STABILITY OF MORPHOLOGICAL MARKERS OVER LOCATIONS, FOR THE IDENTIFICATION OF SUGARCANE VARIETIES

By

S. HARTATIK¹, A. MAKMUR², A. SAEFUDDIN² and S. LAMADJI³
¹Faculty of Agriculture, Jember University, East Java-Indonesia
²Faculty of Agriculture, Bogor Institute of Agriculture, Bogor-West Java-Indonesia
³Indonesian Sugar Research Institute, Jl. Pahlawan 25 Pasuruan, East Java, Indonesia

Abstract

Though subject to change due to environmental influences, morphological markers of sugarcane varieties are still widely used at several sugarcane research institutes. To be used effectively and efficiently as genetic markers they must be highly heritable and stable over locations. The present study examined the stability of 23 morphological markers. Sixty-four sugarcane clones consisting of early hybrids, domestic commercial varieties and imported varieties from sugarcane germplasm maintained by the Indonesian Sugar Research Institute, Pasuruan, were used in this experiment to examine the stability of morphological characters. All varieties were grown at three locations in East Java. Two locations represented irrigated areas but differed in their soil types, and one represented a non-irrigated area. The result revealed that, except for the bud furrow, all morphological characters were stable over locations as indicated by non-significant $\chi^2$ values ($p<0.01$). Therefore, morphological markers are sufficiently stable to be used in variety identification, across the tested environments.

Introduction

Identification of varieties, i.e. distinguishing and describing varieties, is important for plant breeding programs. Traditionally, variety identification has been done by using morphological markers (Cross, 1990). This is due to the ease of scoring these markers. They are usually quick and simple to evaluate, cheap to establish, and do not require high levels of specialist knowledge (Guiard, 1995; Lamadji, 1998).

Recently, research has developed molecular markers for plant breeding, genetic engineering and, to a certain extent, for plant material identification (Huckett and Botha, 1995; Powell et al., 1996). The authors noted the merit of working at the DNA level. They described the variation of individuals by differentiation between and within samples in the sequence of nucleotides at the DNA level. In addition, it has been argued that DNA markers with significant linkage to phenotypic characters could be useful in breeding programs since they would facilitate accurate, rapid and early screening of progeny independent of environmental or orthogenic factors. However, the use of molecular markers may not be practical in many breeding programs due to the high cost of establishing and maintaining specialised facilities and labour. Therefore, morphological markers are still a popular choice among taxonomists and plant breeders for characterising and classifying varieties into similar groups. Guiard (1995) reported that the International Union for the Protection of New Varieties (UPOV) uses phenotypic descriptions of varieties for conducting the diversity, uniformity and stability (DUS) test. Morphological characterisation is employed for the genus Arachis (Chandran and Pandya, 2000), and successfully used for developing core collections of alfalfa, chickpea, and clover germplasm (Basigalup et al., 1995; Hannan et al., 1994; Kouame and Quesenberry, 1993).

Morphological characters are assumed to be under complex genetic control, may involve epistatic interaction or pleiotropy, and are subject to genotype x environment interaction. Nevertheless, when carefully chosen, morphological markers can provide a stable tool for germplasm evaluation and description (Erskine and Williams, 1980). Moreover, morphological markers for variety description and classification for several crops, such as common bean (Johns et al., 1997) and wild rose (Debener et al., 1996), have shown good agreement with some molecular markers, such as RAPDs.

The development of morphological characters to identify and distinguish sugarcane (Saccharum spp.) varieties was based on earlier work conducted by van Dillewijn (1952), Skinner (1971) and Artschwager (1949), with some modifications. The use of morphological markers is an effective method for describing and identifying varieties in several sugarcane research institutes, as long as these markers are highly heritable and stable over locations. The objective of this study was to examine, verify and ascertain the stability of a group of morphological markers of sugarcane over locations to be used for variety identification.

Materials and methods

Plant material

Sixty-four sugarcane varieties, of diverse geographical origin, were used to study the stability of 23 morphological markers (Table 1).

