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Genetic diversity in parent lines of sweet sorghum based on agronomical traits and SSR markers

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ABSTRACT

Agronomical traits and SSR markers were used to analyze genetic diversity of 142 parent lines of sweet sorghum. Parent lines were clustered into 5 groups based on agronomical traits and each group is characterized by morphological traits and main origins. 41 selected SSR markers were used to analyze the parent lines. The genetic distance (GD) of the parent lines ranged from 0.558 to 0.858, averaging 0.640. The average specific index of parent lines was 189.0, which ranged from 109.1 to 454.7. According to SSR markers, parent lines were clustered into 7 groups but the agronomical traits of which were not significantly different between each group, and clusters based on SSR markers did not coincide with the analysis results based on agronomical traits. Cluster analysis was failed to distinctly group restorer lines and maintainer lines by both agronomical traits and SSR markers. Parent lines should be selected both with different agronomical traits and distant genetic distance for higher heterosis. Groups clustered by agronomical traits and molecular markers should be taken into account simultaneously in hybrid breeding. Information of this study can be used to select parent lines for development of segregating populations and hybrid breeding of sweet sorghum.

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1. Introduction

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is one of many types of cultivated sorghum, which is noted for its high sugar content in stem and grown for the production of grain, silage, fodder, syrup, and ethanol (Lu, 2008). And its relative resistance to drought and heat may also increase its importance worldwide if the predicted effects of global warming come true. Hybrid sweet sorghum is improved by development of superior parent lines with good combining ability to ensure productive hybrid combination. Therefore, knowledge of genetic diversity of parent lines will facilitate more efficient selection of parental genotypes and help to predict the degree of inheritance and the levels of heterosis in hybrid.

It is well documented that crosses between unrelated and consequently genetically distant parents show greater hybrid vigor than that between closely related parents (Lu, 2008). Information about genetic diversity also permits the classification of parent lines into heterotic groups, which is particularly important for hybrid breeding. The classification of parent lines into heterotic groups can be based on geographical origin, agronomical traits, pedigree data or molecular marker data (Ali et al., 2008). In the past, indirect estimates of genetic diversity based on morphological information and pedigree had been widely used in many species including sorghum (Ayana et al., 2000). Morphological traits analysis is considered to be helpful to identify heterotic patterns of combinations between parent lines for hybrid breeding. In fact, plant breeders often select parental lines in combinations with morphological traits and pedigree information which are readily observable and co-inherited with the desired traits. However, morphological variation does not reliably reflect the real genetic variation because of genotype–environment interactions and the largely unknown genetic control of polygenic inheritance morphological and agronomic traits (Shehzad et al., 2009). Subjectivity in the character evaluation is also linked to developmental stage.

With the advent of molecular genetic technique, genetic diversity was assessed by various types of molecular markers, especially those employing DNA markers, which have been proven to be a powerful tool for fingerprinting and assessing genetic variation of various crops, including maize (Phumichai et al., 2010), sorghum (Li et al., 2010), wheat (Royo et al., 2010), and rice (Xangsayasane et al., 2010). They can reveal genetic differences at the DNA level between plants which are in the absence of environmental effects and are effective to evaluate the genetic diversity of germplasm in breeding programs. On the other hand, molecular markers do not

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require previous pedigree information which is valuable for crops but frequently lacking (Bohn et al., 1999). Molecular markers such as simple sequence repeats (SSRs) (Mutegi et al., 2011), restriction fragment length polymorphism (RFLP) (Deu et al., 2006), random amplified polymorphic DNA (RAPD) (Medraoui et al., 2007), and amplified fragment length polymorphism (AFLP) (Ritter et al., 2007) have been successfully used to estimate genetic diversity in sorghum. Among these markers, SSR markers are increasingly used to assess sorghum genetic diversity because of their uniform genome coverage, high levels of polymorphism, co-dominance, specific PCR-based assays, and the more significant variability in genotyping and genetic diversity studying (Pejic et al., 1998) than other molecular markers. Studies have shown that SSR loci give good discrimination among closely related individuals even when only a few loci were employed (Kong et al., 2000).

Studies have shown that it is better to analyze genetic diversity both with morphological traits and molecular markers (Barro-Kondombo et al., 2010; Dias et al., 2008), however few studies have assessed genetic diversity using morphological traits and molecular markers simultaneously. In this study, parent lines of sweet sorghum have been used frequently for creating segregating populations and hybrid breeding, but the pedigree information for many of them was not available or poorly documented during the procedure of introduction, crossing and backcrossing. Therefore, it is necessary to assess the genetic diversity of these parent lines in order to provide useful information to increase the efficiency in breeding program. The main objectives of this research were to estimate the genetic diversity of parent lines which were used in hybrid breeding program based on agronomical traits and SSR markers, and to compare the classification of parent lines obtained by different methods.

