Improvements in biomass production: Learning lessons from the bioenergy plants maize and sorghum

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Abstract

The use of biomass energy is preferred over fossil fuels as the former is more sustainable and emits a reduced amount of greenhouse gases. Sorghum, a tropical C_4 plant, is known to have a marked drought tolerance. However, very little is known about the mechanisms involved. An allied species *Zea mays*, also a C_4 plant, is far less drought tolerant. Both plants are known to accumulate high biomass during the course of their growth cycle and can be used as renewable energy sources. This article discusses the possibilities of using these two plants for the production of improved biomass in the context of drought tolerance and sustainable food production. Crucial and novel approaches that render Sorghum more tolerant to drought have been reviewed. Novel drought tolerance mechanisms in two sorghum cultivars- Samsorg 17 (more drought tolerant) and Samsorg 40 (less drought tolerant) have also been reviewed.

Key words: Biomass energy, Drought stress, Maize, Sorghum

Introduction

Drought causes massive economic and agricultural losses and is forecasted to be exacerbated in the future due to global climate change (Chen *et al.,* 2012; IPCC, 2013; Ogbaga, 2018). Drought in arid regions is further compounded by competition for land between the industrial and agricultural sectors. Since arable land available for agricultural use has decreased, there is a need to breed crops that can produce maximum yield on limited land. In the tropics, efforts have long been in progress to produce water-use efficient crops that can thrive in marginal lands. Attempts have also been made to understand the drought tolerance mechanisms operative in widely cultivated staple drought-tolerant crops such as Sorghum so as to aid in the improvement of other crops (Turyagyenda *et al.,* 2013; Ogbaga *et al.,* 2017b; Zafar *et al.,* 2017). Breeding of less tolerant staple crops is essential to enable them survive with limited water resources.

Drought causes numerous morphological, physiological and biochemical changes in plants. Morphological effects are visible in the form of wilting of shoots and leaves of green plants. Adjustments in rooting, water uptake, and xylem structure can occur to offer some degree of drought tolerance (Ghannoum, 2009; Ogbaga *et al.,* 2016). With regard to physiology and biochemistry, photosynthesis is altered due to disruption of electron transport chain and Benson-Calvin cycle (Flexas and Medrano, 2002). Drought induces a variety of specific physiological responses in plants such as stomatal reaction ranging from partial reduction in stomatal aperture (leading to a decrease in photosynthesis) to complete stomatal closure (Blum, 1988; Blum, 2011). Stomatal closure is regarded as the earliest primary response of plants to stress, the secondary

responses mostly biochemical manifest only during severe stress (Chaves *et al.,* 2003). The absence of stomatal effects in C_4 plants has been explained as being due to PEPC having a high affinity for CO_2 and thus enables CO_2 fixation for a longer period during drought (Ghannoum, 2009; Chaves et al., 2009; Carmo-Silva *et al.,* 2012). Plants can keep stomata open using various mechanisms to acclimate or tolerate stressful conditions (Chaves *et al.,* 2009; Yamori *et al.,* 2014). During stress, photosynthesis may decline to correspond to the stomatal opening.

This occurs together with the down-regulation of photosystem II and activation of cyclic electron flow to prevent the production of reactive oxygen species and for net ATP production (Takabayashi *et al.,* 2005; Zegada-Lizarazu and Monti, 2013). It is debatable whether such a loss in photosynthesis leads to less accumulation of biomass. In the absence of any form of regulation, water deficit alters the linear electron transport ultimately leading to specific photosystem damage (photoinhibition) or production of reactive oxygen species (Medrano *et al.,* 2002; Asada, 2006). Plant biomass is a renewable energy source that emits a reduced amount of greenhouse gases, and hence is considered sustainable. The high biomass plants have energy stored in them in the form of chemical bonds which can be released biologically or chemically to generate fuel (McKendry, 2002a).

