**Boot stage**	All leaves are fully expanded. The head has developed to nearly full size and is enclosed in the flag-leaf sheath.
GS3	6	**Half bloom**	When half of the plants in a field have started to bloom. It usually represents two-thirds of the time from planting to physiological maturity
	7	**Soft dough**	Half of grain dry weight is accumulated during this period
	8	**Hard dough**	About 75% of the grain dry weight has accumulated
	9	**Physiological maturity**	Maximum total dry weight of the plant has been reached. Physiological maturity can be determined by the dark spot on the opposite side of the kernel from the embryo

1.1.3 Classification et Taxonomie

Sorghum appartient au genre Sorghum dans la famille Poaceae (Clayton et Renvoize, 1986). Snowden (1936) a utilisé des caractères morphologiques, principalement des épillets, pour la classification. Il a divisé le genre dans les sections Eu-sorgho et Para-sorgho (Figure 5). Il a en outre sous-divisé Eu-sorgho en deux sous-sections: Arundinacea et Halapensia. Arundinacea contenait la série spontanea, qui comprenait les espèces sauvages ou races, et la série Sativa qui comprenait les espèces cultivées (Snowden, 1936; Snowden, 1955). Au total, Snowden (1936) distingue 31 espèces cultivées et 17 espèces sauvages apparentées dans le Arundinacea.

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Cette classificaton a été affinée par la suite sur la base de caractères morphologiques supplémentaires, compte tenu en particulier la branche primaire, noeud et épillets morphologies (Garber, 1950; Celarier, 1959), ce qui entraîne une division du genre Sorghum en sections Chaetosorghum, Heterosorghum, Parasorghum, Stiposorghum et Sorgho ou sous-genres (De Wet et Harlan, 1970; De Wet, 1978). Dans le sous-genre Sorghum trois espèces ont été reconnues: deux taxons rhizome, S. halpense et S. propinquum, et un troisième, S. bicolor, qui comprenait tous les sorghos annuelles cultivées, sauvages et adventices (De Wet, 1978; Dahlberg et al ., 2011; Singh et Lohithaswa, 2006; Hariprasanna et Patil, 2015).

Par la suite, S. bicolor ssp. bicolor, S. bicolor ssp. drummondii et S. bicolor ssp. arundinaceum ont été décrits comme trois sous-espèces de Sorghum bicolor (De Wet et Huckabay 1967; De Wet, 1978). S. bicolor ssp. arundinaceum a ensuite été rebaptisé S. bicolor ssp. verticilliforum (De Wet, 1978; Wiersema et Dahlberg, 2007;. Dahlberg et al, 2011;. Kimber et al, 2013).

De nos jours, toutes les variétés de sorgho cultivées appartiennent à S.bicolor ssp bicolor. Basé sur épillet et panicule morphologie, ces variétés sont divisées en cinq races de base: bicolor, caudatum, durah, guinae et kafir (Figure 1), et dix races hybrides qui présentent les caractéristiques d'au moins deux de ces races (De Wet 1978 ; Kimber et al, 2013;.. Dillon et al, 2007; Hariprasanna et Patil, 2015).

1 Bicolor

Le bicolor de course a une panicule ouverte et de taille moyenne. Il a une longue clasping et glumes épais. Les glumes enferment totalement le grain elliptique à l'échéance. Les épillets pédicellées sont persistantes et pédicelles sont courtes. Bicolor a allongé et généralement de petite taille des graines et est généralement faible rendement (De Wet, 1978; Mann et al, 1983;. Hariprasanna et Patil, 2015).

2 Caudatum

La gamme de panicule de compact à ouvrir. Les glumes sont plus courtes que les grains. Il a obovales à elleptical épillets sessiles tandis épillets pédicellées sont caduques. Les grains sont grandes, ressemblant à une tortue, asymétrique, à plat sur un côté et courbé sur le côté oppsite, blanc crayeux ou pigmenté. Cette course est généralement un rendement élevé (De Wet, 1978;. Mann et al, 1983; Hariprasanna et Patil, 2015).

3 Durra

La panicule est dense et compact. L'épillet sessile sont aplatis et ovales et les épillets pédicellées sont importants et persistants. La texture de la pointe de glumelles est différente de celle de leur base. Il est avec pli transversal jusqu'au milieu et coriaces. sorghos Durra ont arrondi graines blanches. Cette course est généralement un rendement élevé (De Wet, 1978;. Mann et al, 1983; Hariprasanna et Patil, 2015).

4 Guinée

La Guinée course a un grand et ouvert panicule. Les glumes sont longues. Les grains ont de taille petite à moyenne, sont plus courtes que les glumes, et sont blanchâtres. Les épillets pédicellées sont à la fois persistantes et à feuilles caduques. L'épillet sessile ouvre à maturité et expose la graine. La course de Guinée tend à être faible rendement (De Wet, 1978;. Mann et al, 1983; Hariprasanna et Patil, 2015).

5 Kafir

sorghos cafres ont une panicule plus ou moins compacte qui est souvent cylindrique et allongée. Glumes sont beaucoup plus courtes que les grains. Les épillets sessiles ont tendance à être velu. Les grains sont symétriques, et plus ou moins sphérique. Cette course est généralement un rendement élevé (De Wet, 1978;. Kimber et al, 2013; Hariprasanna et Patil, 2015).

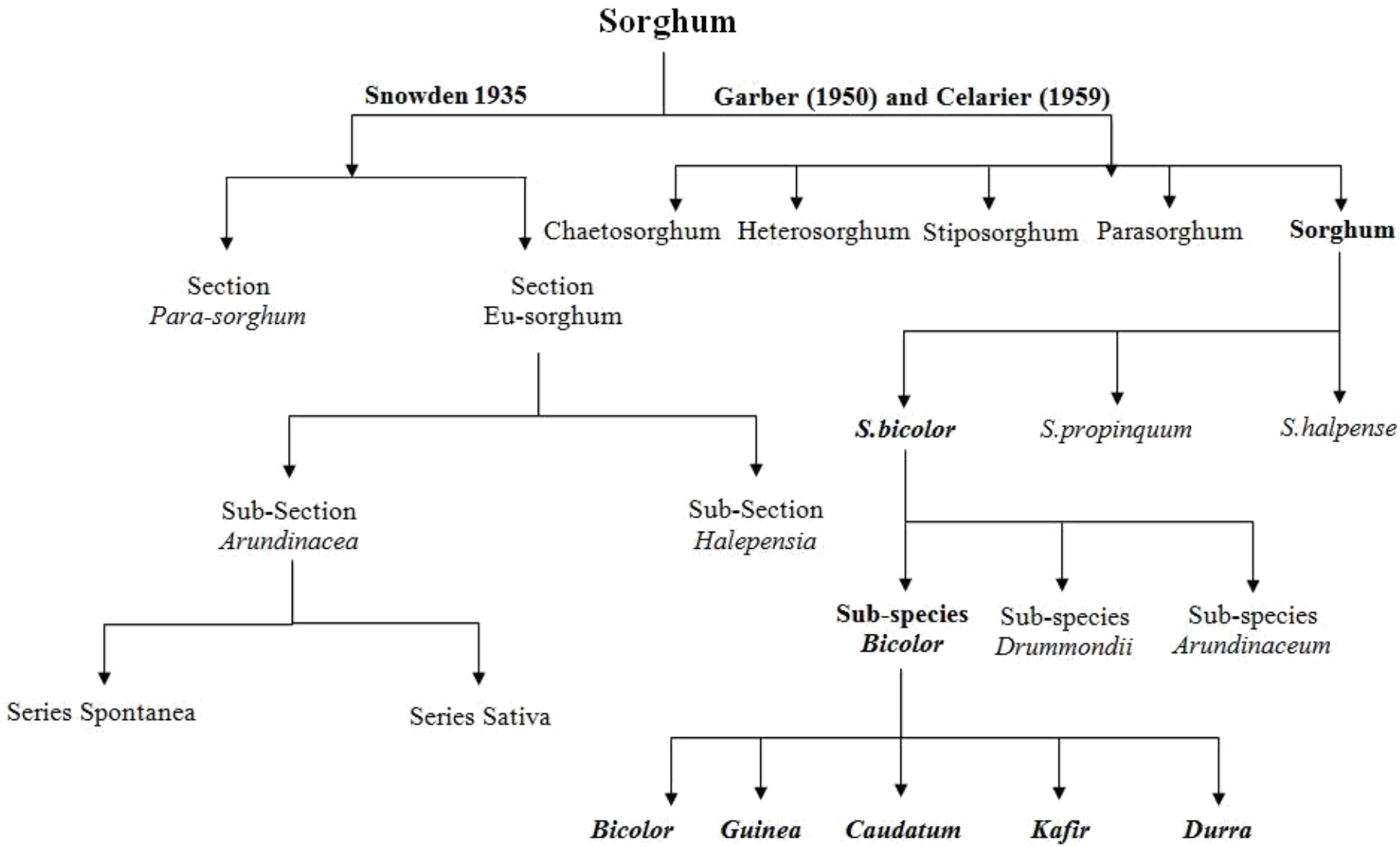

Figure 1. Classification du sorgho (Inspiré de Hari Prasanna et Patil, 2015)

1.1.4 Origine et domestication du sorgho

Le sorgho est une culture ancienne originaire d'Afrique, où effectivement la plus grande diversité du sorgho sauvage et cultivé est trouvé (Kimber et al., 2013). La domestication du sorgho a eu lieu il y a environ 5000-8000 ans dans le nord-est de l'Afrique, dans une région qui correspond à la région frontalière entre l'Egypte et le Soudan (Mann et al, 1983;. Wendorf et al., 1992). céréales carbonisées de sorgho ont été fouillés à un site archéologique Holocène tôt à Nabta Playa près de la frontière égypto-soudanaise (Wendorf et al., 1992; Dahlberg et Wasylikowa, 1996; Jacob et al, 2013;. Hariprasanna et Patil, 2015). Sorghum probablement dispersé avec la migration des personnes à travers les prairies du Sahel-Soudan et au sud de la région de la vallée du Nil le long de la Great Rift. Cette hypothèse est étayée par le modèle de distribution des sorghos sauvages et cultivées en Afrique (Murdock, 1959; De Wet et Harlan, 1970; Kimber et al, 2013.).

Ensuite, il y a plus de 5000 ans, le sorgho propagation de l'Afrique orientale à l'Inde par des orateurs sémitiques qui ont porté leurs cultures avec eux (Figure 2). Sorghum atteint l'Indonésie et la Chine de l'Inde il y a environ 4000 ans (Singh et Lohithaswa, 2006; Kimber et al, 2013.). Il y a environ 3000 ans sorgho est arrivé au Moyen-Orient, et around1500 il y a des années en Extrême-Orient, comme une conséquence de la migration des personnes et le commerce (Figure 5) (Dillon et al., 2007). Enfin, au milieu du 19ème siècle, les esclaves africains portant des semences de sorgho avec eux introduit cette culture aux États-Unis, qui est aujourd'hui le plus grand pays de sorgho à croissance (Singh et Lohithaswa, 2006; Hariprasanna et Patil, 2015).

Comme dans toutes les céréales, humain choisi traits morphologiques et physiologiques spécifiques volontaires ou involontaires au cours du sorgho domestication. Ces caractéristiques comprennent une panicule nonshattering, grandes inflorescences et les graines, à haut rendement, le battage facile, hauteur appropriée [pour faciliter la récolte] et une courte période de dormance des graines (Mann et al, 1983;. Singh et Lohithaswa, 2006). Ces caractéristiques étaient mieux adaptés à la culture pour les besoins humains. Après la domestication initiale, au cours de la dispersion de sorgho à différentes régions, sélection supplémentaire a conduit à l'adaptation à de nouveaux environnements et provoqué de nouvelles caractéristiques. En conséquence, une grande diversité phénotypique et physiologique a surgi qui était la base pour la production de nombreuses variétés locales. Ces variétés locales sont aujourd'hui une ressource génétique précieuse pour l'amélioration du sorgho par la sélection moderne (Singh et Lohithaswa, 2006;. Dahlberg et al, 2011; Kimber et al, 2013;.. Morris et al, 2013).

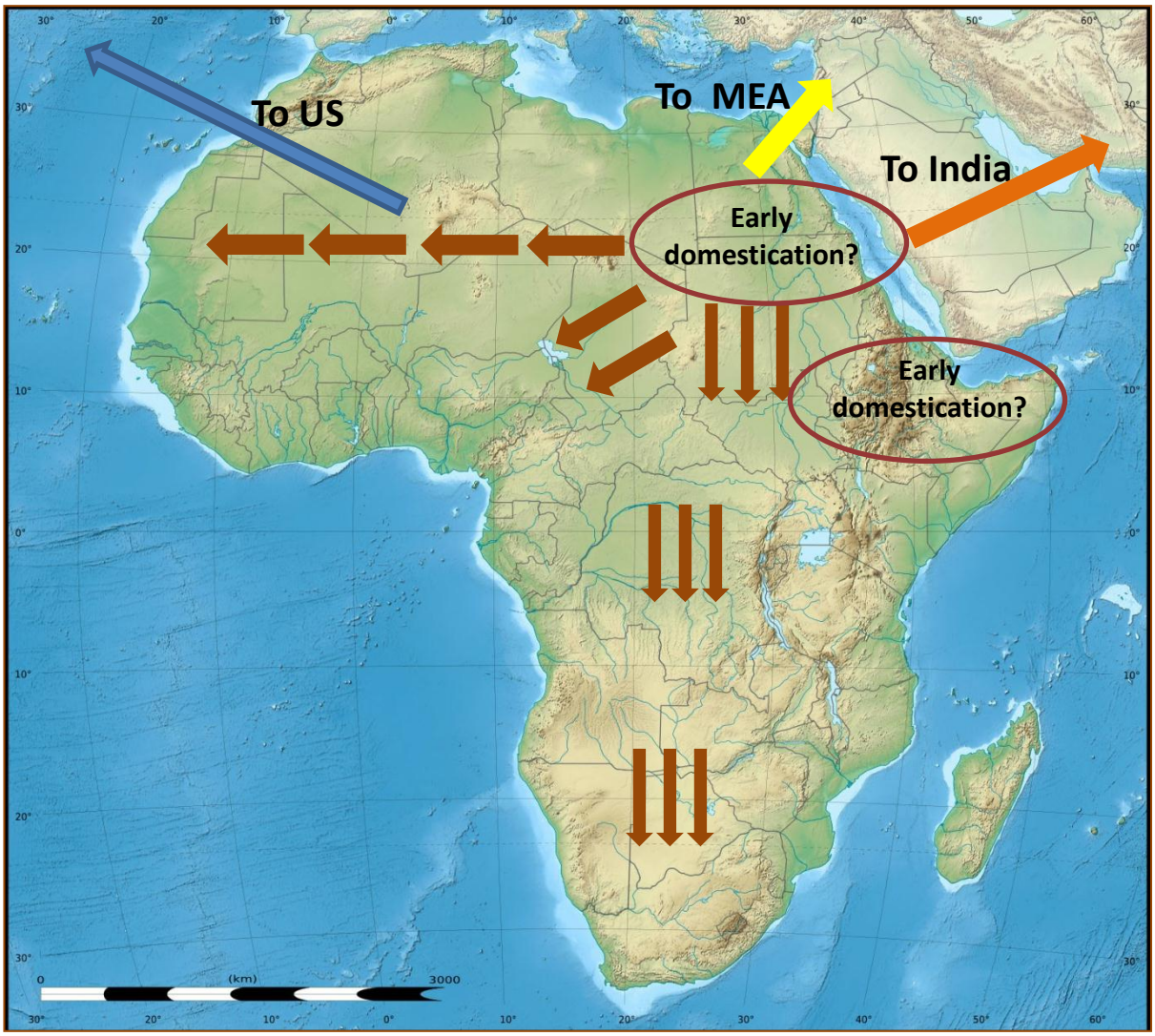

Figure 2. Origine et suggéré mouvements des races domestiquées de Sorghum bicolor (Inspiré de Kimber et al., 2013)

1.1.5 Répartition actuelle du Sorghum bicolor

Sorghum bicolor ssp. bicolor est largement distribué dans le monde entier. races cultivées de sorgho sont dominantes en Afrique. De même, les sous-espèces de sorgho sauvages sont également fréquentes sur le continent africain. S. bicolor ssp. verticilliforum, anciennement connu sous le nom S. bicolor ssp. arundinaceum, comprend les variétés arundinaceum, virgatum, aethiopicum et verticilliforum. La arundinaceum variété est présent dans les forêts tropicales d'Afrique centrale et de l'Ouest dans des environnements qui ne sont pas appropriés pour les courses de sorgho cultivées. Le virgatum de course se produit dans les parties de la vallée du Nil qui ont des inondations saisonnières, ainsi que dans certaines parties du Soudan. Aethiopicum est une herbe de désert, il se produit dans la région de Kassala du Soudan et en Ethiopie.

La course la plus large est distribué verticilliforum; il est présent dans les savanes de l'est et du sud Afrique (De Wet et Harlan, 1970; De Wet, 1978; Kimber et al, 2013.).

