Biocatalysis and Agricultural Biotechnology 3 (2014) 198–206

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Original Research Paper

Diversity studies on soybean accessions from three countries

D. Appiah-Kubi [a,*], J.Y. Asibuo [a], M.D. Quain [a], A. Oppong [a], R. Akromah [b]

[a] CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana
[b] Kwame Nkrumah University of Science and Technology, Faculty of Agriculture, Kumasi, Ghana

ARTICLE INFO

Article history:
Received 11 April 2013
Received in revised form
5 November 2013
Accepted 25 November 2013
Available online 12 December 2013

Keywords:
Diversity
Soybean
SSR markers
PCA
Biplot

ABSTRACT

Experiments were conducted to assess the genetic and morphological diversity among 36 soybean [*Glycine max* (L.) Merrill] accessions from three different countries, namely: Ghana, Nigeria and Brazil using SSR markers and morphological traits. For genetic diversity studies, 20 mapped soybean SSR markers were amplified via PCR and then run on agarose gel. Molecular data was scored and analysed using CLUSTER procedure in SAS. Morphological characterization using quantitative traits were also analysed using principal component analysis (PCA) procedures of SAS. These 20 SSR markers grouped the germplasm into six clusters based on Jaccard similarity coefficient. Three morphological traits namely: plant height, number of seeds per pod and days to maturity were the most important traits that discriminated the germplasm into three clusters based on PCA biplot, which also corresponded to their country of origin. Hybridization of these gene pools could enhance soybean breeding efforts in Ghana.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is a leguminous annual crop belonging to the family *Fabaceae*. It is known to have originated from China (Fukuda, 1933; Vavilov, 1951; Hymowitz and Newell, 1981). According to Hymowitz (1970), the first domestication of soybean was traced to the eastern part of North China in the eleventh century B.C. It is said that, the exchange of soybean seeds through human activities resulted in gene flow from centre of origin to Korea, Japan and other parts of South–East Asia (Abe et al., 2003). Soybeans are grown primarily for the production of seed and has several uses in the food and industrial sectors; more recent is the development of feedstock for biodiesel for which Ghana has the potential (Johnson and Holloway, 2007). It is also one of the few legume crops that has the greatest potential to contribute to employment and income generation in rural communities if its agro-industrial potentials are exploited. Among the grain legumes, soybean currently ranks third after groundnut and cowpea in terms of production and utilization in Ghana (MoFA, 2011).

The demand for soybean as a raw material for oil and cake for the poultry industries has been on the ascendency over the years in Ghana (Gage et al., 2012). The total world soybean production as at 2011 was 260.9 million metric tons (FAOSTAT, 2013) and has been forecasted to be 371.3 million metric tons by 2030 (Masuda and Goldsmith, 2009). According to Masuda and Goldsmith (2009), research investment and effort are needed to generate the yield improvement necessary to meet global demand projections.

Soybean research and production in Ghana are recent, and are besieged with a lot of constraints. These include inadequate funding, marketing, seed viability, low research effort, pest and diseases, narrow genetic base, and pod shattering among others (Wuni, 2011). However, narrow genetic base is one of the major problems that affect soybean breeding and development in Ghana.

The existing soybean varieties in the country were introductions from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; which were evaluated by CSIR-Crops Research Institute (CSIR-CRI) and CSIR-Savannah Agriculture Research Institute (CSIR-SARI) and released as varieties to farmers (Asafo-Adjei and Adekunle, 2001; Tefera et al., 2010). Since 1990, eight commercial soybean varieties namely: Salintuya-1, Bengbie, Anidaso, Quarshie, Jenguma, Nangbaar, Ahoto, and Salintuya-2 have so far been released in Ghana by CSIR-CRI and CSIR-SARI. Since the soybean varieties Ahoto and Nangbaar were released in 2005, no new variety has been reported.

Introducing new soybean lines can increase genetic diversity, thereby facilitating the development of new varieties that can address some of the constraints in soybean production. Thus, there is the need to assemble new germplasm from different geographical regions in order to assess their genetic diversity at both morphological and molecular levels. This will lead to the selection of parental lines with desirable traits for hybridization to produce new varieties that meet both the industrial and consumer needs in Ghana.

Information on genetic diversity and relationships in crop plants are important for efficient selection of parental lines for

* Corresponding author.
E-mail addresses: davekubi@yahoo.co.uk, dadavekubi@gmail.com (D. Appiah-Kubi).

1878-8181/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bcab.2013.11.008

new crosses to be initiated; and also for preservation of germplasm by plant breeders (Tatineni et al., 1996). Traditionally, morphological traits have been used to distinguish crop varieties (Chowdhury et al., 2001), which are highly influenced by environmental factors. For plants with narrow genetic base such as soybean, molecular characterization can provide additional information on their degree of genetic diversity (Acquaah, 2007). The objective of this study was to evaluate the genetic diversity existing between released varieties and exotic germplasm using morphological and molecular tools.

2. Materials and methods

2.1. Morphological analysis

Both field and laboratory experiments were conducted at the CSIR – Crops Research Institute research station at Fumesua – Kumasi, Ghana during the major season of 2010. Experimental field was ploughed and harrowed. Thirty-six soybean genotypes being used in the soybean breeding programme at CSIR–CRI were evaluated for this study. They consisted of 20 lines from IITA, Nigeria; ten cultivars from EMBRAPA, Brazil; and six released cultivars from Ghana (Table 1). The experimental design was laid out using alpha lattice design with three replications. There were four row plots measuring 4 m long and a spacing of 60 cm between rows and 5 cm within rows. Weeds were controlled by hand hoeing at three and six weeks after planting. Pre-flowering insects were controlled using karate (at the rate of 15 g lambda-cyhalothrin per hectare) and against post-flowering insects using 400 g dimethoate per hectare.