The study of C_4 metabolism can improve our understanding of how C_4 plants partition carbon into sugar reservoirs and increase their cell wall thickness (Lawrence and Walbot, 2007). Maize and Sorghum are known genetic C_4 models that are considered as the tropical biomass plants (Lawrence and Walbot, 2007; Paterson *et al.,* 2009b). The genome sequences of both plants have been completed and provide a knowledge base for determining gene functions (Carpita *et al.,* 2008; Pennisi, 2008; Paterson *et al.,* 2009b). The complete sequence of both plants allows for rapid gene identification by nested association mapping (NAM) or analysis of quantitative trait loci (QTL) of the genes important for useful traits, such as plant anatomy, architecture, height, leaf area, density and biomass (Yu and Buckle, 2006; Yu *et al.,* 2008). The advantages of using these plants for genetic studies start with their close evolutionary relationship with bioenergy perennial grasses and C_4 photosynthesis system. In order to increase the drought tolerance of maize and other plants of the same biochemical subtype, it would be crucial to understand the mechanisms by which both plants tolerate drought and accumulate biomass simultaneously. This article examines the possibility of using maize and sorghum plants for biomass production with special emphasis on the crucial and novel traits identified in Sorghum.

Photosynthetic electron transport and biomass production: In Calvin cycle, CO_2 fixation occurs and energy is stored as glucose or sucrose (Zhu *et al.,* 2008). With adequate nutrients, oxygen, water, and plant respiration, these sugars convert to biomass production in plants. In plant physiology, biomass production is assessed by net assimilation. Net assimilation is weight increase per unit area per unit time (Poorter and Remkes, 1990).The reducing power and adenosine triphosphate (ATP) required to drive the reactions of the Calvin cycle is generated from photosynthetic electron transport (Miyake *et al.,* 2004; Ogbaga *et al.*, 2014). The electron transport chain (ETC) contains two reaction centers (photosystems): PS II and PSI and a cytochrome b6f complex (Ogbaga *et al.*, 2018). These are linked by plastoquinone and plastocyanin. PS II and PSI are the first and second protein complexes of ETC and occur in the mesophyll and bundle sheath chloroplasts respectively. The two proteins are regulated during drought with PSII being more susceptible to damage. ETC is responsible for the movement of electrons from water to NADP with H+ translocated from the chloroplast thylakoid membrane to thylakoid lumen in the process. This translocation can generate non-photochemical quenching (NPQ), a photoprotective mechanism, which protects plants from damage during stress (Miyake *et al.,* 2004).

Maize as a biomass plant: Maize is considered a C_4 biomass plant, which can produce ethanol. Annually, over 4 billion gallons of ethanol is generated from maize kernel in the United States (Sticklen, 2006). For this to happen, the starch content is converted to sugar and subsequently by yeast to ethanol through fermentation (McKendry, 2002b). There are two main processes involved in this conversion-thermo-chemical and biochemical (McKendry, 2002b). In the former, the conversion of biomass to energy proceeds though one of the following routes- combustion, pyrolysis, gasification, and liquefaction. In the latter, the options are digestion (*i.e.*, the production of biogas) and fermentation (McKendry, 2002b). The choice of the route depends on the type of bioenergy sought. For instance, in the United States, fermentation seems to be the preferred option for ethanol production in industries. Few strategies that have been shown or suggested to improve either drought tolerance and/or biomass accumulation in maize include- Selection of improved cultivars, increase in atmospheric CO_2 and manipulation of key metabolic enzymes such as fructose-1, 6-bisphosphatase (FBPase), and Sedoheptulose-1, 7-bisphosphatase (SBPase) (Edmeades *et al.,* 1999; Sahrawy *et al.,* 2004; Sticklen, 2006; Hay, 2012; Rosenthal *et al.,* 2011).

Selection of improved cultivars under well-watered conditions and mild water stress at flowering reportedly enhanced biomass partitioning and resulted in higher harvest index in tropical maize populations (Edmeades *et al.,* 1999). Similarly, the increase in atmospheric CO_2 has also been reported to increase biomass production in the plant. However, it should be noted that such an increase might not correlate with high net assimilation. Other factors such as water, nutrients, oxygen, and plant respiration need to be regulated for high biomass accumulation (Sticklen, 2006). The manipulation of key metabolic enzymes such as FBPase and SBPase can also improve biomass accumulation. When pea FBPase was downregulated in transgenic *Arabidopsis*, sucrose production increased suggesting that the production of high sugar may convert to greater biomass in the plant (Sticklen, 2006; Sahrawy *et al.,*

2004). This hypothesis was tested upon inoculation of maize plants with the bacterium *Azospirilium brasilense* containing higher levels of trehalose (Rodríguez-Salazar *et al.,* 2009). It was reported that such inoculation conferred drought tolerance and a marked increase in leaf and root biomass of the plant (Rodríguez-Salazar *et al.,* 2009). Similarly, when SBPase was overexpressed, the yield of tobacco and soybean yield improved in the field (Rosenthal *et al.,* 2011; Hay, 2012).Since SBPase from maize was reported to be like FBPase, it could be speculated that overexpression of both enzymes in maize would potentially improve yield (Michelet *et al.,* 2013).