Le modèle de répartition actuelle des races de sorgho cultivées est en partie expliquée par leurs traits biologiques et en partie par leur dispersion historique à travers les continents (Figure 2) (Kimber et al, 2013;. Hariprasanna et Patil, 2015). Le bicolor course est cultivé à travers l'ensemble des zones de culture de sorgho en Afrique et en Asie, mais est rarement une culture importante. Au lieu de cela, les sorghos bicolor sont principalement cultivés pour leur tige douce et grains amers qui sont utilisés pour aromatiser la bière de sorgho. La distribution de la caudatum course en Afrique est étroitement associée aux zones de peuplement de personnes parlant la langue Chari-Nil. Il est adapté aux régions arides qui reçoivent entre 250 à 1300 mm de pluie par an. La durah course est présent dans les hautes terres d'altitude moyenne de l'Ethiopie, la vallée du Nil du Soudan et de l'Egypte, le Pakistan et régions de l'Inde (De Wet, 1978;. Mann et al, 1983; Kimber et al, 2013.).

La Guinée est essentiellement connu comme une race africaine à l'ouest, mais il est également présent dans les montagnes de l'Afrique orientale, dans les zones à forte pluviométrie. Cette course est préférable dans les régions humides, car il est pas fortement affectée par la moisissure des grains et les insectes, qui sont provoquées par de longues saisons des pluies en Afrique de l'Ouest (Stemler et al, 1977;. De Wet, 1978; Mann et al, 1983. ; Morris et al, 2013;. Kimber et al, 2013).

1.1.6 Génomique de Sorghum

L'analyse génomique des cultures peut jouer un rôle important dans le soutien de l'agriculture durable partout dans le monde, notamment en Afrique, en Asie et dans les régions semi-arides (Morris et al., 2013). Sorghum bicolor est une espèce diploïde (2n = 20) (Singh et Lohithaswa, 2006). Elle est une plante en C4, à savoir, il possède des caractéristiques biochimiques et morphologiques spécifiques qui permettent l'assimilation du carbone élevé à des températures élevées (Edwards et al., 2004). Il est la première culture africaine pour laquelle la séquence du génome complet est disponible. Le génome de Sorghum bicolor est un des plus petits parmi les herbes; elle englobe environ ~ 730 Mb. Cette taille correspond à environ 25% du maïs ou de la canne à sucre génomes (Paterson et al., 2009). En conséquence, le sorgho est devenu une espèce intéressante pour la recherche génétique et pour des études comparatives entre les céréales.

Par exemple, pour faciliter l'identification des gènes d'intérêt agronomique pour l'amélioration du sorgho, Morris et al. (2013) ont caractérisé ~ 265.487 seul nucleotide polymorphismes (SNP) dans un panel de 971 accessions de sorgho choisis à travers le monde et adaptées aux conditions agro-climatiques variées. Les auteurs ont effectué une étude d'association pangénomique (GWAS) pour identifier les gènes variation naturelle sous-jacente dans les traits agro-climatiques, avec un accent particulier sur la hauteur de la plante et de l'architecture de l'inflorescence. Les résultats obtenus par cette étude en analysant le génome du sorgho en utilisant GWAS ont identifié trois principaux QTL pour la hauteur des plantes (Morris et al., 2013). Ces QTL ont des distributions alléliques distinctes entre les accessions de sorgho en Asie et en Afrique révélant ainsi des différences de hauteur de la plante entre accessions.

1.2 L'azote Métabolisme

L'azote (N) est quantitativement la plus importante des éléments nutritifs pour les plantes. Il est un facteur limitant majeur dans la croissance des plantes, la productivité des cultures et le rendement (Hirel et al, 2007;. Lea et Azvedo, 2007). N est absorbé par les plantes à partir du sol sous des formes differrent, soit directement par les racines des plantes ou par des mycorhizes associés aux racines (Andrews et al., 2013). Nitrate (NO3-) est la principale source N disponible pour les plantes dans le sol (Cawford, 1995), mais l'ammonium (NH4 +) peut également être important, en particulier dans les sols non perturbés et non fécondés. Certaines plantes telles que les légumineuses peuvent prendre leur N en faisant symbiose avec des micro-organismes (comme rhizobiums) qui fixent l'azote atmosphérique. Ceci fournit un avantage quand les plantes poussent dans un sol à faible teneur en N (Franche et al, 2009;. Raven et Andrews, 2010; Andrews et al, 2013.). Le cycle N dans les plantes implique de nombreux processus: l'absorption, l'assimilation, la translocation et, en particulier dans le vieillissement des plantes, le recyclage et la remobilisation.

Deux systèmes d'absorption de nitrate différents jouent un rôle clé dans l'absorption d'azote et le transport à travers les plantes. Un système de transport de haute affinité (HATS), est responsable de l'absorption des nitrates lorsque la concentration en nitrate externe est faible (inférieure à ≤ 1mm). Un système de transport à faible affinité (LATS) est plus important à des concentrations de nitrates> 1mM (Forde, 2000). Deux HATS ont été décrits. En plus d'un système constitutif, CHATS, il y a un système inductible, iHATS, qui devient fonctionnelle seulement après exposition au nitrate (Aslam et al., 1993; Forde, 2000; verre, 2003).

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Deux classes de gènes, NRT1 et NRT2, ont été trouvés pour être impliqués dans LATS et HATS respectivement. Dans Arabidopsis thaliana, 53 NRT1 et sept NRT2 membres ont été identifiés (Orsel et al., 2002;. Tsay et al, 2007). AtNRT1.1 a été le premier gène ayant été isolé et étudié de manière approfondie pour sa double affinité. Son produit du gène a une double fonction; il sert de transporteur de nitrate et aussi comme capteur de nitrate pour activer l'expression de gènes liés à nitrate (Forde, 2000; Masclaux-Daubresse et al, 2010;. Bai et al, 2013.). Graminées ont plusieurs étroitement liés co-orthologue à AtNRT1.1. Quatre exemplaires ont été identifiés dans le maïs (ZmNRT1.1A, ZmNRT1.1B, ZmNRT1.1C, ZmNRT1.1D) (Plett et al., 2010) trois du riz (OsNRT1.1A, OsNRT1.1B et OsNRT1.1C), et sorgho (SbNRT1.1A, SbNRT1.1B et SbNRT1.1C) (Plett et al., 2010). Par ailleurs, les résultats effectuées sur la famille de gènes NRT2 a montré la présence de deux gènes NRT2 du riz (OsNRT2.1 et OsNRT2.2), trois dans le maïs (ZmNRT2.1, ZmNRT2.2 et ZmNRT2.3) et de sorgho (SbNRT2 .1, SbNRT2.2 et SbNRT2.3) (Plett et al., 2010).

À la suite de l'absorption par les racines, le nitrate peut être stocké ou réduit en nitrite par nitrate réductase (NR) (figure 3). Le nitrite ainsi obtenu est ensuite réduit en ammonium sous l'action de la nitrite réductase (NIR) (figure 3). NR est la première enzyme dans la voie d'assimilation des nitrates. Ainsi, la réduction du nitrate par NR peut être une étape de limitation (Srivastava, 1980). Nitrate et la réduction de nitrite peuvent avoir lieu dans les racines ou les pousses en fonction du génotype et des conditions environnementales, en particulier des concentrations de nitrates dans les sols (Figure 3) (Lea et Azevdo, 2007; Masclaux-Daubresse et al, 2010;. Andrews et al. , 2013).

Ammonium, la deuxième source d'azote pour les plantes, peut être absorbée à partir du sol, produit par la réduction du nitrite ou du recyclage de la photorespiration d'acides aminés (figure 3) (Andrews et al., 2013). Il est assimilé en acides aminés par la glutamine synthétase (GS) / glutamate synthase (GOGAT) voie (Andrews et al., 2013). GS catalyse une réaction dépendant de l'ATP qui fixe l'ammoniac (NH3) au glutamate, en formant glutamine (figure 6). Les plantes possèdent deux isoformes majeures de GS: GS1 et GS2 (Cren et Hirel, 1999). GS1 est situé dans le cytosol et présent dans une variété d'organes et de tissus tels que les racines, les feuilles et les cellules de phloème. GS2 est situé dans les chloroplastes des tissus photosynthétiques et les plastes des racines.

Les deux isoformes peuvent se produire dans différents rapports en fonction des organes et des espèces (Martin et al., 2006; El-Omari et al, 2010; Hirel et al, 2011; Andrews et al, 2013.). GS1 est impliquée dans l'assimilation de l'ammonium repris à partir du sol et joue un rôle clé dans la N recyclage et remobilization, tandis que GS2 est responsable de l'assimilation de l'ammonium provenant de la réduction des nitrates et photrespiration (Tabuchi et al, 2007; El-Omari et al., 2010).

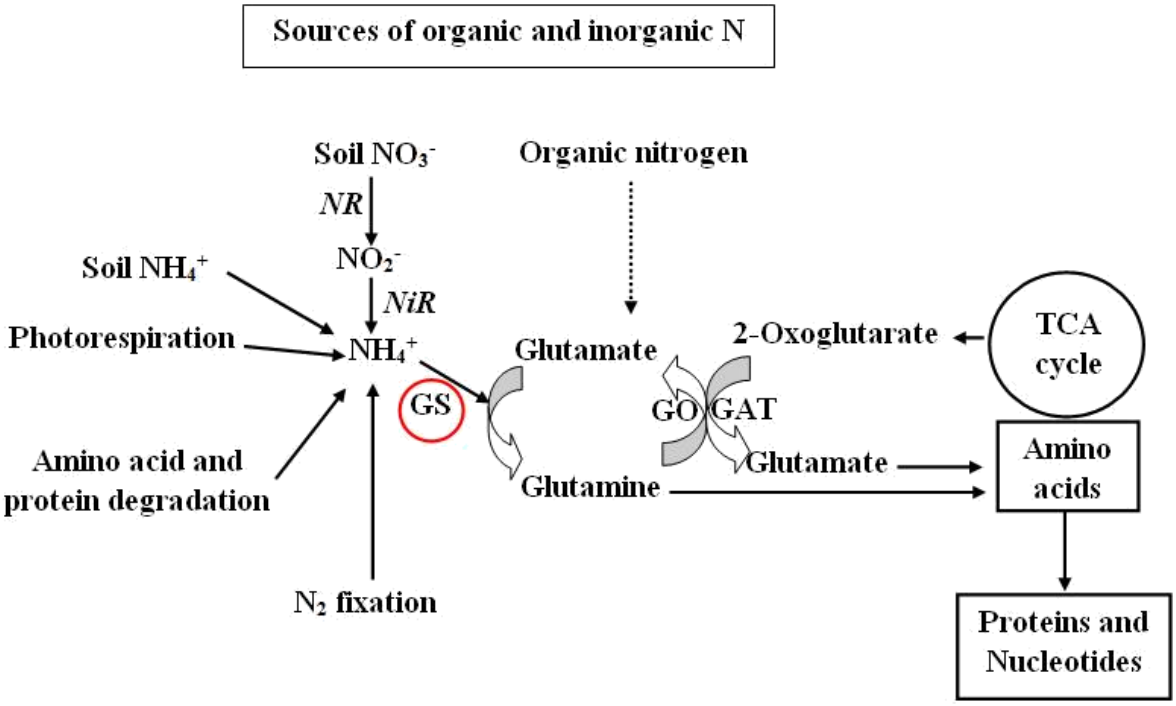

Figure 3. Les réactions principales de l'assimilation de l'azote dans les plantes. Modifié à partir de Hirel et al.2011.

GOGAT (glutamine 2-oxoglutarate amino transférase) catalyse la conversion de glutamine et 2-oxoglutarate à deux molécules de glutamate (Figure 3) (Cren et Hirel, 1999; Forde et Lea, 2007; Masclaux-Daubresse et al., 2010). GOGAT dispose également de deux isoformes: une forme de ferrédoxine-dépendante qui est, en conjonction avec GS2, impliqués dans l'assimilation de l'ammonium provenant de la réduction des nitrates et photorespiration. La seconde isoforme est la pyridine nucléotide-dépendante, et est impliqué dans la synthèse du glutamate dans les deux tissus photosynthétiques et non photosynthétiques (Hirel et Lea, 2001; El-Omari et al, 2010;. Hirel et al, 2011;. El- Omari et Nihiri., 2015).

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La glutamine et le glutamate sont les principaux donneurs pour les groupes amino à toutes les molécules contenant de l'azote, y compris d'autres acides aminés, des protéines et des nucleotides pour la synthèse d'ARN et d'ADN (Hirel et al., 2011). Ainsi, GS / GOGAT est d'une importance clé pour N assimilation dans les plantes. Tout le N dans une plante, sans tenir compte de sa source, est canalisée par la réaction catalysée par GS / GOGAT qui est, par conséquent, considéré comme un point de contrôle pour la croissance et la productivité des plantes (Hirel et al, 2005b;.. Tabuchi et al, 2005;. Martin et al, 2006).

Au cours de la sénescence des plantes annuelles, des protéines de feuilles et en particulier les protéines sont dégradées photosynthétiques pour servir comme source de carbone, d'azote et d'autres éléments destinés à l'alimentation des organes de retard de croissance, notamment les graines. Tous les organes qui absorbent et assimilent l'azote inorganique pendant le démarrage de la croissance végétative de fonctionner comme une source N au satges développement en retard, un phénomène appelé N remobilization (Hirel et al, 2001;., 2007; Masclaux-Daubresse et al, 2010). Ce phénomène est important pour acheminer N organique aux semences pendant le remplissage des grains de céréales, car l'absorption d'azote et d'assimilation sont généralement insuffisantes pour répondre à la forte demande pour cet élément dans le développement des semences (Masclaux-Daubresse et al, 2010).

1.3 utilisation de l'azote effieciency

Pour assurer le rendement élevé des cultures d'engrais synthétiques sont fréquemment utilisés. L'ingrédient principal de ces engrais est le nitrate, car la disponibilité de ce nutriment est généralement limitée dans le sol (Andrews et al., 2013). Cependant, l'excès de l'apport d'engrais minéraux a un impact négatif sur l'environnement, en particulier le sol et la pollution de l'eau. Comme un anion, le nitrate est facilement lavé sur le sol, dans les couches profondes du sol et dans les eaux souterraines, ou dans les ruisseaux, les rivières et les lacs. Lixivier peut provoquer une perte de 50% à 70% de N entrées (Raun et Johnson, 1999). En outre, l'utilisation d'engrais de synthèse peut conduire à l'émission d'oxyde nitreux (N2O) par les bactéries du sol qui utilisent le nitrate comme substrat. N2O est un vert plus puissant gaz à effet de que le dioxyde de carbone (CO2) (Galloway et al., 2003; Butterbach-Bahi et al, 2013.).

De la fois écologique et un point de vue économique, et à la lumière d'une population mondiale croissante, le développement de variétés de cultures productives qui nécessitent une faible apport d'azote est de haute priorité.

Introduction en français traduite par Google-Translate

Hautement cultures productives sont d'une grande importance pour répondre à la forte demande de la population mondiale croissante. Ainsi, il est nécessaire de comprendre comment l'azote peut être utilisé efficacement dans les cultures (Canas et al, 2009;. Gupta et al, 2012)..

En général l'utilisation d'azote Efficiency (NUE) est définie comme la production de céréales par unité de N disponible dans le sol (Moll et al, 1982;. Hirel et al, 2001;. Gallais et Hirel, 2004). Deux facteurs contribuent à NUE: (i) l'efficacité de l'azote d'absorption, qui est la capacité d'une plante à prendre N du sol sous forme de nitrate et / ou d'ammonium, et (ii) l'efficacité d'utilisation de l'azote, qui est la capacité à utiliser N acquise pour la production de grains (Good et al., 2004). Les composants de NUE sont des espèces-et spécifiques du génotype, mais peuvent également être influencées par des interactions génotype-environnement (Hirel et al., 2007).

Différentes approches ont été utilisées pour étudier la base de NUE dans les cultures à un agronomique, métabolique, niveau biochimique, moléculaire ou génétique. La plupart des recherches ont été menées dans le maïs (Hirel et al, 2001;. 2005a; 2005b; Gallais et Hirel, 2004; Canas et al, 2009;. 2010; Amiour et al, 2012;. Gupta et al, 2012).. Les marqueurs biochimiques sont fréquemment utilisés comme indicateurs de l'état nutritionnel des plantes à différents stades de développement. Thesemarkers comprennent l'activité GS, la teneur en nitrates, teneur totale en protéines, la teneur en chlorophylle et la teneur totale en N. Ces marqueurs ont été utilisés pour comprendre ce qui limite N assimilation et remobilisation dans le maïs (Gallais et Hirel, 2004; Hirel et al, 2005a;. 2005b; Canas et al, 2009;. 2010; Amiour et al, 2012.), Mais aussi dans d'autres cultures telles que le blé (Kichey et al, 2005;.. Ping et al, 2011).

l'activité GS est l'un des principaux facteurs qui contrôlent la croissance des céréales, comme mentionné précédemment (Hirel et al, 2005b;. Tabuchi et al., 2005; Martin et al., 2006). Il a été utilisé comme marqueur pour l'assimilation à la fois N et N inorganique recyclage (Hirel et al., 2001). Une étude génétique quantitative en mettant l'accent sur la productivité du grain de maïs a montré une forte coïncidence entre QTL pour feuille activité GS et le rendement en grains (Hirel et al., 2001), et il existe une corrélation positive entre l'activité GS et le rendement en grains parmi les génotypes de maïs (Gallais et Hirel, 2004). De même, dans le blé, il y avait une forte corrélation positive entre l'activité GS et végétale teneur totale en N (Kichey et al., 2005).