KEYWORDS: Sugarcane, Morphological Marker, Variety Identification.
All varieties were planted at three locations in East Java. Two locations represented irrigated areas, but with different soil types, i.e. regosol and grumosol. The other location represented a dry land area. The experiment followed a randomised complete block design with three replications. Crop management followed the standard management at the Indonesian Sugar Research Institute (ISRI). Q

Data were collected for 23 morphological markers referred to by Lamadji (1998) and presented in Table 2. The observations were made during the month of November and December of 1998, when the plants were at the age of 6–7 months after planting (Clements, 1980).

Table 1—Sugarcane varieties used in the present study, and their country of origin.

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>NA56-30, NA56-79, TUC73-3, TUC 72-51</td>
</tr>
<tr>
<td>Australia</td>
<td>Q58, Q67, Q80, Q81, Q83, Q85, Q86, Q89, Q110, Q113, Q114, Q118, Regnar, Triton</td>
</tr>
<tr>
<td>Barbados</td>
<td>B5814, B42271, B76718</td>
</tr>
<tr>
<td>Brazil</td>
<td>IAC58-480, SP71-355, RB725143, RB725033</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>CR67-274</td>
</tr>
<tr>
<td>India</td>
<td>Co214, Co218, Co261, Co331, Co453, Co419, Co277, Co973, Co1007</td>
</tr>
<tr>
<td>Indonesia</td>
<td>PS80-442</td>
</tr>
<tr>
<td>Mauritius</td>
<td>M442-51</td>
</tr>
<tr>
<td>Philippines</td>
<td>Phil56-226, Phil56-07</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>PR1013, PR1048, PR1117</td>
</tr>
<tr>
<td>Reunion</td>
<td>RP35-370</td>
</tr>
<tr>
<td>South Africa</td>
<td>N15, NCo283, NCo310, NCo376</td>
</tr>
<tr>
<td>Taiwan</td>
<td>F146, F153, F154, F155, F169, F170, F171, F172, PT43-52</td>
</tr>
</tbody>
</table>

Data analysis

Statistical analysis of the morphological data was performed using SPSS 9.0. As morphological characters are descriptive characters, all data were transformed to binary fashion, i.e. '1' indicating the presence, and '0' absence of a described character (Lamadji, 1998).

The Cochran test for k related samples was used to estimate the stability of all morphological characters (Siegel, 1988). It provides a test for whether three or more attached sets of frequencies differ significantly among themselves, including matches based on relevant characters where the same subjects are tested in different environments. The equation is as follows:

\[
Q = \frac{(k-1) \left[ k \sum_{j=1}^{k} G_j^2 - \left( \sum_{j=1}^{k} G_j \right)^2 \right]}{k \sum_{i=1}^{n} L_i^2 - \sum_{j=1}^{n} L_j^2}
\]

where, Q = $\chi^2$, which is distributed as $\chi^2$ with df = k - 1; Gj = total number of '1' for all accessions at each location; k = number of locations (k = 3); Lj = total number of '1' for each accession over all locations; n = number of varieties.

The morphological characters are confirmed stable over locations if $\chi^2$ (calculated) ≤ $\chi^2$ (table) with α = 0.01 (9.21).

Results and discussion

All varieties planted at the three locations showed normal growth. The results of the present study revealed that, except for bud furrow, the $\chi^2$ calculation for all morphological descriptors was less than the critical value (Table 2). This result suggests that the bud furrow character was not stable over locations and therefore should not be used in DUS tests of sugarcane varieties.

Visual observation showed that the bud furrow characteristic was mostly found on varieties that were grown on irrigated areas. Differences in water and soil nutrient supply on irrigated and non-irrigated areas may have caused differential expression for this trait. The result of this study supported that of Jackson and Galvez (1996) which suggested that marginal soil fertility and water supply could cause genotype x site interactions for several sugarcane morphological characters.

Phenotype expression of individual and population are regulated by both genetic and environmental components. The characters that have largely influenced by the genetic component are highly heritable traits and stable over locations. All morphological characters used in this experiment are highly heritable traits (Lamadji, 1998). The results revealed that all morphological characters, except for bud furrow, were stable over locations. This suggests that these morphological markers can be used and are valid for DUS testing and for sugarcane variety identification and characterisation due to simplicity, ease of use, and low cost.