Sorghum as a biomass plant

Sorghum like maize is a C_4 plant but unlike maize has a higher photosynthetic efficiency. The stalk of some varieties, for instance, sweet sorghum, consists of mostly sucrose; thus, has nearly 60% of sugar in its dry matter (Antonopoulou *et al.,* 2008). The overall content of fermentable sugars is therefore considered high in sorghum particularly in sweet sorghum varieties (Calviño and Messing, 2012). It also has the potential to produce not just sugars, but starch and cellulose in higher amount than any other species (Rooney *et al.,* 2007). Industrially, the best-known energy products from sorghum are ethanol, methane and more recently hydrogen (Antonopoulou *et al.,* 2008). Sorghum grown for bioenergy production is regarded as a superior choice over maize because it has high water use efficiency and drought tolerance, gives higher yield and is easier to manipulate genetically using genetic and traditional methods (Rooney *et al.,* 2007). Some strategies that have been shown or suggested to improve either drought tolerance and/or biomass accumulation in sorghum include high photosynthetic efficiency, increase in atmospheric CO_2, stay green trait, high stem sugar and manipulation of small RNAs (Borrell *et al.,* 2000; Antonopoulou *et al.,* 2008, Calviño and Messing, 2012, Ottman *et al.,* 2001).

Sorghum is known to have high photosynthetic efficiency and this is maintained for a long period during drought relative to other C_4 species like maize (Antonopoulou *et al.,* 2008; Ogbaga *et al.,* 2014). High photosynthetic efficiency can translate to higher biomass production, which would be ideal for biofuel production. In terms of the effect atmospheric CO_2 free-air CO_2 enrichment (FACE) when applied to sorghum plants grown in the field has been found to improve biomass accumulation in dry plots (Ottman *et al.,* 2001). This finding suggests that if biofuel companies invest in increasing CO_2 concentration in plots used for sorghum planting, yield could increase. However, this approach is not economical and might not be easy for a company with limited funds. Another strategy involves the use of stay green sorghum hybrids. Such hybrids reportedly produced significantly more grain yield than senescent lines under drought (Borrell *et al.,* 2000). Stay green sorghum lines have been reported to exhibit improved grain yield and mass relative to senescent lines. Therefore, biofuel companies can aim to target sorghum stay green lines in order to improve biomass production (Borrell *et al.,* 2014). Finally, high stem sugar is generally observed in sweet

sorghum compared with grain sorghum (Calviño and Messing, 2012). In addition, RNA, miR169, of the transcriptome from grain and sweet sorghum stems was characterized by Calvino and colleagues (Calviño *et al.,* 2011).

They reported that miR169 expression played a role in drought response and correlated with sugar content. Thus, a greater expression of this small RNA could also have a role in biomass accumulation (Calviño *et al.,* 2011; Calviño and Messing, 2012). Similarly, it has been reported that many cell wall-related or lignin genes can also influence biomass accumulation and degradability of sorghum (Oliver *et al.*, 2005). A comparative study of wild-type sorghum lignin content relative to bmr mutants showed that mutants had lower lignin content relative to wild-type (Oliver *et al.,* 2005). In addition, the proportion of specific components of improved starch contents such as amylopectin and amylose can be genetically modified in sorghum (Sarath *et al.,* 2008). Sorghum with lower lignin and improved starch contents can represent alternative biofuel source (Sarath *et al.,* 2008). In addition, some efforts have also been made earlier to develop photoperiod-sensitive sorghum hybrids, which have a longer vegetative growth period to produce higher biomass (Rooney and Aydin, 1999). With the specific genetic combinations of photoperiod-insensitive parental lines, hybrids have been created with high yield of up to 80 mg ha^{-1} (65% moisture) in a single harvest (McCollum *et al.,* 2005). These genotypes were also higher in drought tolerance and/or drought avoidance in rainfed environments.