2. Materials and methods

2.1. Plant materials

One hundred and forty-two parent lines of sweet sorghum including 118 restoring lines (R-lines) and 24 maintenance lines (B-lines) (Table 1), which from China (55), America (24), India (15), Mexico (17), Russia (16) and Ukraine (15) were used in this study.

2.2. Data collection of agronomical traits

The parent lines were grown at the experimental field of Heilongjiang Academy of Agricultural Sciences (Harbin, China) in 2008 and 2009 for evaluation of days from emergence to flowering, growth period, tillers, stem diameter, sugar content, biomass per plant, plant height, panicle length, panicle weight and 1000-grain weight.

The parent lines were planted in three-row plots of 5.0 m long with a spacing of 15 cm between the plants and 0.67 m between the rows with three replications. Data was recorded from continuous sampling of 10 plants of each middle row, and mean value of each trait was used for analysis. Sugar content was measured with a hand-held refractometer by reading juice samples obtained from the whole stalk.

Analysis variance (ANOVA) for agronomical traits was conducted by the Statistics Analysis System (SAS). Cluster analysis was carried out using genetic similarity (GS) and unweighted pair-group method with arithmetic average (UPGMA).

2.3. DNA isolation and PCR assay

DNA was extracted from leaf tips of three to five seedlings of each line using a CTAB method according to Doyle and Doyle (1990).

103 SSR primer pairs which covered all the ten linkage groups of sorghum genome were selected based on the published information (http://sorgblast3.tamu.edu/Sorghum Genome/Mapping). From which, 41 pairs of primers with high levels of polymorphism and stable amplification were selected to genotype the 142 lines (Table 3).

The PCR reactions were performed in 20 μL reaction volumes containing 2 μL genomic DNA, 1.5 μL Primer, 1.5 μL dNTP (10 mmol L^{-1}), 2 μL reaction Buffer (10×), 2 μL $MgCl_2$ (mmol L^{-1}), 0.5 μL *Taq* DNA polymerase (5 U μL^{-1}) and 10.5 μL dd H_2O. Amplifications were performed on a Bio-Rad S1000 Thermocycler. PCR reaction conditions consisted of 5 min at 94 °C for initial denaturation, followed by 35 cycles of amplification of 20 s at 94 °C, an annealing step of 30 s at the appropriate annealing temperatures, 40 s at 72 °C, and 10 min at 72 °C for final extension. PCR products were separated on 6% non-denaturing polyacrylamide gels in 1× TEB buffer, and then visualized with silver staining (Panaud et al., 1996).

2.4. SSR data analyses

Diversity index of primers and specific index of lines were analyzed by using Genetics Statistics 3.0 (Gao et al., 2009). Polymorphism information content (PIC) was calculated by using the formula developed by Anderson et al. (1993).

Genetic similarities (GS) between pairs of lines were measured by the Dice coefficient based on the proportion of shared alleles (Nei and Li, 1979). Genetic distances (GD) between pairs of lines were estimated as GD = 1 − GS.

Clustering analysis was performed by using Genetics Statistics 3.0 and UPGMA (Gao et al., 2009).

3. Results

3.1. Agronomical traits analysis

142 parent lines were clustered into 5 groups at the average distance of 0.65 (Fig. 1). The agronomical traits between each group were different (Table 2).

Group I consisted 22 parent lines with the main characters of middle growth period, lower plant height and lower biomass per plant. This group mainly included 7 lines from America and 7 lines from China, accounting for 31.8% and 31.8%, respectively, and 4 lines from Mexico. There were 5 B-lines in this group, and others were R-lines.

Group I was again sub-divided into 2 sub-groups according to plant height, panicle weight and tillers. The characters of the first sub-group were lower plant height (104.9 cm), higher panicle weight (45.3 g) and all most non-tiller. The characters of the second sub-group were taller plant height (206.2 cm), lower panicle weight (37.3 g) and 0.24 tillers.

Group II consisted 43 lines with the main characters of long growth period, low plant height, thick stem diameter and high panicle weight. This group mainly included 24 Chinese lines, 12 out of 17 Mexican lines and 4 Indian lines. There were 14 B-lines in this group, and others were R-lines.

Group III consisted 17 lines with the main characters of the shortest growth period and the highest plant height. This group mainly included 6 American lines, 5 Ukrainian lines and 4 Russian lines. All the lines in this group were R-lines.

Group IV consisted 52 lines with the main characters of higher plant height and higher biomass per plant. There were 1/2 each of Indian, Russian and Ukrainian lines, and 1/3 each of Chinese and American lines in this group except Mexican

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