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Genetic Diversity Analysis of Sugarcane (*Saccharum* Sp.) in Thailand Using RAPD Technique

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Abstract Random amplified polymorphic DNA (RAPD) technique was used to study genetic relationship among 56 cultivars of *Saccharum* sp. Ninety-six decamer primers were screened and fifteen primers were able to amplify DNA fragments. Nine primers produced reproducible fragments with easily recordable bands which were selected for genetic diversity analysis. These primers produced multiple band profiles with a number of amplified DNA fragment varying from 7 (OPB17) to 13 (OPC19) with an average of 10. The Pearson's similarity coefficients were used to construct a UPGMA dendrogram. Pair-wise estimates of genetic similarity were found to range from 0.102 to 0.998. The highest genetic similarity was observed in AsaToa, TP07-395, Asawa and SO10-06. The lowest genetic similarity of 0.102 was seen in SP80 and UT3. RAPD fingerprint could be of help to plant breeders in selecting genotypes with diverse genetic base for use in their breeding program to develop the new varieties of *Saccharum* sp. hybrid.

Keywords: Sugarcane, Genetic diversity, RAPD

Introduction

Sugarcane is an important industrial crop in subtropical and tropical region which mainly used for sugar and alcohol production (Tabussum *et al.,* 2010). It is belonging to the genus *Saccharum* which is an important component of the tribe Andropogonea in the grass family (Poaceae) (Nawaz *et al.,* 2010). The genera *Saccharum, Erianthus, Miscanthus, Narenga* and *Sclerostachya* constitute a closely related inbreeding group referred to as the *Saccharum complex* (Mukherjee, 1957). The genus *Saccharum* is composed of six species, namely *Saccharum officinarum, S. spontaneum, S. robustum, S. barberi, S. sinense* and *S. edule* (Daniels and Roach, 1987). *S. officinarum* or the "noble cane" accumulate very high levels of sucrose in the stem but have

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poor disease resistance, while *S. spontaneum* is lower in sucrose content but higher levels of disease resistance, adaptability and stress tolerance (Sreenivasan *et al*., 1987)

Consequently, almost all modern sugarcane varieties presently under cultivation have been derived through artificial crosses from a few common ancestral clones (*S. officinarum, S. spontaneum, S. barberi, S. robustum and S. sinense*) as the source of genetic materials (Tew, 2003). Thus, it is appears to be extremely narrow genetic base of modern sugarcane cultivars which is the serious obstacle to sustain and improve crop productivity. This is reflected in the slow progress in sugarcane breeding at present. A common objective for many breeding programs are expand the genetic base of sugarcane and selection of genetically diverse parents in crossing programs to produced new clone that could give a good character according to demand for agricultural.

Determining genetic diversity can be based on morphological, biochemical and molecular markers of information. In *Saccharum* species, morphological traits are high degree of polymorphism and highly influenced by environment interaction that makes it extremely difficult to distinguish the genotypes on the basis of morphological characters. Modern sugarcane varieties were derived from the interspecific hybridization involving different *Saccharum* species such as between the domesticated species *S. officinarum* (2n = 80) and the wild relative *S. spontaneum* (2n = 40-128) (Irvine, 1999). The genome of modern sugarcane cultivar that found in the present has polyploidy and a somatic chromosome number ranging from 100-130 (Grivet and Arrunda, 2002). Therefore, morphological characters and cytology are not reliable markers for systematic and diversity analyses

The molecular markers are very useful tool for resolved the problem complex genetic diversity of sugarcane where they show genetic differences on a more detailed level without interferences from environmental factors (Tabussum *et al.,* 2010). Genetic diversity had been studied in *Saccharum* complex using molecular markers such as Restriction fragment length polymorphisms (RFLPs) (Rodriguez *et al.,* 2003), Random amplified polymorphic DNAs (RAPDs) (Tabussum *et al.,* 2010; Nawaz *et al.,* 2010), Amplified fragment length polymorphisms (AFLPs) (Ana *et al.,* 2005), and Microsatellites (Giovanni *et al.,* 2003).

