Sugarcane genetic transformation aiming drought tolerance

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According to Food and Agriculture Organization (FAO) data, Brazil is the world largest sugarcane producer with 43% of world production. In the milling season 2009/10 the national sugar ethanol sector handled 56 billion dollars with 610 million tons harvest (JornalCana, 2010). Almost half the sugarcane was used to produce sugar and the reminder was use to produce 29 billion liter of ethanol. This scenario reflects the significant importance of the specie in the country since 1532 when Portugueses brought to Brazil the first buds of sugarcane. For a long time the most targeted products derived from sugarcane was sugar instead of ethanol. In 1931, the Brazil's governmental interest began to change and in 1975 The ProÁlcool program allowed estimate increase production from 3 billion L in 1980 to 7 billion L in 1985. Currently, the two products have great value on the international market. Brazil sugar and ethanol exports generated 10 billion dollars in revenue 2009/10 (UNICA, 2011).

The importance of sugarcane as an energy crop has grown in recent years given to one of its products, ethanol, seems as one of the most viable alternative to replace fossil fuels (Goldemberg, 2007). The implementation of new ways to generate energy is not only to reduce oil imports, due to its increasing prices or exhaustion of their deposits. The moment stands out, among other factors, the high CO_2 emissions generated by burning fossil fuels, contributes to global warming and a series of climate change that claims for alternative forms of energy (Buckeridge et al., 2010). In addition, sugarcane crop have an important social role in the Brazilian agribusiness, improving opportunities for farmers and rural communities. According to UNICA (União da Indústria de Cana-de-açúcar), the sugar ethanol sector creates 4.5 million direct and indirect rural jobs and make account 72 thousand producer in 2009/2010 milling season.

The Savanna's (or Cerrado's) area is promising to expand the production but the irregular rainfall usually leds to severe drought stress in some periods, which affects the normal growth of sugarcane (Singh et al., 2006). The eminent and inevitable increase in the sugarcane cultivated area will require development of new varieties more adapted to marginal areas (poor soils, toxic compounds, water limited) and resistant to the main diseases and pests.

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Currently, modern sugarcane cultivars were generated from crosses between *Saccharum* genus *(*mainly *Saccharum officinarum,* that contributed to increase sucrose in stalks), and *Saccharum spontaneum* (a wild vigorous species which contributed with genes for disease and stress resistance) (Cesnik and Miocque, 2004). The selection of genotypes that are water deficit tolerant and their clonal propagation is the main propose of sugarcane breeding programs aiming to increase productivity leading to great yields of ethanol (Silva et al., 2007). Despite the fact that conventional breeding programs contribute to the release of new drought tolerant varieties (Silva et al., 2007, Inman-Bamber & De Jager, 1986, Vasantha et al., 2005), the time required to release new materials corresponds to almost 12 years. The genetic complexity of the modern varieties of sugarcane, due to the high ploidy level is the main obstacles to the generation of improved varieties. In the case of characteristics controlled by multiple genes and influenced by environment, such as drought, the efforts to clone selection become even more difficult (Yeo, 1998; Flowers et al., 2000). Moreover, some specific desired traits cannot be introduced into sugarcane by traditional breeding requiring the use of genetic engineering to improve sugarcane.

The use of biotechnology tools to improve breeding programs could bring new genes (characteristics) that could contribute to higher sucrose content, resistance to diseases and pests, better tillering and deeper roots, tolerance to drought and heat, and improved agronomic qualities. Besides obtaining new biotechnology products, the introduction of genes in sugarcane could contribute to understand the molecular and cellular processes and physiological mechanisms of plant responses to drought conditions.

Usually, plants respond to abiotic stress by inducing a range of biochemical and physiological mechanisms, which can significantly reduce the corresponding damages inflicted by water deprivation. These mechanisms involve the activation of a complex cascade of genes that triggers the production of functional and regulatory drought responsive proteins. The functional proteins include molecules such as chaperones, late embryogenesis abundant (LEA) proteins, antioxidant proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, HSPs (*Heat Shock Proteins*), detoxification enzyme, among others. The second group is involved in further regulation of signal transduction and stress-responsive gene expression (Urano et al., 2010). The bio-engineering of these genes that encode these regulatory proteins are the most promising strategy to obtain transgenic plants tolerant to drought.

Genetic engineering to enhance drought tolerance has been done in many plants. Starting for *Arabidopsis* (Fujita et al., 2005; Sakuma et al., 2006; Abdeen et al., 2010) and tobacco (Zhang et al., 2005; Shukla et al., 2006; Trujillo et al., 2008) that are model plants used to study the effects and function of the genes. However, crops with economic importance have been also used to concept-proof for drought tolerance as wheat (Morran et

al., 2011), rice (Huang et al., 2007; Ito et al., 2006; Hu et al., 2006) and maize (Shou et al., 2004).

In the case of sugarcane, stable transformation is not a routine step. Low transformation efficiency, transgene inactivation, somaclonal variation on tissue culture, and the long time required for regeneration and commercial release are some drawbacks facing the crop.

The first published report with sugarcane transformation focusing on drought tolerance used the overexpression of the disaccharide trehalose that functions as a protectant in the stabilization of biological structures, enhancing stress tolerance to abiotic stresses in several organisms. At this experiment transgenic sugarcane plants accumulated high levels of trehalose, up to 12.863 mg/g FW, whereas it was present at undetectable levels in non transgenic isoline. Physiological characterization results when plants were submitted to drought stress, such as rate of bound water/free water, plasma membrane permeability, malondialdehyde content, chlorophyll *a* and *b* contents, and activity of SOD and POD of the excised leaves, suggest that overexpression of trehalose have enhanced drought tolerance in these plants (Zhang et al., 2006)

Another example is the increase of drought tolerance in transgenic sugarcane that overexpress proline in response to stress. Transformed plants were protected against the oxidative stress caused by water deficit. The higher tolerance of those transgenic plants was assessed by higher biomass yields after 12 days of withholding water (Molinari et al. 2007).

Our group has developed transgenic sugarcane plants with the *At*DREB2A gene under control of stress inducible (*Zmrab17*) and constitutive (*ZmUbi-1*) promoters. This gene is one of the most studied transcription factor (Liu et al., 1998; Shinwari et al., 1998). The protein DREB interacts with *cis*-acting dehydration-responsive element/ C-repeat (DRE/CRT) present in the promoter region of many genes related to drought activating the expression of these genes. Microarray and RNA gel blot analyses revealed that DREB2A regulates expression of many water deficit stress inducible genes (Sakuma et al., 2006). Preliminary results show that transgenic events were resistant to 1% of ammonium glufosinate and until now we selected 10 out 69 events carrying *rab17*::DREB2A::T-nos construct. Currently, selected events were maintained under greenhouse facilities for further molecular, physiological and agronomical analyses.

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