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Energy sorghum hybrids: Functional dynamics of high nitrogen use efficiency

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ABSTRACT

The nitrogen use efficiency (NUE) of high biomass energy sorghum hybrid plants increased during 180 days of growth to a maximum of 370 g DW g^{-1} N^{-1}. Shoot N uptake was biphasic and continued for 120 days. Leaf N accumulation was rapid until day 60. Specific leaf nitrogen (SLN) varied from 0.9 to 1.7 g N m^{-2} green leaf area, a typical range for C4 grass canopies. Stem N increased to a maximum at day 120. NUE increased during development in parallel with increasing stem to leaf biomass ratio and as stems decreased from 0.7% to 0.2% N. At the end of the season, green leaves were ∼1% N, represented 17% of total shoot biomass and accounted for 50% of N present in shoots (above ground biomass) while stems were ∼0.2% N, comprised 83% of shoot biomass and accounted for 50% of shoot N. High NUE was due in part to N-remobilization from lower leaves and stem nodes/internodes to upper portions of the canopy. Up to 70% of dry weight and 90% of N was remobilized during senescence of lower leaves and 70% of N was remobilized from lower stem nodes/internodes. The NUE of energy sorghum was similar to *Saccharum officinarum* and *Miscanthus x giganteus*, and higher than grain *Sorghum bicolor*, *Zea mays*, and *Panicum virgatum*. High NUE of energy *S. bicolor* is due to long duration of vegetative growth, high stem to leaf biomass ratio, and very efficient N-remobilization from lower leaves and stem internodes during development.

1. Introduction

The United States Energy Independence and Security Act mandated production of 21 B gals of biofuels from cellulosic biomass and other non-grain sources in the U.S. by 2022 [1]. The U.S. Billion Ton Study Update [2] identified key factors limiting feedstock supply including the amount of land not in competition with food crops and the yield and cost of biomass for economical production of biofuels. The Update used these and other criteria to project that energy crops have the potential to generate 282 M dT of biomass by 2022 and 400 M dT by 2030 under baseline assumptions of energy crop improvement. This amount of feedstock from energy crops, combined with crop and forest residues, could meet U.S. biofuels production goals assuming reasonable conversion efficiency of biomass to biofuels [3–5].

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C4 grasses such as *Panicum virgatum* (switchgrass), *Miscanthus x giganteus* (Miscanthus), *Saccharum officinarum* (sugarcane) and energy *Sorghum bicolor* (sorghum) have been identified as promising energy crops due to their high biomass yield potential [5–9]. *P. virgatum*, *Miscanthus x giganteus*, and *S. officinarum* are perennial C4 grasses with large genomes and complex genetics [10–12]. In contrast, annual energy *S. bicolor* has a relatively small sequenced genome, tractable genetics, and a short generation time [8,13]. Advances in the understanding of *S. bicolor*'s photoperiod sensitive flowering time regulatory pathway also facilitate production of energy *S. bicolor* hybrids with delayed flowering and high yield potential [13–15]. Energy *S. bicolor* plant architecture and biomass yield potential are similar to *Miscanthus x giganteus* and *S. officinarum*, due to long growth duration, efficient light interception and radiation use, and a high shoot biomass harvest index [9]. The genetic tractability of *S. bicolor* and the annual energy *S. bicolor* hybrid production system make this species a useful genetic model for C4 grass bioenergy crops [8,9,16,17].