En outre, dans le sorgho, feuille activité GS observée au nitrate d'ammonium et de la nutrition principalement nitrate est due à une accumulation de chloroplastique GS2 alors que l'activité GS1 cytosolique accumulé dans les racines icreased aussi sous le nitrate d'ammonium, mais il a montré à être plus élevés dans les usines d'ammonium traité (El -Omari et Nhiri, 2015).

Teneur en nitrates peut être considéré comme un marqueur métabolique N capacité d'absorption au cours des premières étapes du développement du maïs parce que, après l'apparition des soies, l'assimilation du nitrate ralentit comme indiqué dans le maïs cultivé en plein champ. Si une corrélation positive significative a été observée entre la teneur en nitrates des feuilles de maïs jeunes plants et le rendement en grains quel que soit le niveau de N alimentation (Hirel et al, 2001;. Gallais et Hirel, 2004).

Le statut N de la plante a été montré à surveiller en utilisant la protéine totale et la teneur totale N au cours des étapes de remplissage des grains de maïs et de blé (Hirel et al, 2005b;. Kichey et al., 2005). Si, au cours des stades tardifs, des protéines de feuilles et le total des feuilles N diminue progressivement en raison de la dégradation des protéines de feuilles pour fournir N pour les grains pendant le remplissage du grain (Waters et al., 1980).

Chlorophylle est l'un des principaux marqueurs utilisés au cours de la sénescence des feuilles, car sa dégradation est l'un des événements majeurs qui se produit pendant le passage de N assimilation à N remobilization (Hirel et al., 2005a). Comme N est un constituant essentiel de la chlorophylle (Kafle et Sharma, 2015), il a été démontré par des études effectuées sur le maïs (Hirel et al., 2005b) et le blé (Kichey et al., 2005) une corrélation positive entre la chlorophylle ant totale N contenu dans les différentes étapes de la feuille.

Au niveau moléculaire, la base de la réponse des plantes à différentes conditions N a été identifié par différents gènes sensibles. Dans Arabidopsis, une analyse de puces à ADN a montré une différence dans l'expression de certains gènes impliqués dans le métabolisme de l'azote en tant que gènes NR NRT1 et GS dans les plantes cultivées sous différentes concentrations en nitrates. NRT1 gène, par exemple, a montré une réponse plus élevée à un faible apport en nitrate avec une certaine diminution lors d'une exposition à des conditions de nitrates (Wang et al., 2000). Dans le maïs, les niveaux de gènes FNR de transcription modifiés sous les niveaux de nitrates réduits dans le sol. Où, les gènes ZmNRT2.1 et ZmNRT2.2 ont été trouvés pour être avec les niveaux de transcription plus élevés que ceux des ZmNRT1.1A et ZmNRT1.1B à N limitant conditions (Garnett et al., 2013). Dans le blé, l'expression de NRT2.1, NRT2.2 et NRT2.3 a diminué sous le N alimentation haute (Ping et al., 2011).

1.5 Thesis objectives

1.5 Thesis objectives

NUE is a complex trait, involving the interaction of genetic and environmental factors (Hirel *et al.*, 2001). NUE variability has been studied extensively in *Arabidopsis thaliana* (Chardon *et al.*, 2010) and cereals such as maize (Hirel *et al.*, 2001; 2005a; 2005b; Gallais and Hirel, 2004; Canas *et al.*, 2010; Amiour *et al.*, 2012) and wheat (Kichey *et al.*, 2005). These studies showed that plants respond to nitrogen availability with a variety of metabolic, cellular and developmental processes. However, little is known about how sorghum metabolism responds to N availability. Gardner *et al*. (1994) hypothesized that sorghum NUE is related to anatomical, morphological, and physiological traits including the production of fewer, larger and thicker leaves, remobilization of N to younger tissues, and lower dark respiration rates. Analyses of transcript patterns in four N stress-tolerant and three N stress-sensitive sorghum genotypes revealed that high affinity nitrate transporter genes were differentially expressed (Gelli *et al*., 2014). The abundance of gene transcripts for high affinity nitrate transporters was higher in the roots of stress-tolerant genotypes under N deficiency (Gelli *et al*., 2014). At the physiological level, studies have mainly focused on a hybrid between sorghum and sudangrass to characterize nitrate and ammonium assimilation, and the regulation of ammonium tolerance by GS in the plant roots (El Omari *et al*., 2010; El-Omari and Nhiri, 2015). Results obtained till now can not depict a comprehensible picture of N sorghum metabolism in one hand and can not help to understand how sorghum can adapt to variable N availability. Our research project is aiming in a long term to understand the genetic, molecular and physiological bases of NUE in sorghum.

This PhD thesis is one of the first that will contribute to this research project and is, therefore, largely explorative. It covers two major tasks, i) an assessment of morphological parameters in a subset of seven sorghum accessions representative of the genetic diversity in sorghum during vegetative development under two different N regimes, and ii) an assessment of different biochemical, physiological and molecular markers during early vegetative development in the same accessions. These markers include glutamine synthetase (GS) activity, nitrate, chlorophyll, protein, total nitrogen and carbon content and were indicative of the nutritional status in different species as maize (Hirel *et al.*, 2005a; 2005b), wheat (Kichey *et al.*, 2005) and *Arabidopsis* (Chardon *et al.*, 2010).

This PhD project will help answer the following questions:

1. Do sorghum accessions vary in their morphological and developmental response to different nitrate regimes?

2. Do sorghum accessions vary in their physiological response to different nitrate regimes?

3. Which of the physiological, biochemical and genetic markers can best explain variability in nitrogen uptake and nitrogen use and could be used as a physio-agronomic indicator for sorghum N status in future studies?

The answer to these questions will help to improve our knowledge about the physiological and molecular response of sorghum leaves and roots to N stress during early vegetative growth. This will also be useful to start filling the gap concerning N metabolism in one of the most important crops in arid regions.

2 Methodology

2.1 Plant Material

Seven accessions of Sorghum (*Sorghum bicolor* L.) were chosen from a panel of 210 accessions from the core collection (Deu *et al*., 2006) representing *world-wide* sorghum diversity and maintained at CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), Montpellier, France. These seven accessions were chosen because they are RNA sequenced for leaves, flowers and grains, belonging to different sorghum races and thus representing sorghum diversity.

Table 3. The main characteristics of the accessions under study as supplied by CIRAD

Variety code	ICRISAT Or CIRAD ID	RACE	Days from sowing to flag leaf	Days from sowing to flowering	Plant height to the tip of panicle (cm)	RNA sequence for leaves, flowers and grains
34	IS 6193	Durra	70	80	260.8	Yes
71	IS 14317	Guinea Roxburghii	56.5	65	315	Yes
153	IS 29407	Kafir-caudatum	45.5	54.5	215	Yes
169	IS 30436	Caudatum	39	45.5	224.2	Yes
199	SSM 973	Durra	83	91	341.7	Yes
201	SSM 1049	Bicolor	59	67	302.5	Yes
202	SSM 1057	Guinea Margeretiferum	50	57.5	277.5	Yes

Sorghum seeds were sterilized with 5% of NaOCl for 15 minutes, washed thoroughly with sterile water, and then germinated on wet Whatman papers in Petri dishes. Germinated seeds were transferred after three days to pots (diameter: 8 cm; height: 10 cm) containing vermiculite (particle size: 1-6 mm) with five seedlings per pot. Plants were transferred after 4 weeks to larger pots (diameter: 15; height: 15 cm) with 3 plants per pot. After two additional weeks, plants were transferred to fresh pots (15cm/15cm) one plant per pot. Plants were grown in a growth chamber under 75% humidity and 10h light and 28°C/ 14h dark and 22° C. The type of lamps was (Lampe a lodure Metallique haute pression, 150W) with light intensity between 90 and 110 µE/m^2/sec. Pots were shifted randomly 3 times per week to avoid light and position effects.

For low (N-) fertilization, plants received twice a week 100 ml per pot of a complete nutrient solution containing 0.5 mM KNO_3, 0.375 mM $KH2PO_4$, 0.125 mM $K2HPO_4$, 0.375 mM $MgSO_4$, 0.1 mM NaCl, 1.25 mM $CaSO_4$, 5mg/L Fe-EDTA and micronutrients (48.5 x 10^{-6} mM H_3BO_3, 10 x 10^{-6} $MnSO_4$, 16.5 x 10^{-5} Na_2MoO_4, 6 x 10^{-6} $ZnCl_2$, and 0.5 x 10^{-6} $CuSO_4$(Arnon *et al*., 1938; El-Omari *et al*., 2010). For optimal (N+) fertilization, plants received the same nutrient solution but containing 10mM KNO_3. 0.5 and 10 mM KNO_3 was previously shown to generate N deficiency stress or provide optimal growth for sorghum plants, respectively (El-Omari *et al*., 2010). Plant height and number of leaves were weekly surveilled.

Sampling for biochemical assays was always conducted between 9:00 and 12:00 am. Three individual plants from different pots were harvested at two, four and six weeks post emergence per accession and N condition. Roots were gently washed with deionized water to remove attached vermiculite and quickly dried. Leaf and root samples were weighted, snap-frozen in liquid nitrogen and stored at -80 °C until further use.

2.2 Metabolite extraction and analyses, enzymatic assay

Each leaf or root sample was ground to a fine powder under liquid nitrogen, and the content of nitrate, total N, total C, total protein, and chlorophyll, as well as GS activity were determined.

2.2.1 Nitrate

10 mg of freshly ground material were dissolved in 200 µl of distilled water and kept on ice for one hour. During this time, each sample was thoroughly mixed with a vortex every 15 minutes. Following centrifugation for 10 min at 4°C with 20000 g, 160 to 180 µl of the supernatant were recovered. N^+ samples were diluted 1:10 and N^- samples were diluted 1:2 with distilled water. These dilutions were used for nitrate quantification as described in Miranda *et al*. (2001), based on reduction of nitrate to nitrite by Vanadium (III) in combination with detection by the Griess reaction (Tasikas *et al*., 2007).

2.2.2 Total N and C

5 mg of ground material were dried using an oven at 70 °C and used to determine total N and total C content based on the combustion method of Dumas, using an N elemental analyzer (Flash 2000, Thermo Scientific).

2.2.3 GS enzymatic assay, Dosage of total proteins and chlorophyll

500 µl of extraction buffer containing 1 mM Na-EDTA, 1 mM $MgCl_2$, and 100 mM Tris-HCl,

10 mM β-mercaptoethanol, 1 mM leupeptin and insoluble Polyvinylpyrrolidone (PVP) were added to 50 mg of ground plant material and mixed twice thoroughly with a vortex for 45 s. Samples were kept on ice until they were used for dosage of chlorophyll and protein, and for the GS essay.

Chlorophyll dosage was conducted according to Arnon (1949). 50 µl from the leaf homogenate were mixed with 950 µl of acetone and kept at 4°C in the dark for 24 h. After a 10 min centrifugation with 13000 g at 4°C, chlorophyll concentration was determined by measuring the absorption of the supernatant at 652 nm by spectrophotometer against pure acetone.

The remaining 450 µl of the homogenate were centrifuged with 20000 g at 4 °C during 15 min and the supernatant were used for total protein quantification and the GS assay. 10 µl of the supernatant were used for protein quantification according to Bradford (1976) using Bovine serum albumin as a standard.

The remaining 440 µL of the homogenate were used for the GS enzymatic essay according to O'Neal and Joy (1973), using AGH (Gamma glutamyl hydroxamate) as a standard. The enzymatic essay was performed on the obtained supernatant by an ATP-dependant reaction catalyzed by GS activity.

2.2.4 Statistical Analyses

Tables of results for metabolite contents and GS activity are presented as means over three replicates with standard errors (SE). Statistical analyses were done using the non-parametric Kruskal-Wallis test, implemented in Statistica version 6.0 (Statsoft), with a significance threshold of $p \leq 0.05$. For quantitative RT-PCR (qRT-PCR) results, simple regression was done using Statistica version 6.0 and the residuals obtained were used for further statistical analyses that evaluated by Kruskal-Wallis test. P and H values represent the significant difference and Kruskal-Wallis coefficient, respectively. For all results, significance at 0.05, 0.01 and 0.001 probability levels is represented by *, **, *** respectively. Possible correlations between variables were investigated with significance levels reported at $p \leq 0.01$ and $p \leq 0.001$ based on Spearman's rank correlation using Statistica. ρ is the Spearman rank coefficient.

2.3 Expression Analyses of *NRT1.1 genes*

Total RNA was extracted from 100 mg of ground leaves or roots using the RNeasy® Plant Mini kit (Qiagen) following the manufacturer's instructions. DNase treatment was performed on-column during extraction using On-Column DNase kit from Qiagen according to manufacturer's instructions. RNA concentration was determined with a Nanodrop 2000 (Thermo Scientific). First strand cDNA synthesis was performed on 300 ng RNA with the long range 2step RT-PCR (Qiagen) kit according to manufacturer recommendations.

Classical PCR was performed in a total volume of 30 µL of adequate buffer containing 0.2 mM dNTPs, 0.2 µM of each primer, 5 % (w/v) DMSO and 1.5 U Taq DNA polymerase from the Taq-DNA core kit (MP Biomedical). PCR cycling conditions consisted of an initial denaturation phase at 94 °C for 4 min, followed by 35 cycles (95 °C for 30 sec, 60 °C for 30 s, 72 °C for 1 min) and a final extension at 72 °C for 10 min. Electrophoresis separation of PCR products was done on 2 % agarose gels.

Amplified PCR products were cloned into the pCR 2.1-TOPO plasmid vector using the TOPO-TA cloning kit (Life Technology). Recombinant plasmid DNA was prepared using the NucleoSpin Plasmid kit (Macherey-Nagel) and sequenced by Cogenics, Beckman Coulter Genomics.

Primers used for amplification of the reference genes *Elongation Factor 1 alpha* (*EF-1*), and *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and for the studied genes *Nitrate transporter 1.1A, B and C* (*NRT1.1A*, *NRT1.1B*, *NRT1.1C*) were designed using Primer3 v.04.0. Primers are listed in Table 3.

Table 4. Primers used for PCR amplification

Gene	Forward Primer (5'-3')	Reverese Primer (5'-3')	Product size (bp)
GAPDH	CTCAAGGGCATTCTGGGTTA	TATCCCCACTCGTTGTCGTA	152
EF	GAGAAGGAGCCCAAATTCCT	AAAGCGACCAAGAGGAGGAT	111
NRT1.1 A	TCGTGGTGGGAAGAAAAATC	AAGCAGCACACATGACATCC	86
NRT 1.1 B	ATGTATTTTGGCACGCAGGT	ACGCTAGCTGCCATTCATTT	144
NRT 1.1 C	GGCAGCCTCCACAAATTCTA	GCGCTTCTCCTTGTAGACGTA	102

The efficiency of the different primers was determined by a calibration experiment using serial dilutions of a recombinant linearized plasmid of the corresponding gene. The quantitative PCR (qPCR) was done with the FastStart Universal SYBR Green Master (Roch) on a StepOnePlus thermocycler (Applied Biosystem) in a total volume of 15 µl with 0.3 µM of each primer and 2 µl of diluted cDNA by 1/2. PCR cycling conditions consisted of initial denaturation at 95°C for 10min followed by 40 cycles (95°C for 15 sec, 64°C for 30 sec and 72°C for 30sec).

3 Results and Discussion

3.1 Variation in plant leaf number and plant height under different N regimes

Plant leaf numbers and plant height were followed as phenotypic markers for plant development at optimal (N+) and low (N-) N-fertilization during 12 weeks of vegetative growth.

In all accessions the mean number of leaves was higher at N+ than at N- at all time points (Figure 4). After 12 weeks, the mean number of leaves was 11.3 (±) at N+ and 9.0 (±) at N- in all accessions. Some minor differences in mean leaf numbers were observed during the first weeks of development. For example, accessions 201 and 202 had on average about half a leaf less than the other accessions at week 2 in the N- condition. This difference persisted in accession 201 for one additional week but disappeared in accession 202 in week 3. Similarly, accession 199 had a slightly higher number of leaves in week 4 at N-. Starting with week 5 there was no statistical indication for differences in mean leaf number among accessions growing under the same N condition. In conclusion, there appears to be little genetic variation for mean leaf number among the tested Sorghum accessions. Nitrate supply has a clear influence on mean leaf number during vegetative development in all accessions, with plants under nitrogen starvation having fewer leaves.

In contrast to the mean leaf numbers, plant height varied not only in response to N conditions but also among accessions (Figures 5 and 6). Furthermore, growth curves for all accessions under both N regimes showed a particularly strong increase in plant height after week 6. This effect is probably caused by transfer of plants to new pots in week 6. Until week 6, plants of the same accession were grown together, and after transfer plants were grown individually. Hence, competition among plants of the same pot may have influenced growth during the first 6 weeks of this experiment.

Plants growing under the N+ condition were, in general, significantly taller than those growing at N- (Figures 5 and 6; Table 5). At week 6, mean plant height varied between 42.6 cm (accession 153) and 58.2 cm (accession 201) at N+ and between 32.2 cm (accession 153) and 48.1cm (accession 169) at N-. At week 12, mean plant height varied between 101.1 cm (accession 199) and 164.8 cm (accession 201) at N+ and between 79.4 cm (accession 34) and 116.3 cm (accession 169) at N-. Remarkably, plant height of several accessions (169, 201, and 202) grown under nitrogen starvation was higher than plant height of 34, 153, and 199 under nitrate optimum.

