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Genetic diversity in NaN_3 -induced common bean mutants and commercial varieties detected by AFLP

CL Chen¹, HH Wang¹, TL Jeng², SJ Chuang³, ML Wei² and Sung Jih Min^{4*}

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ABSTRACT – The analysis of Amplified Fragment Length Polymorphism (AFLP) was used to estimate genetic diversity in common bean (*Phaseolus vulgaris* L.) variety *Hwachia* and in 34 NaN_3 -induced mutants and 11 commercial varieties introduced from China. Eight primer combinations generated 516 DNA fragments of the tested mutants and introduced varieties, of which 448 fragments were polymorphic. The calculated Jaccard similarity coefficients based on AFLP data ranged from 0.47 to 0.84. The molecular profiles obtained from eight AFLP primer pairs indicated a high genetic diversity among *Hwachia*, NaN_3 -induced mutants and introduced varieties. The extent of genetic variation was slightly higher between *Hwachia* and NaN_3 -induced mutants than between *Hwachia* and introduced commercial varieties. These results, supported by cluster analysis, suggest that NaN_3 -induced mutagenesis effectively broadens the genetic diversity of common bean varieties. Some of the produced mutants could be useful as sources of variation to develop new improved common bean varieties.

Key words: genetic similarity, mutagenesis.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a morphologically diverse grain legume, with large detectable variations for traits related to growth habit, pigmentation, pods and seeds and others (Singh 2001, Coan et al. 2010). It was introduced in Taiwan over a century ago, but is not popular with the local farmers because its yield is lower than of other grain legumes. This factor has reduced the germplasm sources used in hybridization and limited the genetic variability available for common bean breeding in Taiwan. Recently, the fact that common bean is rich in many phytochemicals with beneficial effects on human health (Jeng et al. 2010) has rekindled interest in

the crop in Taiwan. But the number of modern varieties with improved agronomic and phytochemical traits is very limited.

Genetic diversity is the basis of genetic improvement of crops and the most important condition in any breeding program. The genetic viability of common bean in Taiwan is rather limited. To broaden genetic variation, it is therefore essential to collect desirable traits from introduced varieties or wild populations (Singh 2001, Newton et al. 2009). Another approach is to use chemical mutagenesis to broaden genetic diversity (Parry et al. 2009). Mutation breeding in crop plants is a well-documented technique, especially in crops with a narrow genetic base. Many mutants have been identified as donors of desirable traits

¹ Department of Agronomy, National Chung Hsing University, Taichung City, Taiwan, ROC

² Division of Biotechnology, Agricultural Research Institute, WuFeng, Taichung City, Taiwan, ROC

³ Division of Biotechnology, Taiwan Seed Improvement and Propagation Station, Taichung City, Taiwan, ROC

⁴ Department of Food Science and Technology, Hung Kuang University, Shalu, Taichung City, Taiwan, ROC. *E-mail: sungjm@sunrise.hk.edu.tw

in breeding programs. Results of chemical mutagenesis in morphological, physiological and genetic changes, as well as variety development has been reported in common bean (Blair et al. 2007).

Recently a chemical mutagenesis program of the common bean landrace Hwachia, which is very popular in central Taiwan was implemented, and many NaN_3 -induced mutants with differing seed traits were produced (Jeng et al. 2010). Nevertheless, the extent of genetic diversity among these mutants and other introduced common bean varieties has not been determined. In this study, the amplified fragment length polymorphism (AFLP) technique was used to assess the extent of diversity among the NaN_3 -induced mutants and the commercial varieties introduced from China. This information would be useful in the selection of parents for hybrid production with maximum heterosis and of combinations of useful genes in improvement programs of common bean varieties.

MATERIAL AND METHODS

In the present study, the variety Hwachia and its 34 stabilized NaN_3 -induced M6 mutants (Jeng et al. 2010) plus 11 commercial varieties introduced from north and southwest China were used. The experiments were performed in the spring of 2008. Sowing was done manually in rows. Fertilization consisted of the banded application of N, P_2O_5 and K_2O (16, 32 and 31 kg ha⁻¹, respectively). Weed control was performed by hand, and plots were maintained pest and disease-free until harvest. The experimental plots consisted of multiple 7-m long rows spaced 0.6 m and plants 15 cm apart.

Genomic DNA of each accession was extracted from freshly harvested young leaves by using CTAB (hexadecyltrimethylammonium bromide) procedure described by Chuang et al. (2009). The samples of bulked leaf tissues (500 mg) collected from five individual plants were used for DNA extraction. DNA concentration was estimated by a full-spectrum UV/VIS spectrophotometer (NanoDrop, ND-1000), diluted with sterile distilled water to a final concentration of 100 ng μL^{-1} . AFLP analysis and electrophoresis were carried out with eight pre-selected primer pairs (Table 1).

