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Selection strategy for indication of crosses between potential sugarcane genotypes aiming at the production of bioenergy

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ABSTRACT

Sugarcane is one of the crops of greatest interest in the search for efficient biomass for the production of bioenergy. In addition to the production of sugar and ethanol from sugarcane juice, the industrialization process obtains the biomass, a lignocellulosic material with high energy content, which can be converted into biofuels. Recognized this importance, the objective of this study was to indicate crosses between potential sugarcane genotypes, aiming at the production of sugar and ethanol, and at the use of biomass for the production of bioelectricity or cellulosic ethanol. Ninety sugarcane genotypes of the Active Germplasm Bank of the Federal University of Viçosa were evaluated for 15 chemical and technological variables that characterize the juice and the bagasse. Crosses were defined by the genotypes which presented the best means using the simultaneous selection index, excluding the crosses between the most similar genotypes based on genetic dissimilarity verified by the analyses of diversity. Thus, it was possible to indicate 40 crosses between potential parents with high quality for production of sugar and ethanol and with efficient biomass for production of bioenergy, 19 crosses being for production of bioelectricity, 15 for production of cellulosic ethanol, and 6 for both purposes.

1. Introduction

Sugarcane (*Saccharum* spp.) is one of the main Brazilian agricultural products, and it takes the first position in the world ranking in relation to production and export of sugar and ethanol (Mapa, 2016). Climate change and depletion of oil reserves easily extracted, combined with intense socio-economic development have stimulated the use of renewable energy sources which can replace fossil fuels (Carvalho-Netto et al., 2014; Dias et al., 2013). Several countries have been engaged in a constant search for alternative energy sources to reduce CO_2 emission. In this sense, Brazil has dominated the foreign market due to the increasing use of biofuels by the world energy matrix. By 2019, Brazil will have produced about 58.8 billion liters of ethanol, more than double of the 2008 production (Mapa, 2016).

In this context, a new developmental stage of the national sugarcane industry was established, known as sugarcane energy sector. Although many species are promising as energy crops, sugarcane is probably the most important due to its high yield (Carvalho-Netto et al., 2014; Matsuoka et al., 2014). Furthermore, its juice can be used for the production of sugar and ethanol, and its biomass can be used for the production of clean and renewable energy (Dias et al., 2013; Sims et al., 2010).

The bagasse, which is lignocellulosic biomass with high energy content, is obtained during the manufacturing process resulting from the extraction of the sugarcane juice (Santos et al., 2012; Santos et al., 2011). This by-product, which used to be discarded, has been increasingly valued in sugarcane energy mills, which have invested in this raw material for domestic and export levels of bioenergy supply. With this investment, it will be possible to significantly increase the production of ethanol and the energy cogeneration performance (Cavalett et al., 2016). This will generate surpluses for the population, and contribute to bioelectricity supply, without the need for increase of the cultivated area (Hofsetz and Silva, 2012).

Genetic resources can be used to meet the new technological expectations, on the development of cultivars with greater sucrose yield potential and ethanol production (Dal-Bianco et al., 2012; Neto

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et al., 2013) and high quality of biomass for production of bioenergy (Carvalho-Netto et al., 2014). In this sense, the characterization of germplasm banks is an essential tool for breeding programs, since it enables studying the genetic diversity, determining the importance of traits in the evaluation of diversity, discarding traits, determining the relationship between traits, and suggesting possible crosses between genotypes (Marim et al., 2009).

The Sugarcane Breeding Program of the Inter-University Network for the Development of Sugarcane Industry (PMGCA/Ridesa) is the largest sugarcane breeding program in Brazil, and it is responsible for the cultivars República do Brasil (RB). The program originated from the works of Brazilian Federal Universities, which took over the activities previously managed by Planalsucar (Barbosa et al., 2012). The network management model of PMGCA/Ridesa involves a public-private partnership (universities and sugar and alcohol industries) for the development of cultivars (Sediyama et al., 2012). Currently, 69% of all the sugarcane cultivated in Brazil are cultivars developed by PMGCA/Ridesa and this sugarcane breeding center is pursuing energy cane cultivars to some extent (Matsuoka et al., 2014). The Federal University of Viçosa (UFV) has a germplasm bank integrated into PMGCA/Ridesa, which generated the most planted cultivar in Brazil, RB867515 (Chapola, 2013). Despite its relevance for the sugar and alcohol sector, the active germplasm bank of UFV has not been fully characterized for bioenergy purposes. This study therefore aimed at estimating the genetic diversity of 90 genotypes of the active germplasm bank of UFV, and to indicate crosses between genotypes with potential to generate varieties with high yield of sugar and ethanol, and to produce a more profitable and efficient biomass for the production of bioelectricity or cellulosic ethanol.