Novel mechanisms used by Sorghum for improved drought tolerance: It is established that Sorghum maintains photosynthesis longer than maize and is accompanied by greater PSII photochemistry (Ogbaga *et al.,* 2014). Also, provided moisture is sufficient, it maintains stable water relations irrespective of temperature (Machado and Paulsen, 2001). In order to elucidate the traits that contribute to improved drought tolerance, two varieties of sorghum, Samsorg 17 (more tolerant) and Samsorg 40 (less tolerant) from the Institute of Agricultural Research, Zaria, Nigeria were recently studied by Ogbaga and co-workers (Ogbaga *et al.,* 2014; Ogbaga *et al.,* 2016).

The traits that enabled the more tolerant Samsorg 17 to tolerate drought longer were presented. These traits include-maintenance of Rubisco large subunit (RbcL), high constitutive leaf sugar accumulation, sugar alcohol accumulation, low levels of glycine betaine and proline production, high expression of stress and late embryogenesis abundant (LEA) proteins (Ogbaga *et al.,* 2014; Ogbaga *et al.,* 2016). Maintenance of RbcL in the more drought tolerant Samsorg 17 can be an indication of a more efficient CO_2 fixation during drought relative to Samsorg 40. High constitutive leaf sugar accumulation seen in Samsorg 17 offered protection during drought by lowering leaf, shoot and root water potential which enabled a more effective water uptake and longer leaf function in the plants (Fig. 1). Specific reengineering of photosynthetic apparatus occurred in less tolerant Samsorg 40. Plants of Samsorg 40 lost proteins with the nitrogen released

used for amino acid production and maintenance of ATP (Fig. 1). Maintenance of photosynthetic proteins and high constitutive sugars were seen in Samsorg 17 in contrast to Samsorg 40 where loss of proteins and sugar accumulation occurred (Fig. 1). In addition, in Samsorg 40 plants, glycine betaine and proline accumulated in high proportions with increasing drought. The absence of such specific reengineering in Samsorg 17 means that the plant probably had a more active ATP resulting from a higher change in thylakoid pH gradient, which led to higher NPQ generation (Ogbaga *et al.,* 2014).

Biochemical analyses of tissues of Samsorg 17 and Samsorg 40 in terms of metabolism and selected proteins revealed that greater accumulation of sugars, sugar alcohols, lipids, organic acids, heat shock proteins, and dehydrins are vital for drought tolerance. The tolerant Samsorg 17 accumulated these metabolites with greater protein induction or expression while less tolerant Samsorg 40 relied on accumulation of amino acid. These are two different approaches of drought tolerance with that of Samsorg 17 being a better approach (Ogbaga and Stępień, 2015). These traits represent a situation where Samsorg 17 plants receive early signals and become equipped as quickly as possible to deal with drought while Samsorg 40 plants do not receive such signals and have to respond to the condition. Samsorg 17 may have a memory of activating and

possibly remembering strategies that can enhance survival under drought (Niu *et al.,* 2014). In addition, other reports have identified further traits that protect sorghum against drought and a combination of drought and heat stress. These traits include specific transcription factors- MYB78 and ATAF1, polyamine metabolic biosynthetic pathway and the genes SbDHN1 and SbCA involved in dehydrin and carbonic anhydrase biosynthetic pathways, respectively (Johnson *et al.,* 2014, Fracasso *et al.,* 2016). Another gene SbNADP-ME is crucial in maintaining drought tolerance and encodes for NADP- malic enzyme expression (Fracasso *et al.,* 2016).