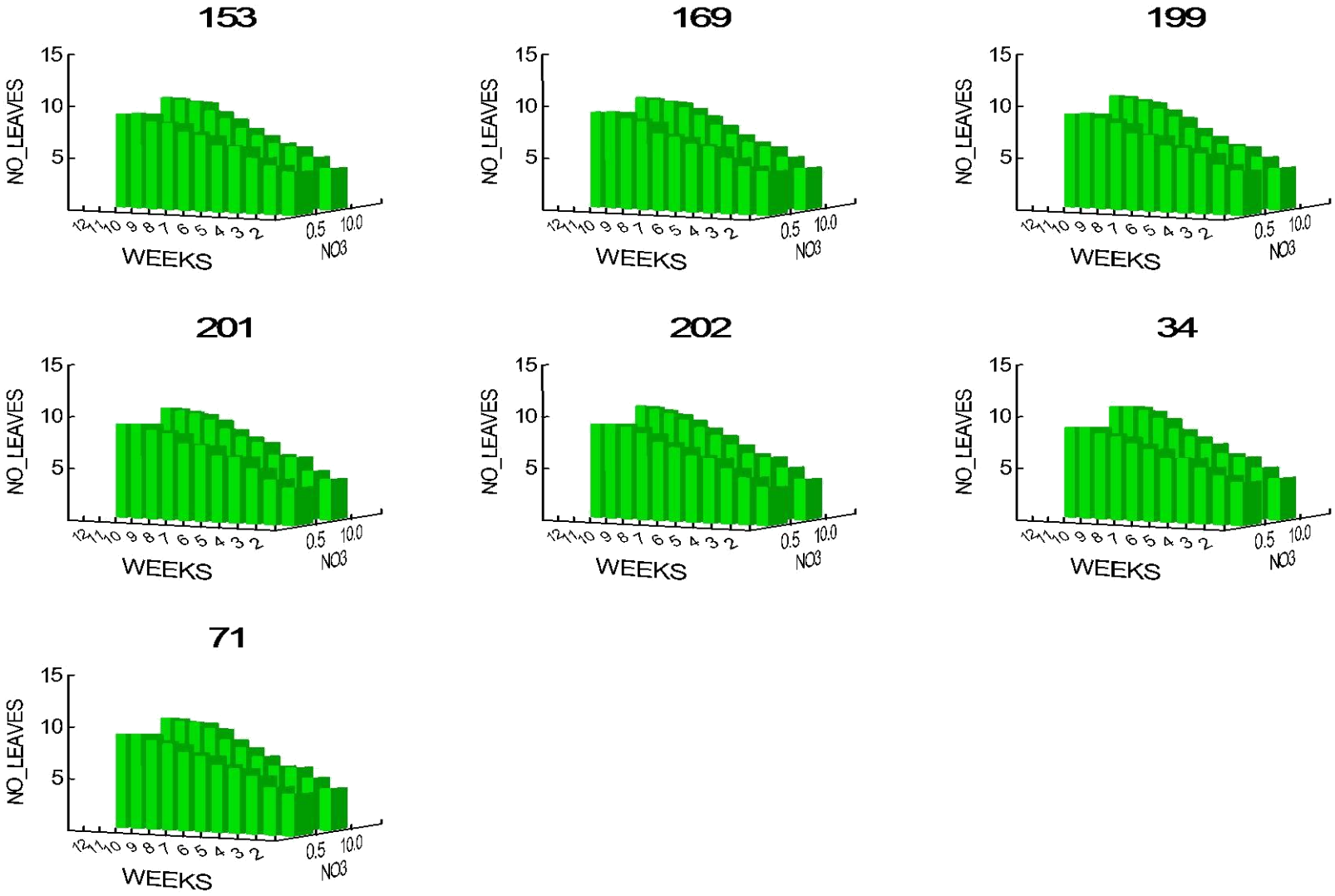

Figure 4. Mean number of leaves per accession under N+ (10mM) and N- (0.5 mM) conditions during the first 12 weeks of growth.

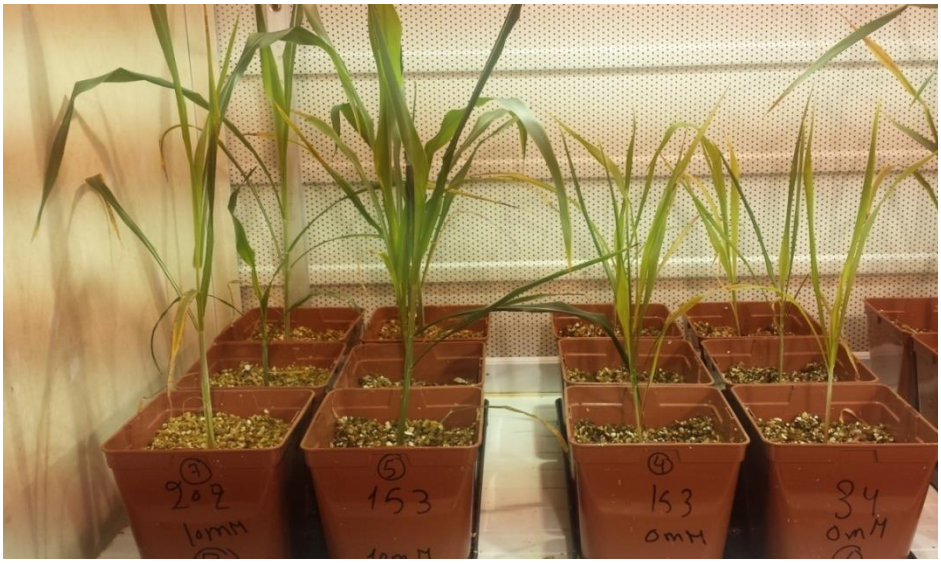

Figure 5. Six-week old sorghum plants growing under different N regimes. Plants with optimal nitrate supply (left) grow taller than plants under nitrogen starvation (right).

Differences in plant height under both N conditions were large, between 40% and 57% (accessions 169, 201 and 202), medium (between 30 % and 40 %; accessions 34, 71 and 153) or small (accession 199). In the latter case, the difference in plant height between N+ and N- was 4.6 % at week 6 and 6.5 % at week 12, but these differences were not significant. It is also noticeable that at week 6, the differences in plant height between both conditions weren't significant after Holm correction but they remained significant at week 12.

Table 5. The influence of nitrogen supply on plant height in different Sorghum accessions at weeks 6 and 12. Statistical significance was inferred by Kruskal-Wallis tests, and a Holm-Bonferroni correction was applied to correct for multiple testing. H is kruskal-Wallis Coefficient, P is the significance value, For each accession: $6 \leq N \leq 9$. Values in italics are those that remained significant after a Holm-Bonferroni correction

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Accession	6 Weeks		12 Weeks	
	H	P	H	P
34	13.5	0.03	7.5	*0.006*
71	9.8	0.01	8.7	*0.003*
153	3.6	0.06	8.7	*0.003*
169	9.8	0.01	8.7	*0.003*
199	1.1	0.35	0.02	0.8
201	9.8	0.01	8.7	*0.003*
202	13.5	0.03	8.7	*0.003*

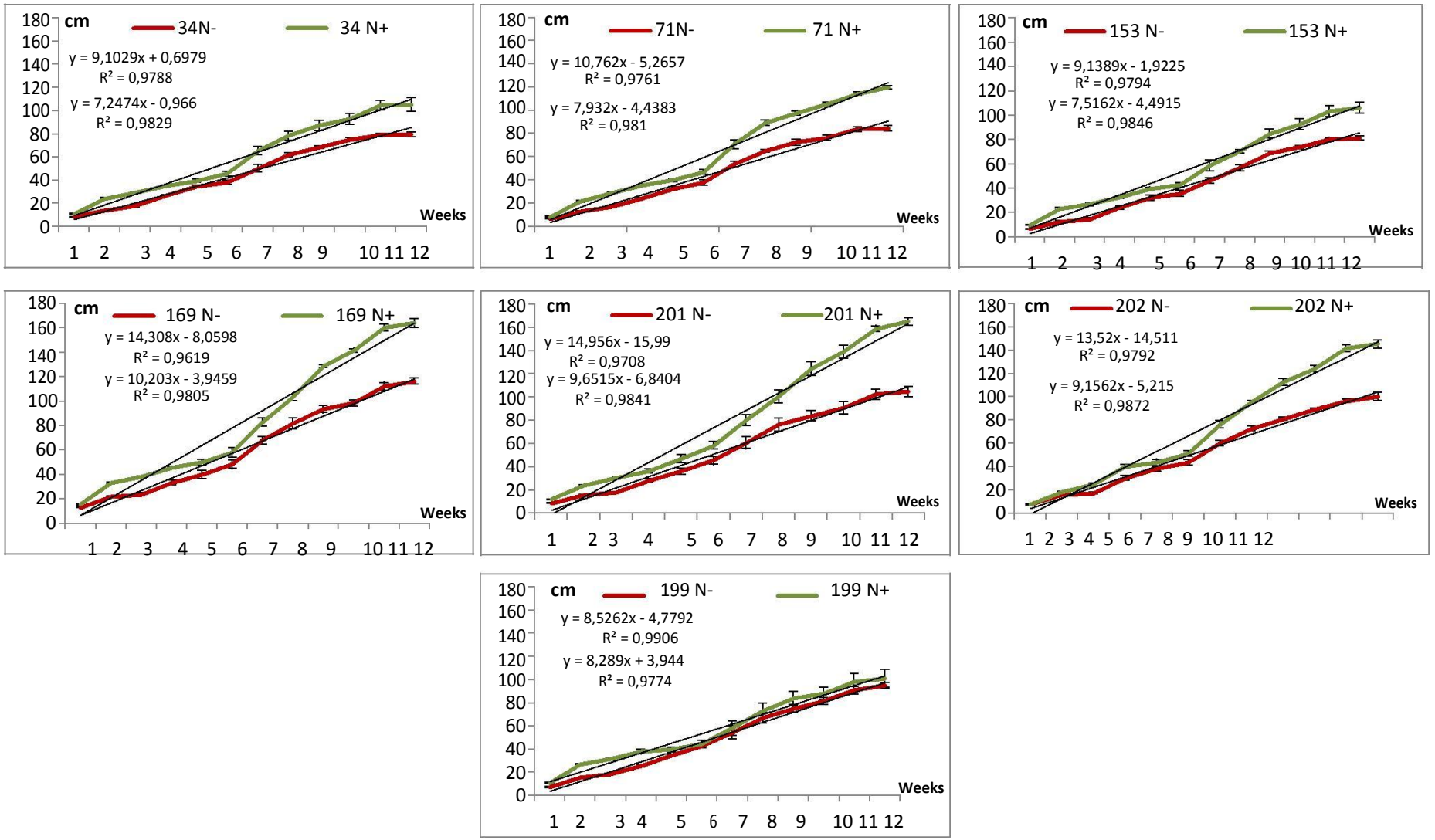

Figure 6. Plant height of 7 Sorghum accessions at N+ and N- during the first 12 weeks of vegetative growth. Shown is plant height at different time points (± standard errors) with optimal (green) and low (red) nitrate supply, as well as linear regression lines (black) for both conditions. Regression equations and determination coefficients (R^2) are indicated.

3.2 Physiological response of sorghum under optimal and deficiency levels of nitrogen fertilization during early stages of development: Leaves and Roots

Seven sorghum accessions were investigated for their C and N content as well as GS activity under two different N regimes. Samples were taken from leaves and roots at 2, 4, and 6 weeks post emergence.

3.2.1 Effect of N on metabolites accumulation and GS activity in sorghum leaves and roots

N had a strong effect on several physiological markers representative of plant N status, including nitrate, total N, chlorophyll and protein content as well as GS activity (Tables 6 A & 6B). In general, these markers had significantly higher values under optimal N supply than under N starvation in both leaves and roots (Figure 7, Tables 10, 11, 12, & 13). Likewise, GS activity was higher under N+ than under N- in both leaves and roots. By contrast, total C content was more or less independent from N conditions, except for accession 153, which had significantly less total C under N- than under N+ in leaves at all three points of sampling.

Although differences between N+ and N- condition were in general significant, there were a few exceptions. These include nitrate content in leaves of accession 169, protein content in leaves of accession 71, protein content in leaves and roots of accession 169, protein content in roots of accession 202, total N in leaves of accession 202 and in roots of accession 169 (Tables 6A & 6B). Of these parameters, total N content in leaves of accession 202 was particularly remarkable because of its very high level under N- which was comparable to or even exceeded leaf total N of other accessions under N+.

Table 6. Effect of N condition on physiological markers in sorghum leaves (A) and roots (B). Statistical differences were evaluated with Kruskal-Wallis tests. H is the Kruskal-Wallis coefficient, P is the significance value; for each accession: $17 \leq N \leq 18$.

A) Leaves

Accession	GS Activity		Nitrate		Proteins		Chlorophyll		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P	H	P
34	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	3.6	0.005 **	12.7	0.0001 ***	1.2	0.27
71	12.8	0.0001 ***	12.8	0.0001 ***	0.2	0.63	6.3	0.010 **	10.1	0.001 **	3.5	0.06
153	12.2	0.0001 ***	2.6	0.0001 ***	6.8	0.009 **	8.6	0.003 **	10.9	0.0001 ***	7.7	0.01*
169	12	0.0001 ***	10.9	0.10	2.4	0.12	6.6	0.009 **	12	0.0001 ***	3.2	0.07
199	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	9.3	0.002 **	2.1	0.15
201	12.8	0.0001 ***	12.8	0.0001 ***	12.2	0.0001 ***	7.7	0.005 **	12.8	0.0001 ***	1.6	0.20
202	12.8	0.0001 ***	12.8	0.0001 ***	9.8	0.001 **	8.2	0.004 **	0.01	0.894	0.09	0.76

B) Roots

Accession	GS Activity		Nitrate		Proteins		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P
34	12.8	0.0001 ***	12.8	0.0001 ***	12 .8	0.0001 ***	10.1	0.001 ***	2.3	0.13
71	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	9	0.002 **	0.11	0.74
153	12	0.0001 ***	12.8	0.0001 ***	4.9	0.026 *	10.1	0.001 ***	0.2	0.64
169	12.8	0.0001 ***	9.8	0.001 ***	0.23	0.63	1.4	0.24	2	0.16
199	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	10.1	0.001 ***	0.68	0.41
201	12.8	0.0001 ***	12.8	0.0001 ***	12.8	0.0001 ***	10.1	0.001 ***	0.88	0.35
202	12.8	0.0001 ***	12.8	0.0001 ***	0.15	0.69	9	0.002 **	0.18	0.67

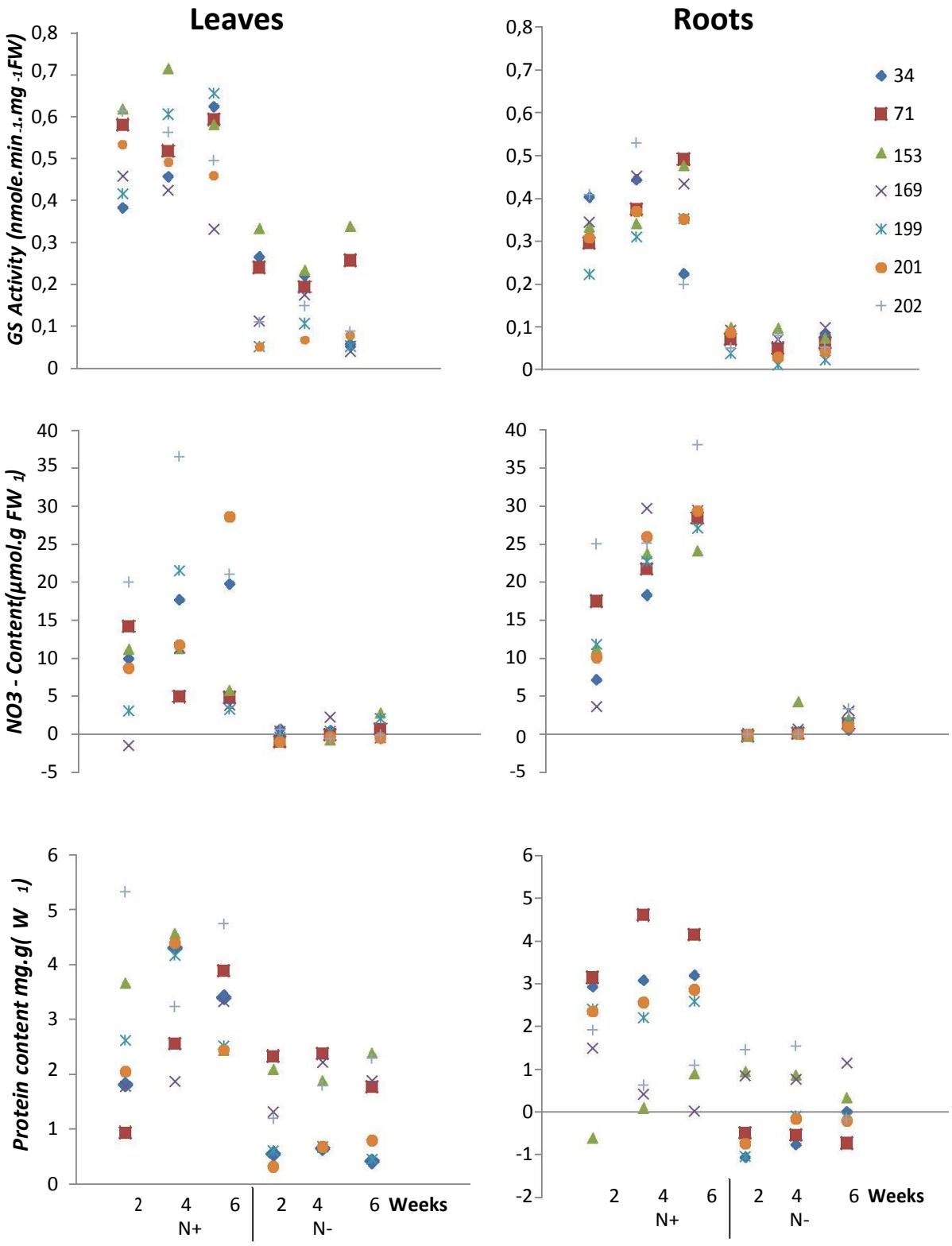

Figure 7. GS activity and metabolite content in leaves (left) and roots (right) of seven sorghum accessions grown under two different N regimes. Each graph depicts results for N+ (left) and for N-(right) at 2, 4 and 6 weeks post emergence.