The percentage of polymorphism, polymorphism information content (PIC_i) and Jaccard's similarity coefficient were calculated using the methods detailed by Roldán-Ruiz et al. (2000) and Jaccard (1908), respectively. The similarity coefficient matrix was subjected to cluster analysis by the Un-weighted Pair Group Method of arithmetic

Averages (UPGMA). A dendrogram was constructed using NTSYS-PC software (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological traits are frequently used to quantify the genetic diversity in common bean genotypes (Ceolin et al. 2007). However, the major limitation of morpho-agronomical characterization is that this kind of evaluation often involves a high number of descriptors, many influenced by the environment, particularly those conditioned by many genes (Chiorato et al. 2007). Therefore, DNA markers, such as RAPD (random amplified polymorphic DNA) (Alzate-Martin et al. 2001; Chiorato et al. 2007) and AFLP (amplified fragment length polymorphism) (Kumar et al. 2008), are also used to determine the genetic diversity in common bean. In this study, the genomic DNA collected from wild type variety Hwachia and its 34 NaN_3 -induced mutants and 11 commercial varieties introduced from China were compared by AFLP analysis. All AFLP primer pairs produced well-defined and scorable DNA fragments with polymorphism among the 46 common bean accessions (Table 1). The average number of scored fragments per primer pair was 56, which was higher than reported by Maciel et al. (2003), who obtained a mean of 38 fragments for common bean; polymorphic fragments were generated with every primer pair.

The level of variation detected by AFLP depends on the number of primer pairs and the genetic distance between the varieties analyzed. From the 46 common bean DNA samples amplified with polymerase chain reaction (PCR) with 8 pre-selected primer pairs, a total of 516 fragments were obtained, of which 448 were polymorphic (Table 1). The higher number of polymorphic fragments confirms the polymorphism detection efficiency of AFLP (Maciel et al. 2003). The polymorphic banding patterns of these primer pairs averaged from 69.23 % for *E-AAC/M-CAA* to 97.65 % for *E-ACG/M-CAT*. The average percentage of polymorphic fragments, across eight primer pairs, was 86.82 %. The calculated values of polymorphism information content (PIC_i) for the eight primer pairs ranged from 0.1861 (*E-ACT/M-CAG*) to 0.2410 (*E-ACG/M-CAT*) (Table 1). The average PIC_i value for the amplification products, across eight primer pairs, was 0.2065.

Kumar et al. (2008) and Svetleva et al. (2006) used the AFLP technique, each with different primer pairs, to analyze the genetic diversity of common bean varieties and landraces collected at different locations, and found an average similarity coefficient of 0.52 and 0.91,

Table 1. AFLP primer combinations with the respective number of scored fragments

AFLP primer-pair	Nr. Monomorphic fragments	Nr. polymorphic fragments	Total produced fragments	Percent polymorphic	PIC _i
<i>E-AGC/M-CTC</i>	11	65	76	85.53	0.2025
<i>E-ACT/M-CAG</i>	11	63	74	85.14	0.1861
<i>E-ACT/M-CAA</i>	10	46	56	82.14	0.1975
<i>E-ACG/M-CAT</i>	2	83	85	97.65	0.2410
<i>E-ACC/M-CTC</i>	6	69	75	92.00	0.1990
<i>E-ACC/M-CAT</i>	8	60	68	88.24	0.2097
<i>E-AAC/M-CAA</i>	8	18	26	69.23	0.1863
<i>E-AAC/M-CAG</i>	12	44	56	78.57	0.2112
Total	68	448	516		
Mean	8.5	56	64.5	86.82	0.2065

respectively. However, only very limited AFLP data are available for evaluating the genetic diversity in crop mutants (Lu et al. 2007, Rashid et al. 2009) obtained by induced mutation. In the present study, Jaccard genetic similarity coefficients were calculated between pairs of common bean accessions. Pair-wise similarities ranged from 0.47 (SA-34 vs. PI-Agro-07) to 0.84 (SA-19 vs. SA-21). An average similarity coefficient of 0.65 was found, which was higher than the average similarity coefficient (0.52) of Kumar et al. (2008), but lower than the average similarity coefficient (0.91) reported by Svetleva et al. (2006).

The similarity matrix further indicated that the similarity coefficients between wild type Hwachia and NaN₃-induced mutants ranged from 0.49 (Hwachia vs. SA-031) to 0.77 (Hwachia vs. SA-03), with an average of 0.68 across all pair-wise comparisons. These variations in Jaccard similarity coefficients were wider than the range obtained for Hwachia and introduced varieties (0.511-0.699). Thus, NaN₃-induced mutagenesis appears to be more effective in generating and broadening genetic diversity than variety introduction (Al-Qurainy and Khan 2009), at least in the case of the commercial varieties introduced from China. However, relatively higher Jaccard similarity coefficients were found between some NaN₃-induced mutants and introduced varieties. For instance, a similarity coefficient of 0.73 (higher than the average of 0.68) was obtained for SA-25 and PI-43. Thus, it appears that the NaN₃-induced mutants SA-07, SA-25 and SA-31 were genetically close to some of the introduced commercial varieties, and were more distant from the wild type Hwachia and other NaN₃-induced mutants.