Chapman (1989) stated that variety identification and characterisation should be based on highly heritable and stable traits, which are easily visible so as to simplify the descriptive work performed by the curator. The markers selected for this study fulfill the above criteria and therefore, with the exception of ‘bud furrow’, can be used as sugarcane variety descriptors.
Table 2—Estimated $\chi^2$ value for Cochran's test for 23 morphological markers measured on 64 sugarcane varieties, across three environments in Indonesia.

<table>
<thead>
<tr>
<th>Morphological markers</th>
<th>Code</th>
<th>Description</th>
<th>$\chi^2$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A: LEAF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bend of leaf blade</td>
<td>D1.1</td>
<td>Less than $1/3$ leaf blade from the top</td>
<td>4.500</td>
</tr>
<tr>
<td>D1.2</td>
<td>More than $1/3$ leaf blade from the top</td>
<td></td>
<td>2.800</td>
</tr>
<tr>
<td>D2.1</td>
<td>Absent</td>
<td></td>
<td>6.580</td>
</tr>
<tr>
<td>D2.2</td>
<td>Present, with length $&lt; 3$ times width</td>
<td></td>
<td>5.515</td>
</tr>
<tr>
<td>D2.3</td>
<td>Present, with length $&gt; 3$ times width</td>
<td></td>
<td>7.000</td>
</tr>
<tr>
<td>Growth type of auricles</td>
<td>D3.1</td>
<td>No auricle</td>
<td>6.320</td>
</tr>
<tr>
<td>D3.2</td>
<td>Vertical</td>
<td></td>
<td>4.267</td>
</tr>
<tr>
<td>D3.3</td>
<td>Falcate</td>
<td></td>
<td>3.176</td>
</tr>
<tr>
<td><strong>B: LEAF SHEATH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width of leaf sheath hair area</td>
<td>P1.1</td>
<td>Absent (no hair)</td>
<td>0.261</td>
</tr>
<tr>
<td>P1.2</td>
<td>Narrow ($&lt; 1/3$ leaf sheath width)</td>
<td></td>
<td>0.869</td>
</tr>
<tr>
<td>P1.3</td>
<td>Wide ($\geq 1/3$ leaf sheath width)</td>
<td></td>
<td>1.736</td>
</tr>
<tr>
<td>Distance of the leaf sheath hair area from the dewlap</td>
<td>P2.1</td>
<td>Absent (no hair)</td>
<td>0.000</td>
</tr>
<tr>
<td>P2.2</td>
<td>Short ($&lt; 1$ cm)</td>
<td></td>
<td>0.231</td>
</tr>
<tr>
<td>P2.3</td>
<td>Long ($\geq 1$ cm)</td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>Length of leaf sheath hair</td>
<td>P3.1</td>
<td>Absent (no hair)</td>
<td>0.000</td>
</tr>
<tr>
<td>P3.2</td>
<td>Short ($&lt; 2$ mm)</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>P3.3</td>
<td>Long ($\geq 2$ mm)</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Position of leaf sheath hair</td>
<td>P4.1</td>
<td>Absent (no hair)</td>
<td>0.000</td>
</tr>
<tr>
<td>P4.2</td>
<td>Straight</td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>P4.3</td>
<td>Incline</td>
<td></td>
<td>1.182</td>
</tr>
<tr>
<td>P4.4</td>
<td>Down</td>
<td></td>
<td>2.438</td>
</tr>
<tr>
<td>Density of leaf sheath hair</td>
<td>P5.1</td>
<td>Absent (no hair)</td>
<td>0.000</td>
</tr>
<tr>
<td>P5.2</td>
<td>Rare ($&lt; 75/cm^2$)</td>
<td></td>
<td>0.788</td>
</tr>
<tr>
<td>P5.3</td>
<td>Dense ($\geq 75/cm^2$)</td>
<td></td>
<td>0.836</td>
</tr>
<tr>
<td>Hair of sheath edge</td>
<td>P6.1</td>
<td>Absent (no hair)</td>
<td>1.000</td>
</tr>
<tr>
<td>P6.2</td>