Limitations of using Maize and Sorghum for biomass purposes: The use of both Maize and Sorghum for biomass purposes is promising and environmentally friendly. However, their use have certain limitations which include: the cost of fuel from plants, competition of plants grown for food and biomass/bioenergy and availability of plants. It is argued that bioethanol is more expensive than petrol (Nguyen and Prince, 1996). This is particularly due to the high cost of processing biomass into liquid fuel rather than cost or availability of feedstock (Lynd *et al.,* 2008). Nevertheless, mixed cropping involving a variety of biomass crops can be helpful in optimizing production (Nguyen *et al.,* 1996). In addition, breeding of traits from more drought tolerant varieties, in this case, sorghum into less tolerant maize is
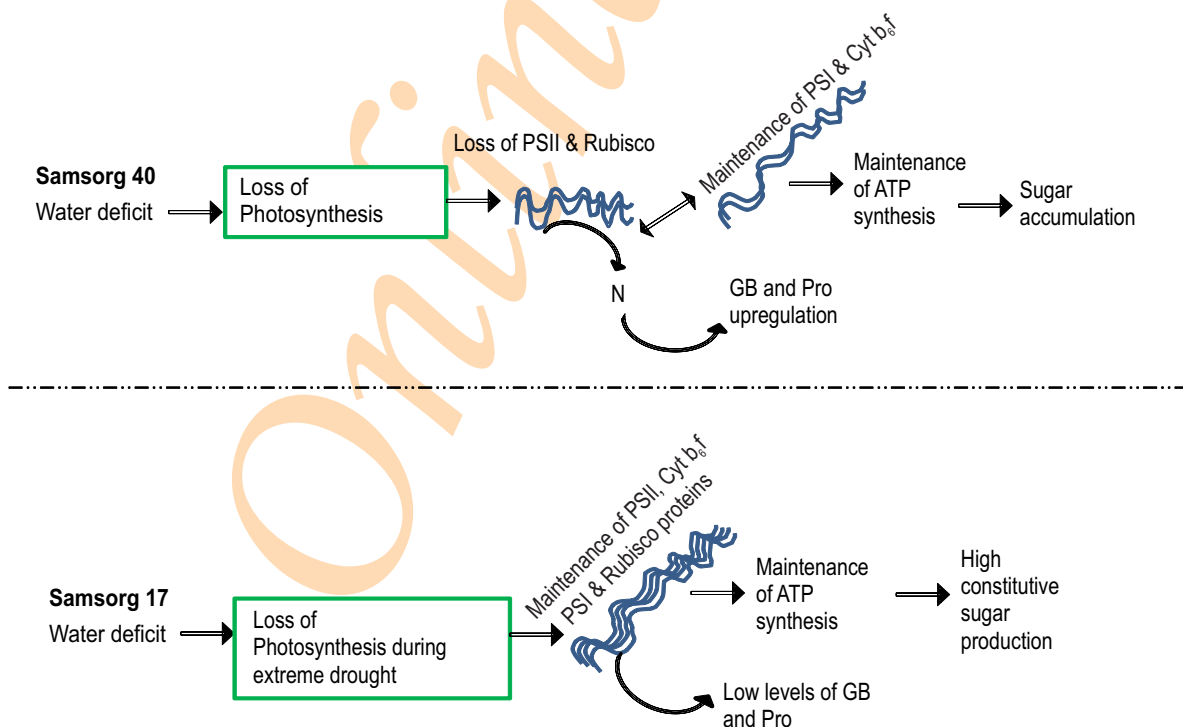

Fig. 1: Overview of the novel drought tolerance mechanisms in two sorghum cultivars- Samsorg 17 (more drought tolerant) and Samsorg 40 (less drought tolerant).

expected to further increase the plant's productivity. In terms of competition of plants for food and biomass/bioenergy, there is a need for the production and growth of both crops, specifically developed for biomass and bioenergy production. Such crops can be grown in regions currently unsuitable for food crops, thus, minimizing the competition between food and biomass /bioenergy (Rooney *et al.,* 2007). Similarly, competition with other renewable sources such as wind, solar and wave power falls under this limitation (McKendry, 2002b). The third limitation is that the plants need to be harvested in large quantity regardless of conversion technology used to provide adequate biomass feedstock. Given the current scenario, the pressing question is - will harvesting huge amount of plants not cause erosion, reduction in food production and depletion of soil carbon and nutrients? (Rooney *et al.,* 2007).