A dendrogram obtained from the similarity matrix showed two clusters, separating at a similarity value of

roughly 0.54 (Figure 1). Cluster I contained four NaN₃-induced mutants (SA-07, SA-08, SA-30, SA-31) and five introduced varieties (PI-Agro-05, PI-Agro-06, PI-Agro-07, PI-Agro-09 and PI-40) (Figure 1). Wild type Hwachia and other 30 mutants and six introduced varieties were grouped in cluster II. These results confirmed that the mutants SA-07, SA-08, SA-30 and SA-31 were more distinct from the wild type Hwachia. The agronomic traits observed (unpublished data) showed that the dry seeds of NaN₃-induced mutants and introduced commercial varieties grouped in cluster I generally were relatively smaller than of Hwachia, NaN₃-induced mutants and introduced varieties grouped in cluster II. Most cultivated common bean varieties are reported to originate from two separate gene pools, the Mesoamerican and Andean, with marked morphological differences (Singh 2001). The seeds of the Andean type varieties are generally larger (> 40 g per 100 seed) than of Mesoamerican type varieties (< 40 g per 100 seed) (Rodiño et al. 2006, Chiorato et al. 2007). The origins of the wild type Hwachia and introduced varieties from north and southwest China are unknown; however, they might be derived from different gene pools. In this regard, a follow-up study to verify the genetic origin should be conducted.

Genetic diversity, resulting from natural selection, spontaneous mutation and artificial selection, is the most important aspect in any plant breeding program. In this study, the molecular profiles obtained from eight AFLP primer pairs demonstrated high genetic diversity among the wild type Hwachia, 34 NaN₃-generated common bean mutants and 11 introduced commercial varieties. However, the degree of genetic diversity in NaN₃-induced mutants was slightly higher than of the Chinese commercial

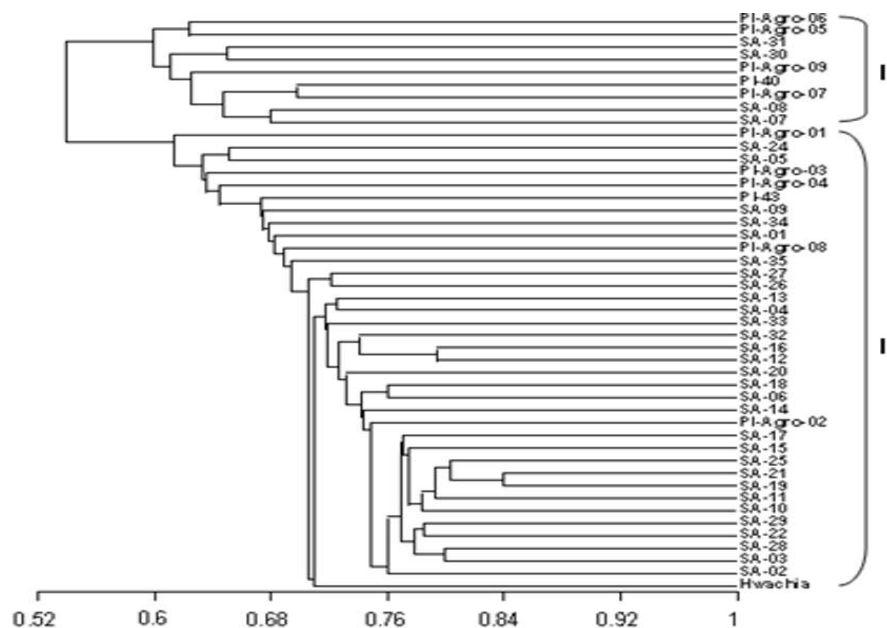


Figure 1. Dendrogram showing variation among variety Hwachia and its 34 NaN_3 -induced mutants and 11 commercial varieties introduced from China based on the calculated Jaccard coefficients using AFLP fragments obtained by eight primer pairs.

varieties. These results, supported by cluster analysis, indicate that NaN_3 -induced mutagenesis could effectively broaden the genetic diversity of common bean varieties. Some of the produced mutants could be useful as sources of variation for the development of new improved common bean varieties.

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Diversidade genética revelada por AFLP em mutantes induzidos por NaN_3 e variedades comerciais de feijão

RESUMO – Marcadores AFLP foram utilizados para analisar a diversidade genética em feijão (*Phaseolus vulgaris* L.) na variedade Hwachia, em 34 mutantes induzidos por NaN_3 e 11 variedades comerciais chinesas. Oito combinações de primers geraram 516 fragmentos de DNA dos mutantes e variedades chinesas, dos quais 448 fragmentos foram polimórficos. Os coeficientes de similaridade de Jaccard calculados com base nos dados de AFLP variaram de 0,47 a 0,84. Os perfis moleculares de AFLP indicaram alta diversidade genética entre Hwachia, mutantes induzidos por NaN_3 e variedades chinesas. A extensão da variação genética foi ligeiramente maior entre Hwachia e mutantes induzidos por NaN_3 do que entre Hwachia e introduziu variedades comerciais chinesas. Estes resultados, apoiados pela análise de cluster, sugerem que a mutagênese induzida por NaN_3 efetivamente amplia a diversidade genética das variedades de feijão. Alguns dos mutantes produzidos podem ser úteis como fontes de variação para desenvolver novas variedades melhoradas de feijão.

Palavras-chave: similaridade genética, mutagênese.

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