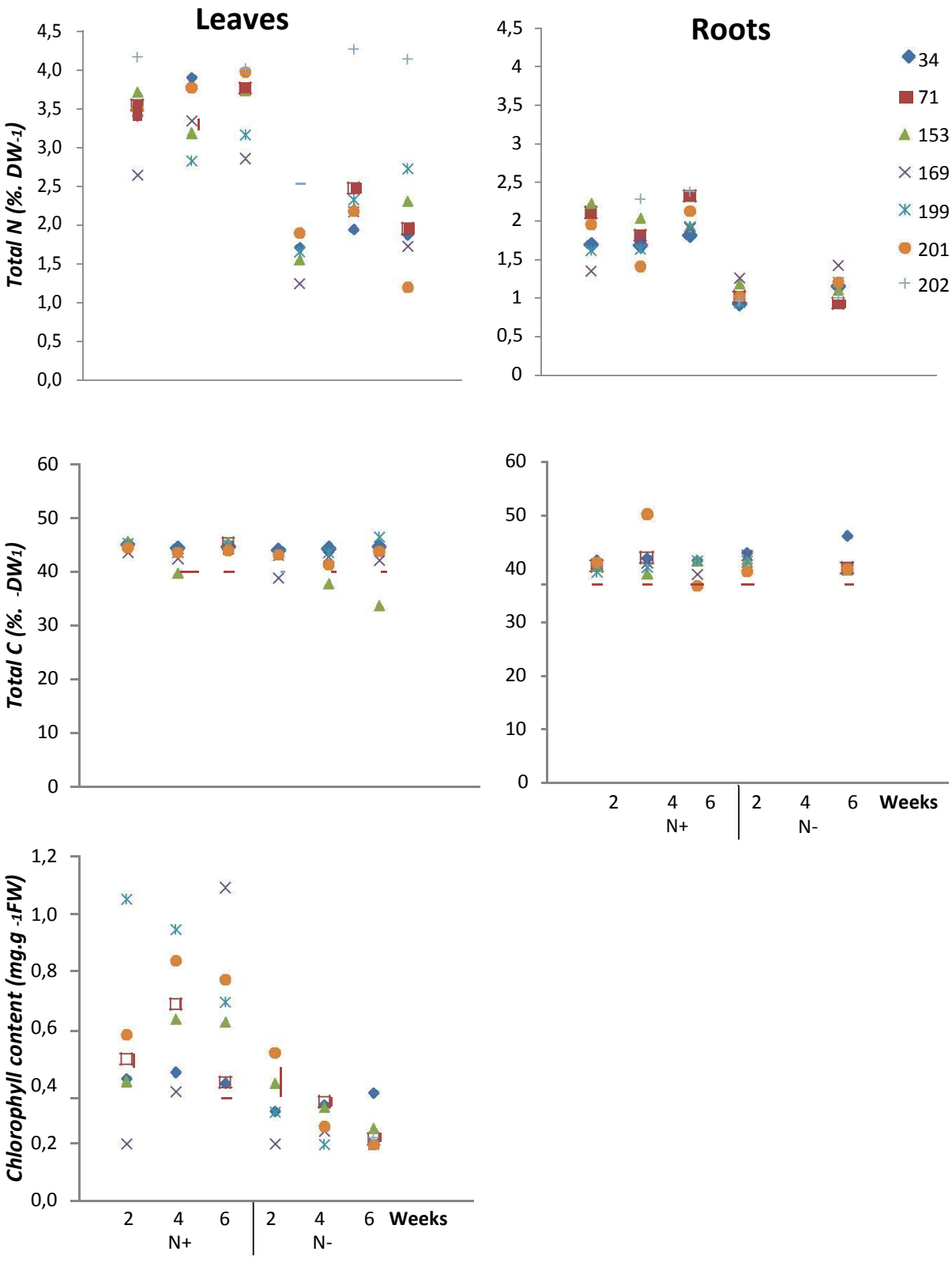

Figure 7 (continued). GS activity and metabolite content in leaves (left) and roots (right) of seven sorghum accessions grown under two different N regimes. Each graph depicts results for N+ (left) and for N- (right) at 2, 4 and 6 weeks post emergence.

3.2.2 Effect of genotype on metabolite content and GS activity in sorghum leaves and roots

The different accessions varied for most of the physiological markers in both leaves and roots, indicating genotypic differences for the response to N regimes among accessions (Figure 7). Table 7 depicts the results from a statistical analysis for genotype effects using the data from the three time points of sampling.

The effect of genotype was significant for most markers in leaves, except nitrate content under N- and protein content under N+ condition. In roots, significant differences were observed for protein and total N under N+, and for GS activity, protein and total C content under N- condition.

Furthermore, rank order of accessions varied for the different physiological parameters between both N regimes for both leaves and roots, indicating genotype-by-environment interaction.

Table 7. Effect of genotype on physiological markers in sorghum leaves (A) and Roots (B). Statistical differences were evaluated by Kruskal-Wallis tests**.** H is Kruskal-Wallis coefficient, P is the significance value. For each accession: 60 ≤N≤63

A) Leaves

		GS Activity	Nitrate	Proteins	Chlorophyll	Total N	Total C
N+	H	13.5	26.9	8.1	16.9	20.8	25.5
	P	0.03 *	0.0001 ***	0.23	0.009 **	0.002 **	0.0001 ***
N-	H	34	10.3	45.3	12.8	19.8	31.1
	P	0.0001 ***	0.10	0.0001 ***	0.05 *	0.003 **	0.0001 ***

B) Roots

		GS Activity	Nitrate	Proteins	Total Nitrogen	Total Carbon
N+	H	9	8.7	54.6	21	9.4
	P	0.17	0.19	0.0001 ***	0.0001 ***	0.15
N-	H	28.7	4.4	38.2	5.7	13.6
	P	0.0001 ***	0.63	0.0001 ***	0.45	0.033 *

3.2.3 Time-dependent patterns of GS activity and Metabolites accumulation in sorghum leaves and roots

GS activity, nitrate content, protein content and total N content displayed considerable variation in both leaves and roots among the three time points of sampling (2, 4 and 6 weeks of plant age) and among genotypes (Figure 7). Likewise, leaf chlorophyll content was quite variable among time points and genotypes. In general, this variation was more pronounced under N+ than under N-. By contrast, total C content remained – with few exceptions (please see above) – relatively stable among time points of sampling, N conditions and accessions.

In leaves, no general effects of plant age on GS activity or metabolite patterns were discernible (Figure 7, Tables 8, 10 & 11). Nonetheless, some accessions appeared to display significant, age-dependent differences in GS activity or metabolite content (Table 8). For example, GS activity increased in accessions 34 and 199 under N+, nitrate content in accession 34 under N+, protein content in accession 71 under N+ and chlorophyll content in accession 169 under N+ (Table 10). Similarly, nitrate content increased in accession 71 under N- and total N in accession 199 under N- (Table 11). Other accessions, however, displayed a different pattern, with a significant decrease in total C in accession 202 under N+ (Table 10) and in GS activity in accession 34 under N- (Table 11). Finally, some parameters first increased and then declined, *e.g.*, nitrate in accession 169 under N+, protein content in accession 34 under N+, total N in accession 169 under N+, GS activity in accession 169 under N-, nitrate content in accession 201 under N- or total N in accession 169 under N-, or first declined and then increased, *e.g.*, total C in accession 199 under N+, GS activity in accession 71 under N- and nitrate content in accession 153 under N- (Figure 7, Tables 10 & 11).

Table 8. Effect of plant age on physiological markers in sorghum leaves under N+ (A) and N- (B). Statistical differences were evaluated with Kruskal-Wallis tests. H is Kruskal-Wallis coefficient. P is the significance value. (For each accession 8 ≤N ≤9)

A) Leaves under N+ condition

Accession	GS Activity		Nitrate		Proteins		Chlorophyll		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P	H	P
34	7.2	0.03 *	5.9	0,05 *	7.2	0.02 *	0.6	0.73	3.5	0.18	0.6	0.72
71	1.8	0.30	5.4	0,06	7.2	0.03 *	5.6	0.06	3.8	0.15	1.1	0.56
153	4.2	0.10	5.6	0,06	2.4	0.28	5.4	0.06	1.9	0.39	3.8	0.15
169	5.1	0.07	6.2	0,04 *	3.8	0.15	6.2	0.04 *	6.3	0.04 *	0.8	0.67
199	7.2	0.03 *	5.4	0,06	5.6	0.06	3.5	0.17	1.9	0.39	7.2	0.03 *
201	0.32	0.80	5.6	0,06	3.2	0.19	1.4	0.49	1.7	0.43	3.8	0.15
202	0.62	0.70	5.4	0,06	1.9	0.39	5.6	0.06	2.7	0.20	7.2	0.03 *

B) Leaves under N- condition

Accession	GS Activity		Nitrate		Proteins		Chlorophyll		Total N		Total C	
	H	P	H	P	H	P	H	P	P	H	H	P
34	7.2	0.03 *	3.6	0.16	0.35	0.84	1.2	0.56	3.8	0.15	2.2	0.33
71	5.9	0.05 *	6	0.05 *	2.48	0.28	5.06	0.08	0	1.00	0	1.00
153	0.8	0.60	7.2	0.03 *	3.2	0.20	4.4	0.11	5.6	0.06	1.7	0.43
169	7.2	0.03 *	6.4	0.04	4.3	0.11	3.5	0.18	5.92	0.05 *	0.62	0.73
199	5.4	0.07	5.6	0.06	6.6	0.73	5.6	0.06	7.2	0.03 *	4.35	0.11
201	2.2	0.33	5.9	0.05 *	1.6	0.43	4.4	0.11	3.8	0.15	5	0.08
202	3.8	0.15	2.7	0.25	4.6	0.10	3.5	0.18	3.3	0.19	4.6	0.10

By contrast, roots showed a general tendency for an increase in GS activity, nitrate content and, to a lesser extent, in protein content under optimal nitrate supply, while root total N and total C content appeared to be unaffected by plant age (Figure11, Table 9A & 12).

Under N+, root GS activity increased from week 2 to week 4, except for accession 153 where GS activity was nearly unchanged. A further increase occurred from week 4 to 6 in accessions 71 and 153, while GS activity remained more or less constant in accessions 169, 199 and 201 and declined in accessions 34 and 202 (Table 12).

Root nitrate content under N+ increased in all accessions, with particularly high values for nitrate content in accession 202 (Table 12). Statistical analysis supported the presence of an age effect on root nitrate content under N+; nitrate content differed significantly (accessions 34, 169 and 201) or marginally not significantly (accessions 153, 169, 199 and 202) between sampling dates (Table 9A).

Likewise, protein content tended to increase over time in roots of plants grown with optimal N supply (Table 12), even though this pattern appeared to be more strongly influenced by genotype effects than root nitrate content (Figure 7). In particular, accession 169 deviated from the general trend of an increase in protein content and this parameter decreased almost linearly over time in this accession (Figure 7, Table 12).

Under N-limiting conditions, there appeared to be no general pattern across genotypes for any of the investigated parameters (Figure 7, Table 13). Nonetheless, some genotypes showed significant age-dependent differences for some of the parameters (Table 9B). For example, total N content increased in accessions 169, 199 and 291 from week 2 to week 6[1], while it decreased in accession 153, and GS activity decreased slightly in accession 71 and more strongly in accession 202 from week 2 to week 4 (Tables 9B & 13). Nitrate content increased over time in accessions 71 and 199 from week 2 to week 6 (Tables 9B & 13). In accession 153, it first increased from week 2 to 4 and then declined from week 4 to 6 (Tables 9B & 13). Soluble proteins were undetectable in the majority of accessions at all three sampling dates, except for accession 153 where protein content appeared to decline over time, accession 169 where protein content remained more or less constant, and accession 202 at weeks 2 and 4 (Table 13).

[1] Root material was limited for week 4, so that analyses for total N and total C content could not be conducted.

Table 9. Effect of plant age on physiological markers in sorghum roots under N+ (A) and N- (B). Statistical differences were evaluated with Kruskal-Wallis tests. H is Kruskal-Wallis coefficient. P is the significance value. (For each accession $6 \leq N \leq 9$)

A) Roots under N+ condition

Accession	GS Activity		Nitrate		Proteins		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P
34	7.2	0.03 *	7.2	0.03 *	1.2	0.56	1	0.59	0.8	0.84
71	7.2	0.03 *	7.2	0.03 *	7.2	0.03*	5	0.08	5.4	0.07
153	5.4	0.66	5.6	0.06	4.4	0.11	1.2	0.56	2.7	0.25
169	5.9	0.05 *	5.4	0.06	3.2	0.2	5.6	0.06	3.2	0.19
199	5.6	0.06	5.6	0.06	3.2	0.19	2.2	0.33	2.7	0.25
201	1.86	0.39	6.4	0.04*	2.1	0.34	0.8	0.67	3.4	0.18
202	7.2	0.03 *	5.4	0.07	7.2	0.03 *	1.2	0.54	1.2	0.54

B) Roots under N- condition

Accession	GS Activity		Nitrate		Proteins		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P
34	5.4	0.06	5.6	0.06	5.9	0.05 *	1.1	0.28	0.42	0.51
71	7.2	0.03 *	6 .4	0.04 *	1.6	0.43	3	0.08	0	1
153	0.04	0.99	7.2	0.03 *	5.2	0.07	1.2	0.03 *	3.8	0.05 *
169	4.8	0.09	4.3	0.11	1.4	0.49	3.8	0.05 *	2.3	0.13
199	2.8	0.24	6	0.05 *	5.6	0.06	3.8	0.05 *	1.2	0.28
201	7.2	0.03 *	5.6	0.06	5.4	0.07	3.8	0.05 *	0.42	0.51
202	2.8	0.24	5.4	0.06	6.2	0.04 *	0.4	0.51	0.04	0.83

Table10. GS activity and metabolite content of sorghum leaves under optimal N supply (N+). Given are the means from three replicates (± SE) per accession and time point

Accession	Plant age (Weeks)	GS Activity nmole.min^{-1}.mg FW^{-1}		Nitrate µmol.g FW^{-1}		Proteins mg.g FW^{-1}		Chlorophyll mg.g FW^{-1}		Total N %.DW^{-1}		Total C %.DW^{-1}	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
34	2	0.38	0.01	10.00	1.50	1.82	0.18	0.43	0.08	3.41	0.14	44.98	0.15
	4	0.46	0.02	17.74	1.52	4.31	0.10	0.45	0.05	3.90	0.18	44.38	0.61
	6	0.63	0.02	19.83	0.40	3.41	0.27	0.41	0.02	3.74	0.10	44.65	0.45
71	2	0.58	0.05	14.25	0.97	0.94	0.24	0.49	0.02	3.55	0.13	44.74	0.18
	4	0.52	0.04	4.98	0.72	2.56	0.05	0.69	0.03	3.31	0.13	44.87	0.40
	6	0.59	0.01	4.86	0.71	3.89	0.35	0.41	0.08	3.77	0.13	45.24	0.31
153	2	0.62	0.03	11.28	2.15	3.67	0.35	0.42	0.01	3.72	0.26	45.59	0.15
	4	0.72	0.16	11.33	0.63	4.57	1.36	0.63	0.04	3.19	0.42	39.73	5.52
	6	0.44	0.05	5.87	0.95	2.44	0.44	0.62	0.18	3.74	0.20	45.29	0.25
169	2	0.46	0.01	-1.41	0.30	1.79	0.55	0.20	0.11	2.65	0.01	43.61	1.69
	4	0.43	0.04	11.41	3.00	1.88	0.18	0.38	0.04	3.35	0.06	42.42	1.83
	6	0.33	0.00	3.93	0.80	3.33	0.48	1.09	0.00	2.86	0.02	44.21	0.29
199	2	0.42	0.02	3.19	0.36	2.62	0.64	1.05	0.07	3.46	0.21	45.22	0.11
	4	0.61	0.01	21.55	0.60	4.17	0.05	0.94	0.12	2.83	0.51	43.57	0.49
	6	0.66	0.02	3.34	0.44	2.52	0.01	0.69	0.13	3.17	0.07	44.92	0.06
201	2	0.53	0.07	8.76	0.92	2.05	0.24	0.58	0.10	3.50	0.21	44.46	0.16
	4	0.49	0.07	11.78	2.61	4.40	0.49	0.84	0.21	3.78	0.31	43.55	0.15
	6	0.46	0.14	28.68	1.13	2.45	0.97	0.77	0.23	3.98	0.20	43.91	0.41
202	2	0.61	0.07	20.06	1.16	5.33	0.70	0.55	0.03	4.17	0.05	43.98	0.11
	4	0.56	0.07	36.54	2.26	3.24	0.70	0.60	0.07	3.41	0.36	43.10	0.32
	6	0.50	0.12	21.10	4.09	4.74	1.92	0.37	0.04	4.02	0.41	40.76	0.82

Table 11. GS activity and metabolite content of sorghum leaves under N limitation (N-). Given are the means from three replicates (± SE) per accession and time point.