In Thailand, the RAPD marker technique has been used to assess genetic diversity in some sugarcane cultivars and germplasm. This marker is the use of a single arbitrary oligonucleotide primer to amplify template DNA without prior knowledge of the target sequence that provide fast results detailing genetic diversity. The main objective of this study was to characterize the extent and pattern of genetic diversity among a collection of *Saccharum* species

and hybrid using RAPD markers. In this context, information on the phylogeny and genetic diversity of available germplasm is essential for the identification of potential germplasm groups and clone selection for hybridization that will be useful for developing a successful breeding program in future.

Materials and methods

The fifty-six cultivars of *Saccharum* sp. were collected from field crops research center at Suphanburi and Khonkhaen province where were maintained of sugarcane germplasm (Table 1). The fresh young leaves were collected from individual cultivars and used for DNA extraction using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The yield of the extracted DNA was quantified by spectrophotometry and DNA concentration was also checked by agarose electrophoresis method on 1 % agarose gel in 1X TBE buffer. The extracted genomic DNA in each clones were diluted with TE buffer to a concentration of 50 ng/ µl.

RAPD primers (Decamer) were used for the amplification that described by William *et al.* (1990). The twenty microliter of mixture was consisted of 1 unit of *Taq* DNA polymerase, 4.5 mM Magnesium chloride, 0.4 mM deoxynucleotide triphosphates, 1.5X PCR buffer, 2 mM RAPD primer and 250 ng DNA template. Amplification was done using thermal cycler with following program: initial denaturation at 94°C for 5 min, 40 cycles were run with denaturation at 94°C for 1 min, primer annealing at 36°C for 1.30 min and extension at 72°C for 2 min and final extension at 72°C for 2 min. RAPD products were separated by agarose electrophoresis on 1.5 % agarose gel in 1X TBE buffer along with 100 bp of DNA marker. The gel were stained with 10 µg/ml ethidium bromide solution about 10 min, then rinsed the gel with sterile water about 10 min. The gel was examined under UV light transilluminator and photographed using gel documentation system.

Table 1. Description 56 genotypes of *Saccharum* sp. used in this study

Code	Cultivars	Species	Code	Cultivars	Species
Sa 01	SP 80	Hybrid	Sa 29	TP07-424	Hybrid
Sa 02	KK 80	Hybrid	Sa 30	TP07-426	Hybrid
Sa 03	UT 84-10	Hybrid	Sa 31	E-heaw	Hybrid
Sa 04	UT 84-11	Hybrid	Sa 32	ThS97-48	*S. spontaneum*
Sa 05	UT 8	Hybrid	Sa 33	KK-1	Hybrid
Sa 06	LK92-11	Hybrid	Sa 34	K88-92	Hybrid
Sa 07	Jeandang	Hybrid	Sa 35	ThS97-45	*S. spontaneum*
Sa 08	Co-8232	Hybrid	Sa 36	ThS97-42	*S. spontaneum*

Sa 09	85-2-353	Hybrid	Sa 37	TPJ03-425	Hybrids
Sa 10	ROC1	Hybrid	Sa 38	ThS97-41	*S. spontaneum*
Sa 11	K84-200	Hybrid	Sa 39	ThS97-51	*S. spontaneum*
Sa 12	Mauritius	Hybrid	Sa 40	ThS97-10	*S. spontaneum*
Sa 13	Co290	Hybrid	Sa 41	Chumporn 2	Hybrid
Sa 14	Egypt	Hybrid	Sa 42	SO10-08	Hybrid
Sa 15	UT 1	Hybrid	Sa 43	TP07-305	Hybrid
Sa 16	UT 2	Hybrid	Sa 44	KU60-3	Hybrid
Sa 17	UT 3	Hybrid	Sa 45	B37-16	Hybrid
Sa 18	UT 5	Hybrid	Sa 46	Mahasarakham	Hybrid
Sa 19	SP50	Hybrid	Sa 47	TP07-124-1	Hybrid
Sa 20	Trojan	Hybrid	Sa 48	B47-419	Hybrid
Sa 21	Badilla	*S. officinarum*	Sa 49	Thai 127	Hybrid
Sa 22	B37-161	Hybrid	Sa 50	SO10-06	Hybrid
Sa 23	TP07-428	Hybrid	Sa 51	TP07-395	Hybrid
Sa 24	KK-3	Hybrid	Sa 52	AsaToa	Hybrid
Sa 25	B43-62	Hybrid	Sa 53	Asawa	Hybrid
Sa 26	KU60-5	Hybrid	Sa 54	AIWA	Hybrid
Sa 27	B41-227	Hybrid	Sa 55	B34-164	Hybrid
Sa 28	Songkonphureau	Hybrid	Sa 56	B40-98	Hybrid