Table 1
Soybean accessions used for the study.

Serial number	Soybean cultivar/lines	Source/institution	Country
1	TGX 1805-31F	IITA	Nigeria
2	TGX 1903-7F	IITA	Nigeria
3	TGX 1903-8F	IITA	Nigeria
4	TGX 1904-2F	IITA	Nigeria
5	TGX 1904-3 F	IITA	Nigeria
6	TGX 1904-6F	IITA	Nigeria
7	TGX 1909-3F	IITA	Nigeria
8	TGX 1910-16F	IITA	Nigeria
9	TGX 1835-10E	IITA	Nigeria
10	TGX1903-2 F	IITA	Nigeria
11	TGX 1903-1 F	IITA	Nigeria
12	TGX 1842-18E	IITA	Nigeria
13	TGX 1843-29E	IITA	Nigeria
14	TGX 1844-4 E	IITA	Nigeria
15	TGX 1844-18E	IITA	Nigeria
16	TGX 1910-2F	IITA	Nigeria
17	TGX 1910-3F	IITA	Nigeria
18	TGX 1910-6F	IITA	Nigeria
19	TGX 1910-14F	IITA	Nigeria
20	GMX 92-6-10	IITA	Nigeria
21	BOAVISTA	EMBRAPA	Brazil
22	CELESTE	EMBRAPA	Brazil
23	TRACAJA	EMBRAPA	Brazil
24	PIRARARA	EMBRAPA	Brazil
25	FLORA	EMBRAPA	Brazil
26	BRAZILLIA	EMBRAPA	Brazil
27	MG 68	EMBRAPA	Brazil
28	RAMUNDA	EMBRAPA	Brazil
29	MG/BR 46	EMBRAPA	Brazil
30	SAMBAIBA	EMBRAPA	Brazil
31	JENGUMA	CSIR-SARI	Ghana
32	SALINTUYA-1	CSIR-SARI	Ghana
33	SALINTUYA-2	CSIR-SARI	Ghana
34	QUARSHIE	CSIR-CRI	Ghana
35	NANGBAAR	CSIR-SARI	Ghana
36	ANIDASO	CSIR-CRI	Ghana

Both quantitative and qualitative data were collected using morphological and phenological descriptors based on United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Germplasm Resources Information Network (GRIN) (USDA-ARS, 2010). Field data collection was limited to the two central rows of each plot. Qualitative traits scored were flower colour, pod colour, pubescence colour (when plants had dried), pubescence density, pubescence form, general shape of seed (near round, oblong, oval), seed-coat colour, hilum colour, maturity group, visual nodulation score (from 1 = no nodule to 5 = ample large, active nodules) and lodging (the tendency of stem to lodge was measured at maturity) and was scored visually on the scale 1 (erect) to 5 (prostrate).

For quantitative traits, flowering date was measured by counting the number of days to the date 50% of plants flowered after planting. Days to maturity is the date 95% of the pods reached final colour (when pod colour changes from green to tawny). Mean plant height (centimetres) was measured at the field by taking the heights of three randomly selected plants in each plot from the ground to the stem tip at maturity. Similarly, mean pod length (millimetres) per genotype was measured by randomly selecting five pods and each length measured with the vernier calliper (Tricle brand). Mean seed shape (based on height/length ratio and height/thickness ratio) was also measured with the vernier calliper using five pods per genotype per plot. Number of branches per plant was counted with a counter (Brannan tally-counter) using five randomly selected plants for each genotype. Mean number of pods per plant and mean number of seeds per plant was counted using same five plants for each genotype. A hundred (100) – seed weight from each genotype per plot was also measured with the analytical balance (aeADAM AEP-6000G) and the mean weight recorded. Biomass per plant was taken using the same five plants randomly selected, (i.e. each whole plant with stem, branches, and pods) weighed and a mean weight (grams) recorded using the analytical balance.

Genomic DNA was extracted from leaves of three weeks old soybean seedlings using Qiagen extraction kit following the manufacturer's instructions (QIAGEN Sciences). Twenty mapped soybean SSR markers (Table 3) were used to amplify the genomic DNA via polymerase chain reaction (PCR). A 10 μL PCR reaction mixture (Biolabs regents) comprised 6.15 μL nuclease free water, 1 μL of 10× PCR buffer, 0.9 μL of 25 mM $MgCl_2$, 0.4 μL of 10 mM dNTPs, 0.25 μL of each 5 μM primer (F/R), 0.05 μL of 5 U/μL *Taq* polymerase and 1 μL of 30 ng/μL genomic DNA. The amplification processes were carried out using thermocyler (Gene Amp® PCR system 9700 version 3.09) at 95 °C/2 min as initial denaturation; followed by 92 °C/1 min, 47 °C/1 min, 72 °C/1 min for 33 cycles; and 72 °C/10 min as the final extension. After the amplification process, 2 μL of 6× loading dye (Fermentas) was added to each PCR product; and electrophoresed on 2% Metaphor agarose gel.

The origin of soybean SSR markers used for this study was obtained from University of Georgia, USA; and were chosen based on previous work reported in literature that shows high polymorphism at marker locus (Hwang et al., 2008; Shultz et al., 2007; Hisano et al., 2007; Grant et al., 2013).These 20 mapped SSR markers used (Table 2) were fairly distributed within the genome as documented in soybean genetic linkage mapping database (Grant et al., 2013).

2.2. Statistical analysis for quantitative and molecular data

For the quantitative traits, genetic similarity between pairs was calculated according to Euclidian coefficient and the centroid method of hierarchical cluster analysis (SAS, 2007). Quantitative data were