Accession	Plant age (Weeks)	GS Activity		Nitrate		Proteins		Chlorophyll		Total N		Total C	
		nmole.min^{-1}.mg FW^{-1}		µmol.g FW $^{-1}$		mg.g FW $^{-1}$		mg.g FW $^{-1}$		%.DW $^{-1}$		%.DW^{-1}	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
34	2	0.27	0.01	1.14	0.53	0.56	0.20	0.31	0.01	1.71	0.04	44.02	0.17
	4	0.22	0.00	0.50	0.40	0.65	0.18	0.33	0.07	1.95	0.11	44.25	0.17
	6	0.06	0.00	-0.42	0.15	0.42	0.39	0.38	0.05	1.88	0.13	44.62	0.43
71	2	0.24	0.01	-0.95	0.02	2.33	0.21	0.45	0.07				
	4	0.19	0.00	0.00	0.42	2.38	0.45	0.34	0.05	2.47	0.06	43.18	0.88
	6	0.26	0.02	0.70	0.54	1.77	0.17	0.22	0.04	1.95	0.14	44.50	2.57
153	2	0.33	0.01	-0.26	0.03	2.09	0.25	0.41	0.03	1.56	0.03	34.34	0.49
	4	0.24	0.10	-0.70	0.09	1.89	0.03	0.33	0.05	2.22	0.09	37.80	2.13
	6	0.34	0.05	2.90	0.36	2.39	0.27	0.25	0.04	2.31	0.28	33.72	3.26
169	2	0.11	0.01	0.48	0.24	1.32	0.13	0.20	0.02	1.25	0.05	38.89	2.14
	4	0.18	0.01	2.36	0.22	2.22	0.57	0.24	0.01	2.17	0.25	42.77	0.27
	6	0.04	0.01	-0.38	0.33	1.88	0.04	0.20	0.02	1.73	0.11	42.12	0.59
199	2	0.05	0.01	-0.35	0.06	0.61	0.08	0.31	0.00	1.66	0.08	43.01	0.25
	4	0.11	0.02	0.39	0.47	0.68	0.18	0.20	0.05	2.33	0.17	43.53	0.34
	6	0.05	0.01	2.15	0.66	0.46	0.42	0.20	0.01	2.73	0.10	46.45	2.48
201	2	0.05	0.01	-0.95	0.02	0.32	0.24	0.52	0.07	1.90	0.09	43.12	2.31
	4	0.07	0.01	-0.29	0.02	0.68	0.49	0.26	0.09	2.18	0.16	41.30	0.32
	6	0.08	0.01	-0.42	0.08	0.80	0.13	0.20	0.05	1.20	0.39	43.76	0.21
202	2	0.11	0.02	0.70	0.72	1.20	0.10	0.41	0.03	2.58	0.87	42.73	0.34
	4	0.15	0.01	-0.56	0.29	1.80	0.39	0.27	0.10	4.27	0.52	42.36	0.31
	6	0.09	0.01	-0.03	0.18	2.29	0.09	0.22	0.03	4.14	0.17	43.71	0.23

Table 12. GS activity and metabolite content of sorghum roots under optimal N supply (N+). Given are the means from three replicates (± SE) per accession and time point.

Accession	Plant age (Weeks)	GS Activity		Nitrate		Proteins		Total N		Total C	
		nmole.min^{-1}.mg FW $^{-1}$		µmol.g FW $^{-1}$		mg.g FW $^{-1}$		%.DW $^{-1}$		%.DW $^{-1}$	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
34	2	0.40	0.00	7.15	1.88	2.94	0.15	1.70	0.06	41.51	0.29
	4	0.44	0.01	18.34	0.54	3.09	0.28	1.69	0.16	41.87	0.44
	6	0.23	0.03	28.21	2.89	3.20	0.16	1.82	0.13	41.47	0.77
71	2	0.30	0.02	17.48	0.54	3.15	0.08	2.11	0.13	40.47	0.93
	4	0.37	0.01	21.72	0.47	4.61	0.11	1.81	0.21	41.97	0.13
	6	0.49	0.01	28.45	1.61	4.16	0.05	2.33	0.05	40.05	0.69
153	2	0.33	0.02	11.27	1.51	-0.60	0.24	2.23	0.18	40.48	1.24
	4	0.34	0.03	23.80	3.93	0.11	0.42	2.04	0.21	39.02	1.01
	6	0.48	0.01	24.12	1.70	0.90	0.37	1.93	0.22	41.52	1.24
169	2	0.35	0.02	3.68	1.07	1.50	0.04	1.36	0.02	40.89	0.71
	4	0.45	0.01	29.75	4.13	0.42	0.61	1.77	0.20	41.15	1.01
	6	0.44	0.02	29.45	1.59	0.03	0.35	1.91	0.09	38.94	0.52
199	2	0.22	0.01	11.86	1.98	2.41	0.07	1.62	0.09	39.37	0.94
	4	0.31	0.00	22.75	0.72	2.22	0.23	1.64	0.02	40.32	0.58
	6	0.35	0.06	27.15	3.73	2.60	0.06	1.93	0.17	41.39	0.56
201	2	0.31	0.02	10.12	1.75	2.36	0.13	1.96	0.13	41.12	0.22
	4	0.37	0.03	25.97	1.05	2.57	0.01	1.42	0.69	50.18	5.80
	6	0.35	0.06	29.40	1.72	2.87	0.43	2.13	0.12	36.79	2.97
202	2	0.41	0.01	25.03	1.18	1.92	0.07				
	4	0.53	0.03	25.11	1.81	0.64	0.09	2.29	0.25	39.71	1.09
	6	0.20	0.01	38.07	0.51	1.10	0.02	2.38	0.27	38.88	1.26

Table 13. GS activity and metabolite content of sorghum roots under N limitation (N-). Given are the means from three replicates (± SE) per accession and time point.

Accession	Plant age (Weeks)	GS Activity		Nitrate		Proteins		Total N		Total C	
		nmole.min^{-1}.mg FW^{-1}		µmol.g FW^{-1}		mg.g FW^{-1}		%.DW^{-1}		%.DW^{-1}	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
34	2	0.08	0.001	-0.19	0.02	-1.05	0.14	0.94	0.05	42.93	0.44
	4	0.04	0.001	0.33	0.06	-0.76	0.22				
	6	0.08	0.005	0.64	0.35	0.02	0.19	1.16	0.13	46.09	4.99
71	2	0.07	0.001	-0.25	0.09	-0.48	0.10	1.02	0.09	41.50	0.69
	4	0.05	0.004	0.10	0.17	-0.53	0.24				
	6	0.06	0.003	1.43	0.22	-0.72	0.08	0.94	0.03	39.97	2.23
153	2	0.10	0.005	-0.24	0.04	0.95	0.07	1.19	0.09	41.78	0.44
	4	0.01	0.006	4.21	0.43	0.87	0.01				
	6	0.07	0.040	2.05	0.05	0.35	0.34	1.11	0.15	39.72	0.71
169	2	0.09	0.005	0.08	0.09	0.86	0.11	1.26	0.34	42.50	0.38
	4	0.07	0.020	0.65	0.72	0.77	0.21				
	6	0.01	0.002	3.11	0.56	1.15	0.24	1.43	0.35	40.14	2.05
199	2	0.04	0.001	-0.07	0.14	-1.04	0.04	0.94	0.07	41.37	0.17
	4	0.01	0.020	0.22	0.10	-0.08	0.04				
	6	0.02	0.003	1.62	0.42	-0.13	0.01	1.21	0.03	39.73	1.18
201	2	0.09	0.003	-0.01	0.21	-0.73	0.02	1.03	0.10	39.50	1.74
	4	0.03	0.005	0.01	0.05	-0.15	0.38				
	6	0.04	0.004	0.97	0.16	-0.19	0.11	1.21	0.15	39.90	0.32
202	2	0.05	0.009	0.02	0.29	1.47	0.07	0.97	0.05	40.12	1.11
	4	0.08	0.100	-0.05	0.01	1.55	0.03				
	6	0.05	0.030	3.33	0.27	-0.16	0.10	1.01	0.14	37.88	1.94

3.2.4 Variation in GS activity and metabolite content between sorghum leaves and roots during early development

Most parameters showed significant differences between sorghum leaves and roots under both nitrate regimes (Tables 14), with values typically higher for leaves than for roots. However, there were some remarkable exceptions.

Under optimal nitrate supply, no significant differences between organs were detectable for GS activity in accessions 34 and 169, for nitrate content in accessions 34, 201 and 202, and for protein content in accessions 34, 199 and 201 (Table 14A). Under N limitation, no significant differences were detectable for GS activity in accessions 169 and 201 and for protein content in accessions in accession 202 (Table 14B).

Under N limitation, accession 201 was the only one that displayed a significant difference between leaves and roots (Table 14B).; this, however, may be an artifact because nitrate was undetectable in this accession under N limitation in both organs except for roots at week 6 (Tables 13).

Total N content was typically higher in leaves than in roots under both nitrate regimes except for accessions 169 and 201(Figure 7). Finally, even though total C content was significantly different between leaves and roots (except for accessions 71, 153 and 169 under N-) (Tables 14A & 14B), these differences were relatively small, with leaves containing on average approximately 4 to 8 % more total C than roots.

3.2.5 Statistical relationships between plant height and physiological markers

Spearman rank correlations were evaluated to assess the level of statistical linkage between plant height on one hand and physiological markers on the other. Plant height was used as an estimator for plant fitness due to lack of data for grain yield. Of course, the statistical power of these analyses is limited because of the small sample sizes.

These analyses showed that plant height was correlated with several physiological markers in leaves and/or roots, dependent on accession and N condition.

At optimal nitrate supply, plant height was positively correlated with leaf GS activity in accessions 34 ($\rho = 0.88$, $p < 0.01$) and 199 ($\rho = 0.9$, $p < 0.001$) and with root GS activity in accession 153 ($\rho = 0.8$, $p < 0.01$). Plant height was also positively correlated with leaf nitrate

content in accession 34 ($\rho = 0.8$, $p < 0.01$) and with root nitrate content at a significance threshold of $p < 0.01$ in all the accessions except for accession 169. In accessions 71 and 169 this positive correlation between plant height and root nitrate content was even conserved at $p < 0.001$. Accession 169 showed a positive correlation ($\rho = 0.81$, $p < 0.01$) between total N and plant height.

Plant height was also positively correlated with leaf protein content ($\rho = 0.83$, $p < 0.01$) or chlorophyll content ($\rho = 0.97$, $p < 0.01$) in accessions 71 and 169 respectively. The correlation between plant height and chlorophyll content in accession 169 was also significant at $p < 0.001$ ($\rho=0.98$). Interestingly, plant height and leaf total carbon content were negatively correlated ($\rho = -0.95$, $p < 0.001$) in accession 202.

At N-, there was a positive correlation between plant height and leaf nitrate content in accession 199 ($\rho = 0.81$, $p < 0.01$) and between plant height and root nitrate content in accessions 71 ($\rho = 0.86$, $p < 0.01$) and 201 ($\rho = 0.92$, $p < 0.01$). In addition, accession 34 showed a significant positive correlation between plant height and root protein content ($\rho = 0.8$, $p < 0.01$) and a negative correlation between plant height and leaf GS activity ($\rho = -0.9$, $p < 0.01$).

Table 14. Effect of plant organ on physiological markers in sorghum plants under N+ (A) and N- (B) conditions. Statistical differences were evaluated with Kruskal-Wallis tests. H is Kruskal-Wallis coefficient. P is the significance value. (For each accession 17≤N≤18)

A) Under optimal N supply

Accession	GS Activity		Nitrate		Proteins		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P
34	3.6	0.06	0.04	0.82	0.32	0.56	12.8	0.0001 ***	12.8	0.0001 ***
71	8.7	0.003 **	12.8	0.0001 ***	5.4	0.01 *	12.8	0.0001 ***	12.8	0.0001 ***
153	6.7	0.009 **	7.7	0.005 **	12.8	0.0001 ***	11.5	0.0001 ***	7.7	0.05 *
169	0.009	0.92	4.5	0.03 *	8.3	0.003 **	12	0.0001 ***	6.7	0.009 **
199	10.9	0.0001 ***	5.5	0.02 *	3.6	0.06	11.5	0.0001 ***	12.8	0.0001 ***
201	6.3	0.01 *	1.2	0.26	0.01	0.9	12.8	0.0001 ***	3.9	0.05 *
202	5.4	0.02 *	1.03	0.309	11.5	0.0001 ***	9.3	0.002 **	7.3	0.006 **

B) Under N limitation

Accession	GS Activity		Nitrate		Proteins		Total N		Total C	
	H	P	H	P	H	P	H	P	H	P
34	3.9	0.05 *	0.43	0.5	10.9	0.0001 ***	10.1	0.001 **	4.5	0.03 *
71	12.8	0.0001 ***	1.6	0.2	12.8	0.0001 ***	7.5	0.006 **	2.7	0.1
153	7.8	0.005 **	2.6	0.1	12	0.0001 ***	10.1	0.001 **	3.6	0.06
169	1.2	0.27	0.04	0.8	9.8	0.01 *	1.3	0.24	0	1
199	9.8	0.001 ***	0.02	0.9	11.5	0.0001 ***	10.1	0.001 **	10.1	0.001 **
201	1.2	0.27	10.3	0.001 ***	8.7	0.003 **	3.1	0.07	6.7	0.009 **
202	7.7	0.005 **	2.1	0.15	1.6	0.2	7.2	0.007 **	10.4	0.001 **

3.3 Analysis of the expression of *SbNRT1.1* gene copies in leaves and roots of sorghum accessions 199 and 202

Nitrate is the most important source of inorganic N for crop plants. Its absorption from soil is the first step in the N uptake and transport pathway. This pathway is usually mediated by combined activities of high (HATS) and low-affinity (LATS) transporter systems (Forde, 2000). The plant developed HATS and LATS to cope with low (<1mM) or high (>1mM) nitrate concentrations respectively in soil plant roots. The two transporter systems LATS and HATS are encoded by the *NRT1* and *NRT2* gene families, respectively (Forde, 2000).

NRT1.1, a member of the *NRT1* gene family, has both low and high affinity capacities (Tsay *et al.,* 2007). The expression of *NRT1.1* is highly dependent on N availability (Kant *et al.,* 2008). *In silico* analysis of sequenced genomes identified one *NRT1.1* gene in *Arabidopsis* and poplar while some grass genomes possess three or four homologous gene copies (Plett *et al.*, 2010). In sorghum, three co-orthologous to *AtNRT1.1* were identified and named *SbNRT1.1A*, *SbNRT1.1B,* and *SbNRT.1C* (Plett *et al.,* 2010).

In the present study, the expression of sorghum *SbNRT1.1* genes was investigated in leaves and roots harvested from 2 and 6 weeks plants of accessions 199 and 202 grown under N+ and N- conditions. These two accessions were chosen because of their difference in plant height under both conditions. Accession 199 had a very small difference in height between N+ and N- while 202 was among those accessions that displayed a large difference in plant height difference between both conditions

First, a semi-quantitative RT-PCR experiment was conducted with leaf and root cDNA from 2-week old plants grown under N optimal condition to test whether the designed primers were specific for the three *SbNRT1.1* copies and worked for the *EF* and *GAPDH* reference genes (Figure 8).

This experiment showed that all genes were expressed in both organs of 2-week old sorghum plants grown under N optimal supply. However, *SbNRT1.1C* expression was only very faintly detectable in leaves (Figure 8).

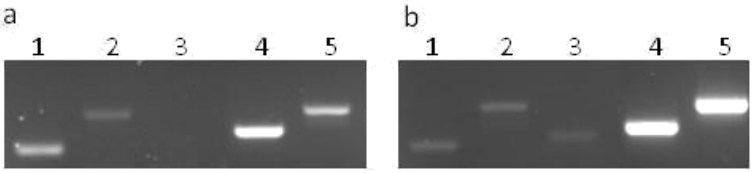

Figure 8. Gel electrophoresis of RT-PCR products from a) leaf and b) root cDNA samples. **1**:*NRT1.1A*, **2**: *NRT1.1B*, **3**:*NRT1.1C*, **4**: *EF*, and **5**: *GAPDH*.

Next, primer binding efficiencies were investigated with quantitative RT-PCR (qRT-PCR), based on plastids that contained the different genes as inserts. This experiment showed that the primer pairs for *SbNRT1.1A*, *SbNR1.1B*, *EF* and *GAPDH* all had similar efficiencies (Table 15), but that amplification of *SbNRT1.1C* was not sufficiently efficient for a quantitative analysis.