Data scoring and analysis of RAPD: Clear and well resolved bands of the sugarcane samples were compared with each other and DNA fragments were scored as present (1) or absent (0) from each primer. The data were used to estimate genetic similarity among the genotypes based on similarity index. This proximity matrix was cluster analysis derived the dendrogram using unweighted pair group method with arithmetic mean (UPGMA), and all these computations were carried out using NTSys Version 2.0e.

Results

Initially 96 decamer primers were screened, out of which only 9 primers showed reproducible fragments with easily recordable bands and gave polymorphisms between the different of 56 cultivars of *Saccharum* sp. (Table 2.). These distinguish banding patterns can be successfully, especially in primer OPC02 which could be amplify all cultivars. The number of RAPD bands per primer ranged from 7 (OPB17) to 13 (OPC19) with an average 10, size of the amplification products ranged from 350 (OPC02 and OPD08) to 1300 bp (OPB17, OPC19, OPD02 and OPD08). In total 89 polymorphic bands were scored which OPC19 primer gave the highest number of polymorphic fragments.

Table 2. Primer codes and sequences of the RAPD primers used and fragment sizes of the generated RAPD markers

Primer	Sequence(5'-3')	Fragment size (bp)
OPB17	AGGGAACGAG	400,550,600,800,1000,1100,1300
OPC02	GTGAGGCGTC	350,450,500,600,700,800,900,1000,1200
OPC18	TGAGTGGGTG	600,700,800,900,1000,1050,1100,1150,1200,1250
OPC19	GTTGCCAGCC	400,450,550,600,650,700,800,900,1000,1050,1100,1150,1300
OPD02	TCGGACGTGA	450,500,600,700,800,900,1000,1100,1200,1300
OPD08	GTGTGCCCCA	350,450,500,600,700,800,900,1000,1100,1200,1300
OPH05	AGTCGTCCCC	550,600,700,800,900,1000,1100,1150,1200
OPU03	CTATGCCGAC	500,600,700,900,1000,1100,1150,1200
OPZ04	AGGCTGTGCT	450,500,600,700,800,900,1000,1100,1150,1200

The Pearson's similarity coefficients among 56 sugarcane genotypes based on the RAPD fragments was used to construct a dendrogram (Figure 1) by UPGMA analysis. Pair-wise estimates of genetic similarity among the 56 genotypes ranged from 0.102 to 0.998. The highest genetic similarity of 0.998 was seen in AsaToa, Asawa, TP07-395 and SO10-06 that are more closely related. The lowest genetic similarity of 0.102 was seen in SP80 and UT3.

The cluster analysis based on similarity value classified 56 cultivars of *Saccharum* sp. into two major groups (Figure 1). The first major groups (I) consisted of the 12 sugarcane cultivars namely SP80, UT8411, Mauritius, UT8410, 85-2-352, Jeandang, Co8232, ROC1, UT8, LK9211, K84-200 and Co290. Those samples were come from Suphanburi field crops research center which could be subdivided into 4 subgroups (IA, IB, IC and ID). Considered of ROC1 (IB) and Co290 (ID) were separated branch from each other in these group. The other major groups, most of them were come from Khonkhane field crops research center (except: UT1, Eqypt, UT2, UT3, UT5, SP50, Trojan and Badilla) which could be subdivided into 6 subgroups (IIA, IIB, IIC, IID, IIE and IIF). The similarity value of ThS97-10 and ThS97-48 were the lowest genetic similarity between cultivars. This would select the parental in hybridization process to creating the new sugarcane cultivars.