Optimization of biomass conversion to sugars: Biotechnological industries can focus on optimizing biomass conversion to sugars in order to boost ethanol and energy production. However, this requires a lot of innovation, investment, and manpower (Lynd *et al.,* 2008). The use of improved corn and sorghum crop varieties can be considered for this purpose. These crops can be genetically manipulated to produce more cellulose hydrolyzing enzymes capable of breaking down cellulose in favor of sugar production (Lynd *et al.,* 2008). Prior to sugar production, the crops would go through the normal biomass conversion cycle that entails feedstock harvest and storage, thermochemical, physical or biological pre-treatment and enzymatic hydrolysis (Himmel *et al.,* 2007). The use of thermochemical pre-treatment for hydrolysis is fast in breaking down the polymer chains (cellulose, hemicellulose and lignin) relative to physical and biological pretreatments. However, the limitation to its use for sugar production is the high cost of processing and formation of toxic substances (Kumar *et al.,* 2009). Physical pre-treatment involves mechanical power *i.e.,* grinding or milling to reduce cellulose crystallinity with a major disadvantage being the consumption of high energy for the process. In contrast, in biological pre-treatment, fungi, bacteria, and protozoans produce cellulase which catalyzes the hydrolysis of cellulose. Biological pre-treatment is time-consuming but uses less energy. During pre-treatment, the polymer chains contained by the plants would be solubilized and made enzymatically digestible and accessible to saccharifying enzymes (Himmel *et al.,* 2007). This is usually followed by the enzymatic breakdown of cell wall made up of lignin.

Three major saccharifying enzymes involved in this process are celluloses, hemicelluloses and accessory enzymes such as phenolic acid esterase and enzymes capable of degrading and modifying lignin (Himmel *et al.,* 2007). For large-scale production of biofuel, it is pertinent to consider sustainable feedstock production. This would undoubtedly involve production of the two crops in large amount with the crops processed using the best pre-treatment option from limited available land.

Summary: In conclusion, it is believed that the traits summarised in this article have provided new insights into both known and novel mechanisms that enhance drought tolerance and/or biomass accumulation in sorghum. These traits when genetically manipulated and transferred into maize may improve the use of limited agricultural land and lead to sustainable biofuel production. Particularly it has been shown that improved breeding and manipulation of photosynthetic enzymes are crucial for improving drought tolerance and biomass. It was also shown in the article that NPQ generation, high constitutive leaf sugar accumulation, sugar alcohol accumulation, maintenance of specific photosynthetic proteins, greater induction of drought-induced proteins, low levels of glycine betaine and proline and accumulation of various metabolites in sorghum are important for survival during drought. In addition, specific transcription factors-MYB78 and ATAF1, polyamine metabolic biosynthetic pathway and genetic contributions such as genes SbDHN1, SbCA, SbNADP-ME involved in dehydrin, carbonic anhydrase and NADP-malic enzyme pathways are crucial for the maintenance of drought tolerance. As some of the traits discussed were obtained from plants mostly grown in pots and/or growth rooms under laboratory conditions, it is proposed that plants should be tested in greenhouse and in field to confirm reproducibility. Also, during breeding, selection of specific traits highlighted could be a good strategy to improve crop production during drought and potentially enhance biomass accumulation in a closely related species like maize. Currently, the use of both plants for large-scale commercial purposes has not been achieved due to the limitations mentioned in the review. Thus, future research should address the limitations to promote the commercialization of both plants for biomass/bioenergy purposes.

Future Research: Laboratory experiments use artificial conditions to study plants' responses to stresses. Results from such experiments are usually far from what could be obtained when similar experiments are performed in the field. Thus, field experiments in natural plant growth conditions would be useful to test the reproducibility of some of the laboratory-generated traits summarised in this review. In addition, attempts should be made to ensure that the best crop varieties are grown in the fields to produce maximum biomass and that harvests are optimized through pre-treatment to promote the commercialization of biofuel production from the plants (Ogbaga *et al.,* 2018).

In order to uncouple further mechanisms that can improve drought tolerance and biomass accumulation, there is a need to invest in biotechnology and more synergistic studies (Alonge *et al.,* 2017). Genetic studies described in the review suggest that genetic engineering is undoubtedly one way to enhance biomass production of plants. Continued increase in knowledge of molecular pathways that modulate phytochemical composition and genetic variations is needed for better understanding of pathways capable of controlling biomass production for biofuel production (Calviño and Messing, 2012). Crop varieties with interesting traits can be studied further using a combination of approaches-systems biology, imaging and computational tools, phenomics (a high throughput phenotypic screening), genomics, transcriptomics, proteomics and

metabolomics (Lynd *et al.,* 2008; Driever *et al.,* 2013; *Athar et al.,* 2016; Ogbaga, 2017; Ogbaga *et al.,* 2017a). Finally, experimental designs and methods can be enhanced with modeling studies (Niu *et al.,* 2014). These can be used to improve our understanding of the regulatory networks of drought on plants metabolism.

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