Table 15. Efficiency of different primers obtained by the calibration experiment

Gene	Primer Efficiency (%)
GAPDH	91.14
EF	89.95
NRT1.1 A	88.12
NRT 1.1 B	86.69
NRT 1.1 C	110

The qRT-PCR experiment was repeated twice providing technical replicates, with three biological replicates per technical replicate. Mean ΔCTs of biological replicates were calculated using either *EF* or *GAPDH* as a reference. Unfortunately, variation between technical replicates was high, in particular when *EF* was used as a reference gene. To account for this large variation between technical replicates, statistical analyses were not conducted with the ΔCT method but rather with the residuals obtained after simple regression analysis. The results from statistical analyses are presented in Tables 16 – 19, and ΔCT and residuals in Tables 20, 21, 22 & 23. Likewise, leaf and root samples were assessed on different qRT-PCR plates but all common samples that would have permitted simultaneous analysis of these plates failed, precluding a comparison of leaf and root expression for the same accession,

N condition had no detectable effect on *SbNRT1.1A* expression in 2- and 6-week old leaves of both accessions (Table 16). Likewise, no effect was found for roots of accession 202 (Table

16). By contrast, *SbNRT1.1A* expression in roots of accession 199 was significantly higher under N limitation than with optimal N supply at both time points (Tables 16 & 21).

For *SbNRT1.1B*, an effect of N was only observed in accession 202. *SbNRT1.1B* expression was lower in 2-week old leaves and roots under N limitation than with optimal N supply while it was higher in 6-week old roots grown under N limitation than with optimal N supply (Tables 17, 22 & 23). No significant effect was observed for accession 199.

Using plant age as a response variable, *SbNRT1.1A* expression was significantly lower in 6-week old than in 2-week old roots of accession 199 under optimal nitrate regime (Tables 18 & 21). By contrast, the expression of *SbNRT1.1B* varied with plant age in both genotypes under both N conditions. Under optimal nitrate supply expression of Sb*NRT1.1B* was significantly lower at week 6 than at week 2 in the roots of both accessions, 199 and 202 (Tables 19 & 23), while under N limitation, its expression was higher in week 6 than in week 2 in the leaves of accession 199, and leaves and roots of accession 202.

Table 16. Effect of N condition on the expression of *NRT1.1A* in sorghum leaves and roots at 2 and 6 weeks of plant age. Statistical differences were evaluated with Kruskal-Wallis tests. H is the Kruskal-Wallis coefficient, P is the significance value. (For each accession $10 \leq N \leq 12$).

Plant organ	Accession	Plant age (Weeks)	EF		GAPDH	
			H	P	H	P
Leaves	199	2	0	1	0.41	0.52
		6	0	1	1.25	0.26
	202	2	0.23	0.63	3.69	0.05
		6	0.04	0.83	2.9	0.08
Roots	199	2	8.3	0.003 **	8.3	0.003 **
		6	8.3	0.003 **	7.41	0.006 **
	202	2	0.92	0.34	2.56	0.1
		6	0.92	0.34	1.25	0.26

Table 17. Effect of N condition on the expression of *NRT1.1B* in sorghum leaves and roots at 2 and 6 weeks of plant age. Statistical differences were evaluated with Kruskal-Wallis tests. H is the Kruskal-Wallis coefficient, P is the significance value. (For each accession $10 \leq N \leq 12$).

Plant organ	Accession	Plant age (Weeks)	EF		GAPDH	
			H	P	H	P
Leaves	199	2	5.02	0.02 *	3.1	0.08
		6	0.41	0.41	0.41	0.52
	202	2	8.3	0.003 **	8.3	0.003 **
		6	2.9	0.09	0.181	0.67
Roots	199	2	1.64	0.2	0.1	0.75
		6	0.91	0.34	2.07	0.15
	202	2	8.3	0.003 **	7.41	0.006 **
		6	5.76	0.02 *	8.3	0.003 **

Table 18. Effect of plant age on the expression level of *NRT1.1A* in sorghum leaves and roots under N+ and N- conditions. Statistical differences were evaluated with Kruskal-Wallis tests. H is the Kruskal-Wallis coefficient. P is the significance value. (For each accession: $10 \leq N \leq 12$).

Plant organ	Accession	N Condition	EF		GAPDH	
			H	P	H	P
Leaves	199	N+	0.64	0.42	0.64	0.42
		N-	0.23	0.63	0.1	0.75
	202	N+	0.72	0.39	0.72	0.39
		N-	3.69	0.05 *	1.25	0.26
Roots	199	N+	5.76	0.02 *	5.76	0.02 *
		N-	8.3	0.003 *	3.1	0.08
	202	N+	1.64	0.2	0	1
		N-	2.07	0.15	3.1	0.08

Table 19. Effect of plant age on the expression level of *NRT1.1* B in sorghum leaves and roots under N+ and N- conditions. Statistical differences were evaluated with Kruskal-Wallis tests. H is the Kruskal-Wallis coefficient. P is the significance value. (For each accession: $10 \leq N \leq 12$).

Plant organ	Accession	N Condition	EF		GAPDH	
			H	P	H	P
Leaves	199	N+	0.1	0.75	0.02	0.87
		N-	5.76	0.02 *	3.69	0.05
	202	N+	0.72	0.39	0.18	0.67
		N-	8.3	0.003 **	8.3	0.003 **
Roots	199	N+	4.33	0.04 *	5.76	0.02 *
		N-	0.025	0.87	0.03	0.87
	202	N+	8.3	0.003 **	8.3	0.003 **
		N-	7.41	0.006 **	5.02	0.03 *

Table 20. Calculated ΔCT and residuals (±SE) obtained from two experiments for *NRT1.1A* in sorghum leaves under high and low N.

	1st Experiment										
Leaves	**Accession**	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals ±SE**		**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	11.72	1.15	0.37	1.15	10.72	1.15	1.73	1.15
		6		13.20	0.62	1.85	0.62	12.20	0.62	3.21	0.62
		2	N-	11.95	0.64	0.60	0.64	11.16	0.30	2.17	0.30
		6		12.82	0.63	1.47	0.63	10.75	0.62	1.76	0.62
	202	2	N+	10.38	0.78	-0.97	0.78	8.23	0.77	-0.76	0.77
		6		9.32	2.21	-2.03	2.21	6.93	1.85	-2.06	1.85
		2	N-	9.62	0.44	-1.73	0.44	5.65	0.18	-3.34	0.18
		6		9.10	0.10	-2.25	0.10	4.95	0.52	-4.04	0.52
	2nd Experiment										
	Accession	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals ±SE**		**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	2.80	1.28	-4.91	1.28	-2.52	0.51	-8.75	0.51
		6		4.61	1.13	-3.10	1.13	-1.20	0.68	-7.43	0.68
		2	N-	3.08	1.17	-4.62	1.17	-1.86	0.46	-8.08	0.46
		6		4.03	0.72	-3.67	0.72	-2.37	0.74	-8.60	0.74
	202	2	N+	7.14	0.74	2.80	1.28	8.16	0.82	1.94	0.82
		6		5.89	1.34	4.61	1.13	6.95	0.60	0.72	0.60
		2	N-	8.34	0.21	3.08	1.17	5.83	0.10	-0.40	0.10
		6		5.31	0.76	4.03	0.72	5.00	0.23	-1.23	0.23

Table 21. Calculated ΔCT and residuals (±SE) obtained from two experiments for *NRT1.1A* in sorghum roots under high and low N.

	1st Experiment										
Roots	**Accession**	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals**	**±SE**	**ΔCT_{GAPDH}**	**±SE**	**Residuals**	**±SE**
	199	2	N+	16.10	0.98	4.75	0.98	14.10	0.98	5.11	0.98
		6		17.80	0.47	6.45	0.47	15.80	0.47	6.81	0.47
		2	N-	12.14	0.24	0.79	0.24	7.05	0.54	-1.94	0.54
		6		14.51	0.58	3.16	0.58	9.55	0.69	0.56	0.69
	202	2	N+	7.160	2.833	-4.190	2.833	6.974	2.098	-2.016	2.098
		6		9.995	0.487	-1.355	0.487	8.436	0.336	-0.554	0.336
		2	N-	5.746	0.330	-5.604	0.330	3.200	0.545	-5.790	0.545
		6		9.358	0.519	-1.992	0.519	7.467	0.726	-1.523	0.726
	2nd Experiment										
	Accession	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals**	**±SE**	**ΔCT_{GAPDH}**	**±SE**	**Residuals**	**±SE**
	199	2	N+	11.020	0.546	3.318	0.546	12.328	0.527	6.101	0.527
		6		13.615	0.249	5.913	0.249	14.294	0.121	8.066	0.121
		2	N-	7.563	0.445	-0.139	0.445	7.907	0.752	1.680	0.752
		6		11.207	0.385	3.505	0.385	11.661	0.477	5.434	0.477
	202	2	N+	8.40	2.29	0.70	2.29	10.17	1.77	3.94	1.77
		6		11.62	0.41	3.91	0.41	10.77	0.33	4.54	0.33
		2	N-	7.04	0.31	-0.66	0.31	5.59	0.18	-0.64	0.18
		6		10.97	0.23	3.26	0.23	9.17	0.23	2.94	0.23

Table 22. Calculated ΔCT and residuals (±SE) obtained from two experiments for *NRT1.1B* in sorghum leaves under high and low N.

	1st Experiment										
Leaves	**Accession**	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals**	**±SE**	**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	8.26	1.12	-1.61	1.12	7.22	0.57	-0.01	0.57
		6		8.71	1.09	-1.16	1.09	7.47	1.02	0.24	1.02
		2	N-	11.72	1.08	1.85	1.08	10.93	0.75	3.69	0.75
		6		8.86	0.46	-1.01	0.46	6.79	0.30	-0.44	0.30
	202	2	N+	2.711	0.412	-7.156	0.412	0.56	0.42	-6.68	0.42
		6		1.847	0.612	-8.020	0.612	-0.54	0.25	-7.77	0.25
		2	N-	12.867	0.659	3.000	0.659	8.90	0.41	1.66	0.41
		6		3.540	0.272	-6.327	0.272	-0.61	0.27	-7.84	0.27
	2nd Experiment										
	Accession	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals**	**±SE**	**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	6.92	1.32	-0.69	1.32	1.61	0.49	-4.53	0.49
		6		7.54	1.16	-0.07	1.16	1.73	0.98	-4.40	0.98
		2	N-	9.88	1.34	2.27	1.34	4.95	0.62	-1.19	0.62
		6		6.73	0.61	-0.88	0.61	0.32	0.54	-5.81	0.54
	202	2	N+	1.08	0.29	-6.53	0.29	2.10	0.39	-4.04	0.39
		6		1.21	0.70	-6.40	0.70	2.27	0.04	-3.86	0.04
		2	N-	10.77	0.33	3.16	0.33	8.26	0.28	2.13	0.28
		6		2.10	0.25	-5.51	0.25	1.78	0.47	-4.36	0.47

Table 23. Calculated ΔCT and residuals (±SE) obtained from two experiments for *NRT1.1B* in sorghum roots under high and low N.

	1st Experiment									
Roots	**Accession**	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals ±SE**		**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	13.57	0.64	3.70	0.64	9.64	0.99	2.41	0.99
		6		16.17	0.34	6.30	0.34	12.07	0.56	4.84	0.56
		2	N-	14.57	0.35	4.70	0.35	9.47	0.68	2.24	0.68
		6		14.52	1.36	4.66	1.36	9.56	1.16	2.33	1.16
	202	2	N+	3.91	1.64	-5.96	1.64	3.72	0.85	-3.51	0.85
		6		11.93	0.39	2.07	0.39	10.37	0.55	3.14	0.55
		2	N-	11.91	0.16	2.04	0.16	9.36	0.85	2.13	0.85
		6		10.12	0.70	0.25	0.70	8.22	0.57	0.99	0.57
	2nd Experiment										
	Accession	**Plant age (Weeks)**	**N condition**	**ΔCT_{EF}**	**±SE**	**Residuals ±SE**		**ΔCT_{GAPDH}**	**±SE**	**Residuals ±SE**	
	199	2	N+	8.36	0.85	0.75	0.85	9.67	0.96	3.54	0.96
		6		11.10	0.59	3.49	0.59	11.78	0.46	5.65	0.46
		2	N-	9.98	0.11	2.37	0.11	10.32	0.52	4.18	0.52
		6		10.35	0.78	2.74	0.78	10.80	0.91	4.66	0.91
	202	2	N+	4.30	0.70	-3.31	0.70	6.07	0.51	-0.07	0.51
		6		9.96	0.25	2.35	0.25	9.11	0.37	2.98	0.37
		2	N-	10.51	0.16	2.90	0.16	9.05	0.19	2.92	0.19
		6		8.86	0.43	1.24	0.43	7.06	0.42	0.92	0.42

3.4 Discussion

3.4.1 Intraspecific variability at the phenotypic level in response to N nutrition during early development

All sorghum accessions under study that grew under N-limitation exhibited a reduction in mean leaf number in comparison to those plants that grew with optimal nitrate supply. Similar results have been previously described for maize (Zhao *et al.*, 2003; Kafle and Sharma, 2015). This reduction in leaf number was suggested to be a consequence of the reduction of node number under N starvation (Zhao *et al.*, 2003; Kafle and Sharma, 2015). Only minor and temporary variation in leaf number was found among sorghum accessions in the present study, indicating that there is little genetic variation for this trait among the tested sorghum accession, at least not during early development. Of course, this does not exclude the possibility that differences in leaf number among accessions may appear during later stages of development, in particular because the duration of the vegetative phase differs among sorghum genotypes, as shown by Clerget *et al*. (2007).

In contrast to mean leaf number, plant height varied not only between different N conditions but also among accessions growing under the same nitrate regime. The present study showed a strong decrease of sorghum plant growth under N limitation, similarly as in other species like corn (Zhao *et al*., 2003) or castor bean (Reddy and Matcha, 2010). It has been already shown that N deficiency affects both, cell elongation and cell division in plants (Roggatz *et al*., 1999), leading to reduced growth (Reddy and Matcha, 2010).

It is well known that sorghum displays genetic variability for plant height;this has been demostrated as demonstrated by several QTL mapping studies (reviewed in: Fernandez *et al.*, 2009). For example, four classical loci affecting plant height were already identified more than 70 years ago (Quinby, 1975). In addition, three major QTLs for plant height were identified using genome-wide association mapping (Morris *et al.*, 2013).

The sorghum accessions under study displayed strong differences in growth under N limitation, with accessions 169, 201 and 202 being the most sensitive ones; these three accessions showed the largest growth differences between N+ *versus* N-. Lower growth differences between low and optimal N supply in the other accessions may reveal their higher ability for N uptake and/or assimilation. A similar argumentation was put forward by Kant *et al.* (2008) to explain the lower tolerance of *Arabidopsis thaliana* to N limiting condition in comparison to its halophytic relative *Thellungiella halophila.*

3.4.2 Intraspecific variability at the physiological level in response to N nutrition during early vegetative development

a) Effect of plant age

Globally, plant age had no significant effect on GS activity and metabolite accumulation in leaves and roots during early developmental stages under both N conditions, with some accession-specific exceptions. This agrees with previous work on maize (Hirel *at al.*, 2005a; 2005b; Amiour *et al.*, 2012) and wheat (Kichey *et al.*, 2005) showing that biochemical and physiological differences among genotypes typically remain fairly small during early development but become pronounced at later stages, in particular during the transition between vegetative growth and grain filling.

b) Effect of N condition

Low N supply led generally to a reduction of metabolite accumulation and GS activity n all tested sorghum accessions, similar to Arabidopsis (Kant *et al.*, 2008; Chardon *et al.*, 2010), maize (Hirel *et al.*, 2005a;; Amiour *et al.*, 2012) and wheat (Kichey *et al.*, 2005).

Nitrate content, the major N metabolite (Crawford and Glass, 1998), reflects the balance between different processes in the plant, i) the capacity of a plant to absorb N from the soil, ii) the efficiency to transport nitrate between plant organs, iii) the efficiency of nitrate conversion to other N-containing metabolites, and iv) the capacity of the plant to store any excess nitrate for further use during later developmental stages (Hirel *et al.*, 2001). Therefore, nitrate may be a good marker for identifying genotypes with enhanced yield; high nitrate content during early vegetative growth was already confirmed to be useful for this purpose in maize (Hirel *et al.*, 2001; Gallais and Hirel, 2004).

In the present study, differences in leaf and root nitrate content among accessions under both N conditions may likewise be influenced by genotypic variation in N uptake, accumulation and/or assimilation, similar to wheat (Ping *et al.*, 2011)

Under optimal N supply, sorghum accessions displayed two distinct patterns of nitrate accumulation. Four genotypes (accessions 71, 153, 169 and 199) had low nitrate content in leaves but high nitrate content in roots, possibly reflecting a high capacity for nitrate uptake in roots on one hand and high assimilation of nitrate in leaves on the other hand. The other three genotypes (accessions 34, 201 and 202) had high nitrate content in both leaves and roots. This pattern is consistent with a high capacity for nitrate uptake by roots, efficient nitrate transport

from roots to leaves, but reduced capacity for nitrate assimilation in leaves, resulting in an imbalance between the rates of nitrate uptake and nitrate assimilation (Cardenas-Navaro *et al.*, 1999). In rice it was suggested that accumulation of nitrate at high levels in roots and leaves could be due to low reduction activity by nitrate reductase (NR) (Hakeem et al., 2012).

Under N limitation, sorghum plants were able to absorb nitrate differentially among accessions particularly at 4 and 6 weeks even at lower levels compared to N+. This is not surprising as it is well known that sorghum has a higher capacity to uptake nitrate efficiently at N limiting conditions (Lemaire et al., 1996). This higher capacity could be due as suggested to developed and branched root system found in sorghum (Lemaire et al., 1996).