Minimizing the cost and maximizing the yield of grain or biomass from inputs such as nitrogen (N) and water is a high priority [18,19]. N fertilizer can represent up to 15% of the cost of biomass production [20], N fertilizer production is energy intensive, utilizes natural gas [21], and run off from agricultural land can have negative environmental impacts [18,22,23]. Moreover, biomass with low N content is well suited for direct energy generation and for biofuels production using biochemical/microbial processes [24,25]. However, N is essential for production of biomass from energy crops, although N requirements vary widely [7,22,26–28]. Differences in N requirements and the NUE of energy crops are due in part to biochemical differences in C3 and C4 photosynthesis [6], with C4 plants requiring less leaf nitrogen and RuBisCO [7,29]. N uptake, assimilation, recycling, removal rates, mineralization, tillage practices and supply from biological sources also affect N requirements and NUE [23]. The N requirements of grain *S. bicolor* are well known and the functional dynamics that affect N utilization in this crop have been analyzed quantitatively and modeled [30,31]. There is less information available about N requirements for sweet and forage *S. bicolor* production but a study conducted in Lubbock Texas indicated N requirements can be met by $\sim$101–108 kg ha^{-1} [32].

In this study, the functional dynamics of nitrogen use by energy *S. bicolor* plants were analyzed to provide a baseline of information about this critical input for growth of energy *S. bicolor* hybrids and to identify ways to improve the NUE of C4 grass energy crops.

2. Materials and methods

2.1. Plant genotypes, plot design, management, and sampling scheme

The energy *S. bicolor* hybrid TX08001 was used for this study [9]. Prior to planting, diammonium phosphate (10-34-0), was applied and incorporated at a rate of 100 kg N ha^{-1}. Planting occurred on the 23rd of April, 2008. Following planting, plots were irrigated twice prior to the 15th of July, and rain-fed for the remainder of their growth. The field trials were grown at the Texas A&M University Field Station outside College Station, TX. The soil in this field consists of Belk Clay [33]. Sufficient seed was sown to yield an excess of plants, which were thinned to 10 cm spacing following seedling emergence for a final population density of 132,000 plants ha^{-1}. Each row of plants was 50 m in length, with consecutive rows planted 76 cm apart. The plot consisted of 3 adjacent rows which were subdivided into sections of approximately 5 m.

Plants were collected from the field every 15 days. All plants were sampled from the center row of each three-row plot to minimize edge effects. For each sample, nine plants were collected. These plants were chosen by first selecting three random ranges within the row, then selecting three consecutive plants within each of the selected ranges. Random ranges were pre-selected using a random number generator. Each plant was cut at soil level and brought to the laboratory for measurement.

2.2. Plant measurements and processing

The fresh weight of each plant and plant height measured from the ground to the collar of the top fully expanded leaf was recorded. Leaf blades were removed, and green leaf area was measured using a planimetric leaf area meter (Licor LI-3100C, Lincoln, Nebraska, USA). Following area measurement, leaf fresh weight was recorded, and leaves were dried in a drying oven for three days at 60 °C with blowers constantly circulating the air, at which point leaf dry weight was recorded. After leaf blades were removed from the stem, stem fresh weight was measured and the stem was then separated into sections.

For each stem section, length and fresh weight were recorded. Then, the stem sections were dried using the same conditions as those used to dry the leaves, and stem dry weights were recorded. In some instances, stem sections were too large to dry in three days. Such stem sections were left in the drying oven for up to five days until all residual moisture had been removed.

Most plants did not produce tillers; when present, tillers were collected along with the main stem for that plant. Each tiller was processed and measured in the same way as the main stem such that all tiller and main stem data could be combined to yield total plant measurements for fresh weight, dry weight, and green leaf area parameters. Energy sorghum flowered very late in the growing season and produced only negligible amounts of grain.

2.3. Composition analysis

Dried plant tissue was ground before being analyzed for composition. Grinding of dried leaf tissue was done using a Cyclone Sample Mill (Udy Corporation, Fort Collins, Colorado, USA). Grinding of stem tissue was done in a two part process, first using a Total Blender Fourside (Blendtec, Orem, Utah, USA), and then using a Krups F203 Fast-Touch Coffee Grinder (Krups, Shelton, Connecticut, USA). Grinding was carried out until the sample could pass through a 2 mm mesh. N content analysis was carried out using a Leco FP-528 Nitrogen/Protein Determinator (Leco Corporation, St. Joseph, Missouri, USA). As this is a combustion-based method requiring destruction of