Discussions

Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level. The genetic similarity of 56 cultivars of *Saccharum* sp. was observed ranging from 0.102-0.988 with an average value of 0.55, indicating moderate level of genetic diversity. In several other studies, sugarcane germplasm also showed moderate level of genetic diversity. Nawaz *et al.* (2010)

reported the average of genetic distance among 40 *Saccharum* spp. cultivars is 0.51 as well as Tabussum *et al.* (2010) reported in 40 *S. officinarum* and *S. barneri* cultivars with an average of 0.42. However, Pan *et al.* (2004) reported the genetic similarity coefficient in *S. spontaneum* that collected from local sugarcane collection in Louisiana is average 74.5% indicating low genetic diversity.

The use of RAPD analysis presents the advantages of a simple, fast, effective and powerful technology. In the present study was showed the moderate level of genetic diversity that the samples were collected and maintained the sugarcane germplasm in Thailand. This probably due to lack of parental diversity cultivars, so effort to be made diversity the parental genetic base for expand genetic diversity. From result of RAPD fingerprint could be help breeder to identify the diverse cultivars from different clusters and employ them in their future breeding program for selection to generate the new *Saccharum* spp. hybrid.

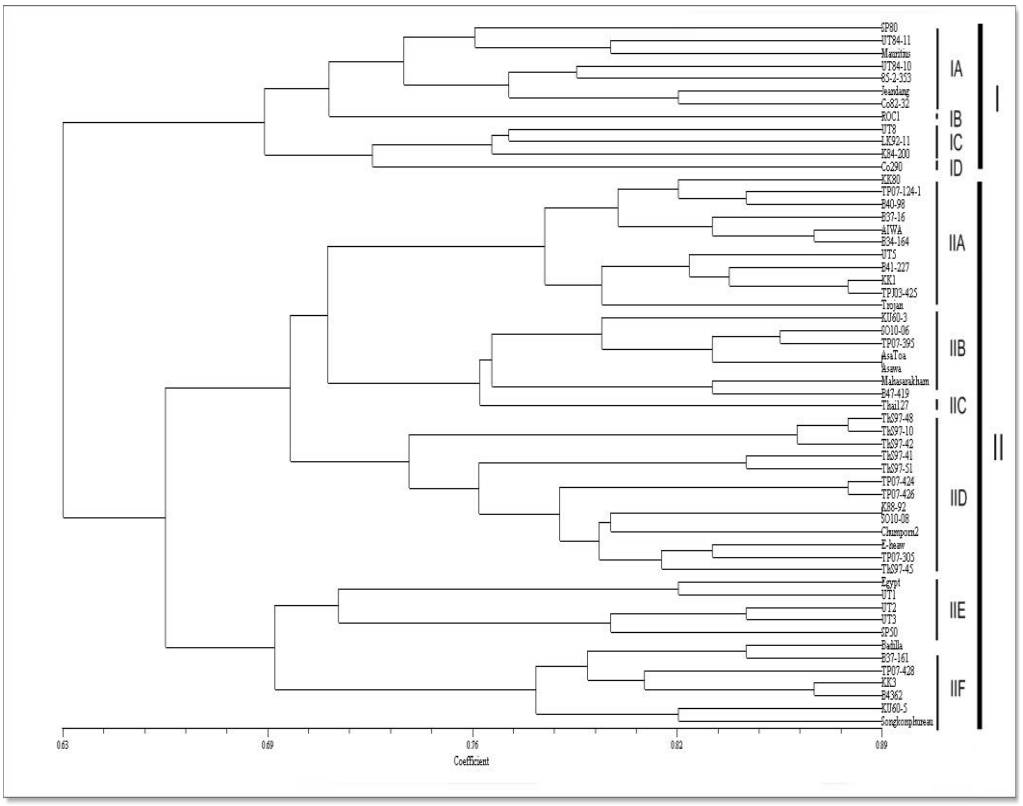

Fig. 1. Dendrogram of 56 cultivars *Saccharum* sp. constructed from RAPD data using UPGMA method based on Pearson's similarity coefficient

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