Molecular and Morpho-agronomic Characterization of NaN_3-induced Common Bean Mutants

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ABSTRACT

In this study, amplified fragment length polymorphism (AFLP) and morpho-agronomic diversities in Hwachia, a common bean (*Phaseolus vulgaris* L.) variety, and its 34 NaN_3-induced mutants (M6 generation) were investigated. Eight primer combinations generated 482 fragments from the tested materials, of which 402 fragments were polymorphic. The calculated Jaccard similarity coefficients based on AFLP data ranged from 0.469 to 0.839. Both clustering and principal coordinate analyses confirmed the presence of molecular diversities among Hwachia and its mutants. Morpho-agronomic variations were also observed among the tested mutants. The relations between Hwachia and its mutants based on AFLP data were correlated with those based on morpho-agronomic characteristics (r = –0.713**). Thus, NaN_3-induced mutagenesis indeed broadened the genetic variation, and possibly produced new varieties in the common bean. Mutants SA-07, SA-08, SA-09, SA-14, SA-30 and SA-31 are useful as sources for deriving new varieties.

Key words: AFLP, Common bean, Genetic similarity, Morpho-agronomic trait.

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疊氮化鈉誘變之菜豆突變系分子與形態農藝特性研究

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摘要

本研究利用擴增增殖片段長度多型性及形態與農藝性狀之差異來探討菜豆品種花莢種及其 34 個疊氮化鈉誘變突變系之遺傳變異。所使用八個引子對在供試突變系共產生 482 個 DNA 片段，其中 402 個 DNA 片段為多形性片段。依據擴增增殖片段長度多型性資料所估算之 Jaccard 相似性係數介於 0.469 與 0.839 之間。群叢及主軸分析確認花莢種及其 34 個疊氮化鈉誘變突變系的確有明顯的分子層次變異。供試材料間也有明顯的形態與農藝性狀差異。此二種特性也有極顯著的相關性 (r = 0.713**)。因此，疊氮化鈉引起的誘變的確擴大遺傳變異，並可能產生新的菜豆品種。其中突變系 SA-07，SA-08，SA-09，SA-14，SA-30 及 SA-31 可做為育成新品種之材料。

關鍵詞：增增殖片段長度多型性、花豆、遺傳相似性、形態與農藝性狀。

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is widely cultivated in the tropics, subtropics and temperate regions for human consumption. It has been introduced into Taiwan for more than a century, but has not favored by the local farmers because of its lower yield compared with other legumes. Recently the common bean is regaining interest because it has been characterized as a healthy food due to its high phenolics content (Broughton *et al*. 2003, Aparicio-Fernández *et al*. 2005). However, the available local landraces are very limited in Taiwan, and no bred varieties have been released to common bean growers. Therefore, developing varieties with improved agronomic traits is a primary goal of the common bean breeding program in Taiwan.

Plant breeding requires the genetic variation of useful traits for crop improvement. However, the genetic base of common bean landraces is narrow in Taiwan. To broaden the genetic base, it is essential to collect the favorable alleles from wild populations or alien species (Singh 2001). Another approach is to use mutagenesis to broaden the genetic diversity (Ahloowalia *et al*. 2004, Parry *et al*. 2009). The results of chemical mutagenesis of common beans in morphological, physiological and genetic changes, as well as varietal development were reported (Blair *et al*. 2007, Al-Qurainy and Khan 2009, Campion *et al*. 2009).

Many studies have used morphological and agronomical molecular information to quantify the genetic diversity present in common bean genotypes (Gómez *et al*. 2004, Durán *et al*. 2005, Rosales-Serna *et al*. 2005, Galvan *et al*. 2006). Morphological data are easy to identify but the number of distinctive characteristics is rather limited. Agronomic characteristics are often multi-genic, quantitative or continuous characters, and their expression is environmentally influenced. Different approaches for assessing diversity at the molecular level, which are useful in complementing the morpho-agronomic characterization, are presently available. The molecular markers, including RFLP (restriction fragment length polymorphism) (Metais *et al*. 2000), RAPD (random amplified polymorphic DNA) (Chiorato *et al*. 2007) and AFLP (amplified fragment length polymorphism) (Svetleva *et al*. 2006, Kumar *et al*. 2008), have been explored in common bean diversity studies.

Sodium azide (NaN_3) is an excellent chemical mutagen, with high solubility in water, strong reaction with and low toxicity to biological materials as compared with alkylating compounds that are usually used for mutation induction in plants (Al-Qurainy and Khan 2009). In 2005, Agricultural Research Institute of Taiwan implemented a mutation program on the common bean, and subsequently produced many NaN_3-induced mutants that were different in growth habits and seed traits (Jeng *et al*. 2010). This paper presents a study on the molecular and morpho-agronomic characteristics of these common bean mutants. The objective of the study was to understand the extent of diversity among the tested common bean mutants using AFLP and morpho-agronomic markers. Knowledge of this diversity should provide useful information concerning the potential value of these mutants to breeding programs of the common bean.