<td>Present</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Trashing habit of leaf sheath</td>
<td>P7.1</td>
<td>Difficult</td>
<td>1.734</td>
</tr>
<tr>
<td>P7.2</td>
<td>Not easy</td>
<td></td>
<td>3.566</td>
</tr>
<tr>
<td>P7.3</td>
<td>Easy</td>
<td></td>
<td>0.839</td>
</tr>
<tr>
<td><strong>C. STALKS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internode arrangement</td>
<td>R1.1</td>
<td>Straight</td>
<td>0.667</td>
</tr>
<tr>
<td>R1.2</td>
<td>Zig-Zag</td>
<td></td>
<td>1.500</td>
</tr>
<tr>
<td>Cross section of internode</td>
<td>R2.1</td>
<td>Round</td>
<td>0.000</td>
</tr>
<tr>
<td>R2.2</td>
<td>Oval</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Bud furrow</td>
<td>R3.1</td>
<td>Absent</td>
<td>31.824*</td>
</tr>
<tr>
<td>R3.2</td>
<td>Present (a part of internode)</td>
<td></td>
<td>31.824*</td>
</tr>
<tr>
<td>R3.3</td>
<td>Present (all of internode)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of root primordia</td>
<td>B1.1</td>
<td>Less than two rows</td>
<td>0.667</td>
</tr>
<tr>
<td>B1.2</td>
<td>2–3 rows</td>
<td></td>
<td>0.800</td>
</tr>
<tr>
<td>B1.3</td>
<td>Greater than 3 rows</td>
<td></td>
<td>1.143</td>
</tr>
<tr>
<td>Root primordia above the bud</td>
<td>B2.1</td>
<td>Absent</td>
<td>2.000</td>
</tr>
<tr>
<td>B2.2</td>
<td>Present</td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>Base of bud wing</td>
<td>M1.1</td>
<td>Upper bud centre</td>
<td>0.000</td>
</tr>
<tr>
<td>M1.2</td>
<td>Others</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Size of bud wing</td>
<td>M2.1</td>
<td>Narrow basal</td>
<td>4.762</td>
</tr>
<tr>
<td>M2.2</td>
<td>Others</td>
<td></td>
<td>4.762</td>
</tr>
<tr>
<td>Edge of bud wing</td>
<td>M3.1</td>
<td>Smooth</td>
<td>8.600</td>
</tr>
<tr>
<td>M3.2</td>
<td>Serrated</td>
<td></td>
<td>4.571</td>
</tr>
<tr>
<td>Germ pore</td>
<td>M4.1</td>
<td>Below or at bud centre</td>
<td>2.000</td>
</tr>
<tr>
<td>M4.2</td>
<td>Above bud centre</td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>Hair of basal edge of bud</td>
<td>M5.1</td>
<td>Absent</td>
<td>2.000</td>
</tr>
<tr>
<td>M5.2</td>
<td>Present</td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>Juncture</td>
<td>M6.1</td>
<td>Absent</td>
<td>4.333</td>
</tr>
<tr>
<td>M6.2</td>
<td>Present</td>
<td></td>
<td>4.000</td>
</tr>
<tr>
<td>Shape of bud patterns</td>
<td>BM1</td>
<td>Round</td>
<td>6.333</td>
</tr>
<tr>
<td>BM2</td>
<td>Oval</td>
<td></td>
<td>1.750</td>
</tr>
<tr>
<td>BM3</td>
<td>Ovate</td>
<td></td>
<td>8.818</td>
</tr>
<tr>
<td>BM4</td>
<td>Obovate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM5</td>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Internode</td>
<td>BR1</td>
<td>Cylindrical</td>
<td>0.222</td>
</tr>
<tr>
<td>BR2</td>
<td>Conoidal</td>
<td></td>
<td>7.515</td>
</tr>
<tr>
<td>BR3</td>
<td>Obconoidal</td>
<td></td>
<td>6.488</td>
</tr>
<tr>
<td>BR4</td>
<td>Spool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR5</td>
<td>Barrel</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* signifies different at $\alpha = 0.01$, where $\chi^2$ table = 9.21.
REFERENCES