Moreover, some sorghum accessions accumulated nitrate in their leaves only at particular time points (*e.g.*, accession 169 at week 4; accessions 153 and 199 at week 6) under N limitation. This suggests that the roots of these genotypes can take up nitrate efficiently; because of low nitrate assimilation in leaves in response to N starvation, surplus nitrate is then temporarily stored in the leaves, similar as in Arabidopsis (Chardon *et al.*, 2010).

Generally, synthesis of nitrogenous compounds in plants is reduced under N starvation (Kant *et al.*, 2008). While protein content was indeed on average lower in sorghum under N limitation, some accessions had a remarkably high content in their leaves and/or roots under N starvation; protein concentration was sometimes indistinguishable between the two N conditions (*e.g*, leaves of accessions 71, 153 and 169, roots of accessions 153, 169 and 202). A similar phenomenon was found for *Thellunigella halophila* under N stress (Kant *et al.*, 2008); apparently, *Thellunigella halophila* maintains high nitrate reductase (NR) activity under N limitation to ensure adequate nitrate assimilation, resulting in high protein content under both conditions, optimal N supply and N starvation.

Likewise, total N content in leaves of accession 202 and roots of accession 169 appeared to be more or less uninfluenced by N regime. In the respective organs of these accessions protein was relatively high, which accounts at least partially for high N content.

N is an integral constituent of chlorophyll (Evans, 1983). Thus, it was logical that in all studied accessions, chlorophyll content was lower under N starvation than with high N supply. This resulted in pale green leaf color, as it is also known for maize (Kafle and Sharma, 2015) and wheat (Kichey *et al.*, 2005). But, the results of this work showed that chlorophyll content in leaves of different accessions had no wonsistent time-dependent pattern. This contrasts with studies in maize (Hirel *et al.*, 2005a) and wheat (Kichey *et al.*,

2005) where a significant decrease in chlorophyll content occurred during ageing which is not observed during early development. Therefore, chlorophyll content could be used as N stress marker during early vegetative growth, but could not be used as an effective trait to discriminate between different genotypes at early vegetative growth.

During nitrate assimilation, amino acid and protein synthesis depend not only on the availability of N but also on the availability of C (Kant et al., 2008). The current study found that total C content was independent of N condition in all sorghum accessions, except for leaves of accession 153 which showed consistently a slightly lower total C content under N limitation. This is a remarkable difference to Arabidopsis, where N deprivation led to a reduced content in total C, probably because of a lower demand for C skeletons under N-limiting conditions (Kant *et al.* 2008).

Carboxylating enzymes of C4 plants, Phosphoenolpyruvate carboxylase (PEPcase) and Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) which represent 40 to 50% of the total N in leaves (Maranaville and Madhavan, 2002). Level and activity of both enzymes are typically reduced under N stress conditions in C4 plants. Maranaville and Madhavan, (2002) identified a nitrogen use efficient Chinese sorghum line that maintained high PEPcase under N stress. This line also showed a high C assimilation efficiency index. Accordingly, it was suggested that PEPcase may play a key role in maintaining efficient photosynthesis in this sorghum line (Maranaville and Madhavan, 2002). *Vice versa*, low total C content in the leaves of accession 153 could be related to lower photosynthetic activity in this accession under N starvation. However, growth of this accession did not differ from other accessions that displayed an average content of total C under N limitation. Although plant growth does not rely only on photosynthetic activity, the result found in the accession 153 is therefore still intriguing.

One of the main enzymes involved in N metabolism is glutamine synthetase (GS). This enzyme plays a key role in both, N assimilation and N recycling. It is considered as the check point controlling the N status in plants (Hirel *et al.*, 2001; Gallais and Hirel, 2004; Martins et al, 2006). In the present study, leaf and root GS activities of all accessions were lower under N limitation than under optimal N supply. This strongly suggests that nitrate supply promoted N assimilation capacity and thus increased GS activity. Indeed, nitrate was shown to induce total GS activity in leaves of maize (Hirel *et al.*, 2005a), rice (Hakeem *et al.*, 2012), wheat (Kichey *et al.*, 2005; Ping *et al.*, 2011) and in leaves and roots of the hybrid sorghum sudan-grass (El-Omari *et al.*, 2010; El-Omari and Nihiri, 2015).

Leaf and root GS activity displayed high variability among accessions, especially under optimal nitrate supply. Higher GS activity in leaves and roots of some accessions could be explained by: 1) differences in the efficiency of nitrate reduction in leaves and roots between accessions; moreover, increased nitrate reduction in roots could induce GS activity as a detoxification response to keep ammonium levels low. This has been shown in roots of sorghum-sudangrass, where high levels of ammonium were detoxified by increased levels of assimilation (El Omari *et al.*, 2010; El Omari and Nhiri, 2015) and also in rice (Hakeem *et al.*, 2012); 2) accession-specific differences in the demand for nitrogenous products essential for plant growth, with a feedback on GS expression or activity levels.

On the other hand, higher GS activity in leaves and roots of some accessions growing under N limitation could be due to the ability of these accessions to maintain a sufficient level of nitrate uptake to be transported and enhance nitrate reduction and/or assimilation.

The current work shows that genotypic differences exist in the responsiveness of sorghum plants to different levels of N availability. However, the non-consistent ranking pattern of different sorghum accessions under different N regimes indicates that there is also an interaction between genotype and N condition. Genotype by N condition interaction has also been shown for maize (Hirel *et al.*, 2001; Gallais and Hirel, 2004; Canas *et al.*, 2010) and Arabidopsis (Chardon *et al.*, 2010). In fact, each of the tested sorghum accessions appears to respond in a different way to variable N conditions, employing different physiological mechanisms. These different strategies may entail differences in NUE and may, ultimately, lead to different agronomical performances.

The present study found significant correlations between plant height and physiological markers, in particular nitrate content and GS activity. The strength of these correlations varied with accession and N conditions. In fact, correlations between plant growth and markers for N metabolism were expected, since N is as a major nutrient required for plant growth.

3.4.3 SbNRT1.1 expression under different nitrate supply at early vegetative development

NRT1.1 is a dual affinity transporter (Sun *et al.*, 2014), shifting from low to high affinity for nitrate based on the phosphorylation status of a threonine residue, Thr101, as shown by study done on Arabidopsis (Sun *et al.*, 2014). To some extent, changes in the expression of the

SbNRT1.1 gene in sorghum leaves and roots between N+ and N- conditions varied with the gene copy in question. The expression levels of *SbNRT1.1A* in the roots of accession 199 and

of *SbNRT1.1B* in 6-week old roots of accession 202 appeared to be up-regulated in response to N limitation. These results contrast with previous work that showed significant repression of *NRT1.1* expression in the roots of *Arabidopsis* and its halophytic relative *Thellungiella halophila* (Kant *et al.*, 2008). However, the higher levels of *SbNRT1.1B* expression in the leaves and roots of accession 202 under optimal nitrate supply at week 2 may reflect the ability of this transporter gene to enroll in early acquisition of nitrate when growing roots enter the soil, as suggested previously for Arabidopsis (Guo *et al.*, 2001).

Higher nitrate accumulation in roots of accessions 199 and 202 under N limitation could be ascribed to increased expression of *SbNRT1.1B* at week 6. One possible explanation for increased nitrate accumulation associated with increased level of *NRT1.1* gene expression could be the ability of NRT1.1 to act as a nitrate sensor that enables plants to detect and exploit available nitrate in the soil (Remans *et al.*, 2006). By contrast, under optimal nitrate supply expression levels of *SbNRT1.1A* and *SbNRT1.1B* in the roots of 199 and *SbNRT1.1B* in the roots of accession 202 were found to be reduced at week 6. Low expression of these genes was associated with high content of nitrate in the roots. These results contrast with wheat where expression of *NRT1* increased with increased nitrate provision (Ping *et al.*, 2011).

4 Conclusions and Perspectives

4 Conclusion and Perspectives

Sorghum is one of the most important crops in arid and semi-arid regions (Paterson, 2008). It is a staple food for millions of people in addition to its use for a variety of different purposes, including animal feed, building material, and fuel production (Dahlberg *et al.*, 2011).

This exploratory work was aimed at assessing the basis of NUE in sorghum through the analysis of the variability among sorghum genotypes in their growth and physiological responses to N availability. It was designed as a first step of a larger project that aims at monitoring the variability of nitrogen uptake and use in sorghum and at linking plant developmental control and N nutrition.

Seven sorghum accessions were chosen from a panel of 210 accessions from the core collection maintained at CIRAD (Montpellier) (Deu *et al.*, 2006), representing the *world-wide* sorghum diversity. These seven sorghum accessions were grown under controlled and homogenous conditions. All were grown with two different nutrition regimes, i.e. at optimal and low nitrate supply, in highly controlled conditions. Growth variation between these accessions strongly suggested that there is a genetic variability for this trait during early vegetative development. Moreover, variability between genotypes for several N-related markers also witnesses for genetic variability for physiological processes related to N-nutrition among accessions.

However, genetic by N-regime interaction has been put in evidence in the present study. Like in maize (Hirel *et al.*, 2001; Gallais and Hirel., 2004) and Arabidopsis (Chardon *et al.*, 2010), the results in the present study strongly suggest that the variation of the response to optimal N input among sorghum accessions can mainly be explained by variation in N uptake, while under low N input, differences in N assimilation would be the main underlying factor. But, accessions that respond well at optimal N condition are not always the same that respond well at low N supply. Together, these factors may cause differences in NUE among sorghum genotypes which may be effective at later stages of sorghum lifecycle, in particular during grain filling.

Among the studied markers in the present work, nitrate content was a good marker for nitrate absorption and uptake capacities of the different sorghum accessions grown under both N conditions. A positive correlation between nitrate content of young vegetative plants and grain yield was already described for maize (Hirel *et al.*, 2001). Hence, nitrate content in vegetative tissues appears to be a good indicator for high yield (Hirel *et al.*, 2001).

The obtained results also showed that GS activity is a good marker for sorghum N status during the nitrogen assimilation phase, as already demonstrated for a number of cereal species (Hirel *et al.*, 2001; 2005a; Gallais and Hirel, 2004; Kichey *et al.*, 2005). Also, the current study found a good correlation between GS activity and plant growth.

Other markers as protein and total N content were influenced by N condition but there was also genotype-specific variability. Hence, these markers may be indicative for variability of different accessions in their response to N availability and, thus, their ability to adapt well to N limitation. Chlorophyll content was also sensitive to N limitation, but did not show any consistent changes among accessions. Hence, chlorophyll content might be useful as a marker for N stress during early vegetative development but does not discriminate well between accessions.

Even though there was no general age-dependence of physiological and biochemical markers in the present study during early vegetative development, this does not preclude an age-effect at later developmental stages. In particular, whether these markers vary during flag leaf emergence and grain filling stages should be investigated.

Accessions that are able to uptake and store nitrate at N limitation are probably good candidates for high NUE, in particular during grain filling. In this study two accessions with these properties, 153 and 199, were identified. Furthermore, accession 202 showed a high capacity to absorb and store nitrate at N+ conditions, as shown by its high content of free nitrate in leaves and roots. It remains to be shown whether these properties coincide with high yield under nitrate limitation and optimal nitrate supply, respectively.

At the molecular level, the expression patterns of *SbNRT1.1* genes varied depending on the gene copy, organ, N condition, plant age and genotype. This preliminary analysis provides a first glimpse of potential specific roles for these different gene copies in sorghum, compared to the unique copy present in the Arabidopsis genome. However, this suggestion must be taken with caution as the results of quantitative RT-PCR displayed high technical variation. Therefore, a similar experiment should be repeated, potentially covering a larger time interval, including the grain filling stage, to determine whether results are consistent and whether the observed variation is biologically meaningful.

Obviously, the dynamics of N status in sorghum requires further research. For example, nitrate reductase (NR) activity and glutamate dehydrogenase (GDH) should be investigated

through biochemical essays and at the level of gene expression, because NR activity can be a bottleneck in N metabolism but accessions may vary in their efficiency to reduce nitrate to nitrite. Thus, it will be interesting to monitor NR in leaves and roots of different sorghum accessions. Likewise, GDH plays, together with GS, a key role during the transition from N assimilation to N remobilization. Furthermore, it will be necessary to test the contribution of different GS isoforms (GS1 and GS2) to N metabolism under different N conditions and during the sorghum lifecycle. This appears to be particularly important because GS1 and GS2 appear to respond differently to high concentrations of nitrate or/and ammonium in sorghum-sudangrass (El-Omari and Nihiri, 2015).

Finally, amino acid and ammonium content should also be investigated as other indicators of plant N status as it was already done in maize (Hirel *et al.*, 2005b).

On a larger scale, a potential link between N metabolism and hormonal control of development should be investigated. Indeed, sorghum roots and roots exudates are enriched in strigolactones, a class of hormones involved in the control of plant architecture, when plants are grown under N starvation (Yoneyema *et al.*, 2007b). A first step in such a project could be the analysis of tissue-specific expression patterns for genes that are involved in the strigolactone pathway in response to different N conditions.

This study is one of first to understand the physiological, molecular, and genetic basis of NUE in sorghum as an important crop for arid and semi-arid regions. Ultimately, the aim of these studies is to identify sorghum genotypes with the best performances as a function of N nutrition under different environmental conditions and, thus, to optimize and improve sorghum yield under low N input.

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université PARIS-SACLAY | ÉCOLE DOCTORALE Sciences du végétal : du gène à l'écosystème

Titre : Evaluation de la réponse de différents génotypes de sorgho à la disponibilité de l'azote

Mots clés : Sorgho, Azote, Nitrate, Glutamine synthétase (GS), Métabolites, Variabilité intra-spécifique

Résumé : Sept accessions représentant la diversité génétique du Sorgho (*Sorghum bicolor*) ont été cultivées dans une condition de carence (N-) et une condition non-limitante (N+) en nitrate. Les paramètres de croissance (taille de la plante et le nombre de feuilles), les paramètres physiologiques (teneur en nitrate, teneur en protéines, concentrations totales en carbone et azote) et l'activité de la glutamine synthétase (GS) ont été mesurés dans les feuilles et les racines des plantes de sorgho à trois stades précoces de développement végétatif (2, 4 et 6 semaines après germination). Les résultats montrent que : i) les valeurs obtenues pour l'ensemble des paramètres sont généralement plus faibles en situation de carence en nitrate ; ii) la taille des plantes et le nombre de feuilles sont plus grands sous un régime non-limitant ; iii) tous les paramètres étudiés sauf la teneur en Carbone, étaient sensibles à la disponibilité en azote. Cependant, les différents génotypes étudiés affichent de grandes variations dans leurs réponses aux deux conditions de cultures. On observe une variation de la taille des plantes entre les génotypes au cours du développement végétatif précoce, mais pas pour le nombre de feuilles. De même, on observe une grande variation dans les réponses physiologiques entre les différents génotypes. Des corrélations fortes et significatives ont été détectées entre la taille des plantes et la teneur en nitrate. Cependant ces corrélations varient selon les conditions de cultures et les génotypes étudiés. En outre, la teneur en nitrate et l'activité GS mesurés aux stades précoces de croissance, semblent être de bons marqueurs pour discriminer entre les différents génotypes pour leur aptitude à absorber ou assimiler les nitrates dans les deux conditions de cultures. L'expression dans les feuilles et racines de deux génotypes de sorgho, de deux copies d'un gène candidat pour l'efficacité d'utilisation d'azote, *SbNRT1.1* codant pour un transporteur de nitrate, varie en fonction de la disponibilité en nitrate, de l'organe et de l'âge de la plante. Notre étude constitue une première contribution à l'analyse de l'efficacité d'utilisation d'azote chez le sorgho par une approche physiologique et une approche génétique. Les résultats obtenus ouvrent des perspectives pour de futures études fondamentales mais aussi des recherches finalisées qui conduiront à l'identification de génotypes valorisant mieux l'azote.

Title : Assesment of sorghum response to Nitrogen availability

Keywords : Sorghum, Nitrogen, Nitrate, Glutamine Synthetase (GS), Metabolites, Intraspecific variability

Abstract: Seven accessions of *Sorghum bicolor* were grown with low (N-) and optimal (N+) nitrate supply. Growth parameters (plant height and leaf numbers), physiological parameters (nitrate, protein, total N and total C contents) and the activity of glutamine synthetase (GS) were studied in leaves and roots of sorghum plants at three time points of early vegetative growth (2, 4 and, 6 weeks post emergence). Plant height and leaf number were higher with nitrate supply. Except for carbon, all studied parameters were sensitive to N availability and values were typically lower when nitrate supply was low. However, different genotypes displayed considerable variation in their response to N regimes. Variation among genotypes during early vegetative development was observed for plant height, but not for leaf number. Likewise, physiological parameters varied among accessions. A significant and strong correlation, N- and accession-dependent, was detected between plant height and nitrate content. Moreover, nitrate content and GS activity at early growth stages appeared to be good markers to discriminate between nitrate uptake and assimilation capacities of different accessions under both N conditions. In some sorghum accessions, protein and total N content were indicative of high nitrate reduction and assimilation even under N limitation. Chlorophyll content was also sensitive to N availability. Furthermore, expression studies of *SbNRT1.1*gene copies in leaves and roots of two accessions reflected variability in expression dependent on nitrogen condition, plant organ, plant age, and gene of interest. This study is helpful to characterize different aspects of the N metabolism in sorghum and may aid in the identification of sorghum genotypes with enhanced nitrogen use efficiency, a trait that is of key interest in one of the most important crop plants in arid and semi-arid regions.

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