2. Material and methods

2.1. Plant material

Ninety sugarcane genotypes were evaluated from the active germplasm bank of UFV (Supplementary Material, Table S1), which is integrated into PMGCA/Ridesa, located in the Fundão Farm, Viçosa, Minas Gerais (MG) (lat. 20°45′14″ S, long. 42°52′53″W, at 650 m asl). The implementation of the germplasm bank was carried out in 2011, with simple plots consisting of a 3 m long furrow, with no replications, spaced 1.4 m between furrows.

In 2013, for this study, 10 stalks per plot were randomly collected and taken to the laboratory of the experimental station of the Center for Research and Experimentation in Sugarcane (Ceca/PMGCA/UFV), located in the municipality of Oratórios, MG (lat. 20°25′10″S, long. 42°48′15″W, at 494 m asl), where technological analyses were carried out.

2.2. Determination of technological variables

Stalks were crushed and homogenized. Initially, an aliquot of each genotype was weighed in order to obtain the sample fresh weight. After drying this same material at 105° C, the dry weight of the bagasse with juice was obtained. Dry weight was determined by the ratio between the dry weight of the bagasse with juice and the sample fresh weight.

A 500 g subsample previously crushed and homogenized was subjected to hydraulic press at 250 kgf cm^{-2} for 1 min. By this procedure, it was obtained the juice, which was used to determine the total soluble solids content (Brix), with the aid of a digital refractometer. Apparent juice sucrose content (Pol) was determined using a polarimeter. The wet cake weight was obtained by weighing the bagasse of the subsamples removed from the press. The dry cake weight was determined by weighing the same material dried in an oven at 105 °C for 24 h.

From the values obtained, and by the expressions described in the instruction manual Consecana (2006), it was determined the fiber content, the purity and the total recoverable sugars (Trs).

2.3. Determination of the sugar contents in the juice

This analysis was carried out in the Laboratory of Biotechnology and Plant Breeding of the Department of Plant Science of UFV. An aliquot of the juice of each genotype obtained by the hydraulic press was kept at −20 °C and used to determine the contents of glucose, fructose and sucrose by the enzymatic extraction method (Lisec et al., 2006). Duplicates were carried out for each sample. The total sugar content was obtained by the sum of the contents of the three sugars.

2.4. Determination of bagasse cell wall components

Analyses of cell wall composition were carried out in the Laboratory of Biotechnology and Plant Breeding. For these analyses, it was used the previously crushed, pressed and ground bagasse. This material was sieved through a 60 mesh screen for grain size separation and proper homogenization of the material. Subsequently, in a Soxhlet extraction device, samples were extracted with organic solvent for 5 h.

Lignin is highly insoluble in mineral acids, and therefore may be gravimetrically analyzed after cellulose and hemicellulose hydrolysis with sulfuric acid. Thus, the lignin content was determined using the method described in the NREL tests (Templeton and Ehrman, 1995), known as Klason lignin (Tappi, 1998).

Cellulose and hemicellulose contents were determined by gravimetric analysis (Carrier et al., 2011). The procedure used for the preparation of holocellulose involved treatment with sodium acetate solution at 75 °C for 5 h, with the addition of sodium chlorite every hour, for 4 h. For the determination of α-cellulose, the holocellulose sample was subjected to treatment with 17.5% m/m NaOH solution, and subsequently with 10% m/m acetic acid solution. After drying, the material was weighed, and α-cellulose content was determined by gravimetry. Thus, since holocellulose consists of only cellulose and hemicellulose, the hemicellulose content was determined by calculating the difference between the holocellulose content and the α-cellulose.

The ash content of the biomass was obtained by the difference between the weight of the ground and dried sugarcane bagasse, before and after incineration in a muffle furnace at 575 °C for three hours (Santos et al., 2014).

2.5. Determination of cellulose crystallinity content

Crystallinity analysis was carried out in the Physics Laboratory of the Department of Exact Sciences of UFV. Ground and sieved sugarcane bagasse samples were placed in 2 mm depth and 25 mm diameter disk-shaped acrylic cylindrical cells. X-Ray diffractograms were obtained at room temperature by a 6 mm slit monochromator, in an interval of 2θ angles, ranging from 10 to 40°, with an increment of 0.1000°, and speed of 2 s per step. It was also used a Universal X-ray diffractometer (X-XD8 Discover Bruker model), operating at 40 kV power and 40 mA current, with CuKα (λ = 1.5418 Å) radiation. Crystallinity index were estimated based on the areas under the crystalline peaks and the amorphous, after correction of the base line, according to the method of Segal et al. (1959), represented by the equation:

$$CrI = \frac{(I_{002} - I_{am})}{I_{002}} \times 100$$

where, *CrI* is the percentage calculated crystallinity; I_{002} is the diffraction intensity associated with the crystalline cellulose (maximum diffraction between $20° < 2\theta < 25°$); and I_{am} is the amorphous cellulose (minimum diffraction between $15° < 2\theta < 20°$).

It is important to consider that Segal method is not able to provide the exact crystallinity of the material (French and Cintrón, 2013). However, this method is useful for comparing the relative differences among samples (Caliari et al., 2017; Park et al., 2010). In this way, to