LA STABILITÉ DES MARQUEURS MORPHOLOGIQUES À TRAVERS LES SITES, POUR L’IDENTIFICATION DES VARIÉTÉS DE CANNE À SUCRE

S. HARTATIK1, A. MAKMUR2, A. SAEFUDDIN2 et S. LAMADJI3
1Faculty of Agriculture, Jember University, East Java-Indonesia
2Faculty of Agriculture, Bogor Institute of Agriculture, Bogor-West Java-Indonesia
3Indonesian Sugar Research Institute, Jl. Pahlawan 25 Pasuruan, East Java, Indonesia

Résumé

Bien que les marqueurs morphologiques des variétés de canne à sucre soient sensibles au changement dû aux influences de l’environnement, ils sont encore très utilisés dans plusieurs instituts de recherches de canne à sucre. Pour être utilisés efficacement comme marqueurs génétiques ils doivent être très héritables et stables à travers les sites. La stabilité de 23 marqueurs morphologiques a été l’objet d’une étude. Soixante-quatre clones de canne à sucre comprenant des hybrides précoces, des variétés commerciales locales et étrangères du germoplasme entretenus par l’institut de recherche sucrière de l’Indonésie, à Pasuruan, furent inclus dans cette étude sur la stabilité des caractères morphologiques. Toutes les variétés ont été implantées sur trois sites dans l’Est de Java. Deux sites représentèrent les régions irriguées avec différents types de sol et le troisième représenta une région non-irriguée. Les résultats révèlèrent que tous les caractères morphologiques étaient stables à travers les sites et que les valeurs non-significatives χ² (p<0.01), à l’exception du sillon de l’oeilleton. Les marqueurs morphologiques sont donc suffisamment stables pour être utilisés dans l’identification variétale à travers les environnements.

Mots clés: canne à sucre, marqueur morphologique, identification variétale.
ESTABILIDAD DE LOS MARCADORES MORFOLÓGICOS A TRAVÉS DE LOCALIDADES, EN LA IDENTIFICACIÓN DE LAS VARIEDADES DE CAÑA DE AZÚCAR

S. HARTATIK1, A. MAKMUR2, A. SAEFUDDIN2 y S. LAMADJI3

1Faculty of Agriculture, Jember University, East Java-Indonesia
2Faculty of Agriculture, Bogor Institute of Agriculture, Bogor-West Java-Indonesia
3Indonesian Sugar Research Institute, Jl. Pahlawan 25 Pasuruan, East Java, Indonesia

Resumen
Aunque sujetos a los cambios debido a los efectos ambientales, los marcadores morfológicos de las variedades de caña de azúcar son todavía ampliamente utilizados por muchos institutos de investigación de caña de azúcar. Para que sea usado efectiva y eficientemente como marcador genético ellos deben ser altamente heredables y estables a través de las localidades. El presente estudio examinó la estabilidad de 23 marcadores morfológicos. Sesenta y cuatro clones de caña de azúcar entre híbridos, variedades comerciales locales e importadas del germoplasma mantenido por el Indonesian Sugar Research Institute, Pasuruan, se emplearon en este experimento para examinar la estabilidad de los caracteres morfológicos examinados. Todas las variedades crecieron en tres localidades al oriente de Java. Dos localidades representaron áreas con riego pero que se diferenciaban en su tipo de suelo y una tercera representó un área sin riego. El resultado mostró que, excepto el surco de la yema, todas las características morfológicas fueron estables a través de las localidades señalado por la no significancia de los valores $\chi^2$ ($p<0.01$). Por tanto, los marcadores morfológicos son lo suficientemente estables a través de los ambientes evaluados para que sean utilizados en la identificación de las variedades.

Palabras claves: caña de azúcar, marcador morfológico, identificación varietal.