MATERIALS AND METHODS

Plant materials

In the spring of 2005, 1,000 seeds (M0) of Hwachia, a common bean (*Phaseolus vulgaris*) variety, was subjected to NaN_3 soaking (1 mM) for six hours at room temperature and washed with distilled water afterwards. Preliminary test indicated that about 60% of Hwachia seeds were not damaged by NaN_3 soaking (unpublished results). The NaN_3-treated seeds (M0) were then planted on the experimental farm of Agricultural Research Institute at Wufeng, Taichung Hsien, Taiwan. The grown plants from those seeds (M0) were allowed to self-fertilize and produce M1 seeds. In the autumn of 2005, the M1 seeds were harvested and bulked. One thousand randomly-selected M1 seeds were subjected to NaN_3 soaking (1 mM) again and planted in the same experimental field. The harvested M2 seeds were bulked, and then 500 seeds were randomly selected from this M2 population to grow and produce the M3 population in the spring of 2006.

Line selection was initiated in the M3 population. The seeds from the 100 individual plants of the M3 population that differed from the wild type variety Hwachia in various phenotypes

(e.g., the flower color, the pod color, the seed size and the seed color) were harvested and packed separately. In the autumn of 2006, 5 randomly-selected seeds from each package of the selected M3 population were planted as the M4 generation. The pedigree method of line selection was continued in M4 to M5 generations with notable and stable morphological characteristics to make sure that no further segregations had occurred in the selected progenies.

In the present study, the field-grown, wild-type variety Hwachia and its 34 stabilized NaN_3-induced M6 mutants (Table 1) were used. The experiments were performed in the spring of 2008. Sowing was done manually in rows. Fertilization consisted of a banded application of 16 kg N, 32 kg P_2O_5 and 31 kg K_2O ha^{-1}. Weed control was performed by hand, and plots were maintained to be pest and disease free until harvest. The experimental plots consisted of multiple 7-m long rows. The spacing was 0.6 m between rows and 15 cm between plants within rows. The central 5-m long area of each row-plot was harvested to estimate seed yield per plant and seed yield components (the number of pods per plant, the number of seed per pod and the 100-seed weight).

Amplified fragment length polymorphism (AFLP) analyses

Genomic DNA from each accession was extracted from freshly harvested young leaves by using CTAB (hexadecyltrimethylammonium bromide) procedure described by Chuang *et al*. (2009). The bulked leaf tissues (500 mg) collected from 5 individuals of common bean per accession were used for DNA extraction. DNA concentration was estimated by full-spectrum UV/VIS spectrophotometer (NanoDrop, ND-1000), and was diluted with sterile distilled water to give a final concentration of 100 ng μL^{-1}. AFLP analysis and electrophoresis were carried out using eight pre-selected primer pairs (Table 1) as detailed by Chuang *et al*. (2009).

Morpho-agronomical characteristics diagnoses

Plants were characterized using eight morphological characteristics (Table 2) and nine agronomic characteristics (Table 3) developed by Schachl and De la Rosa (2001). Most of the morphological traits were measured on four randomly selected plants sampled at R1 (initiation of flowering) stage of development. Two agronomic characteristics, duration of vegetative growth and total growth duration (from sowing to the date of measurement), were recorded on the basis of average of the entire plot's growth stage. The agronomic characteristics were measured on three replicates, each with seven plants with same size selected at random from each plot, and expressed as a mean of three replicates.

Table 1. AFLP primer combinations used and respective number of scored fragments.

AFLP primer-pair	No. monomorphic fragments	No. polymorphic fragments	Total produced fragments	Percent polymorphism	PIC
E-AGC/M-CTC	12	61	73	83.56	0.1990
E-ACT/M-CAG	11	57	68	83.82	0.1836
E-ACT/M-CAA	11	44	55	80.00	0.1599
E-ACG/M-CAT	2	78	80	97.50	0.2399
E-ACC/M-CTC	9	59	68	86.76	0.1996
E-ACC/M-CAT	10	52	62	83.87	0.2041
E-AAC/M-CAA	10	13	23	56.52	0.1803
E-AAC/M-CAG	15	38	53	71.70	0.1874
Total	80	402	482		
Mean	10.0	50.25	60.25	80.40	0.1981

Table 2. Unique DNA fingerprinting patterns of six common bean mutants based on AFLP fragments generated by various primer pairs.

Primer pair	Mutant					
	SA-05	SA-08	SA-09	SA-30	SA-31	SA-34
M-CAT/E-ACG	2 (125,311)	-	1 (230)	3 (74, 113,240)	1 (103)	-
M-CAT/E-ACC	3 (134,166, 229)	-	1 (180)	-	-	-
M-CAG/E-ACT	-	-	-	1 (361)	1 (108)	-
M-CAA/E-ACT	-	-	-	-	-	1 (60)
M-CTC-E-ACC	-	1 (72)	-	1 (470)	-	-
M-CTC/E-AGC	1 (67)	-	-	1 (529)	1 (83)	-
Total	6	1	2	6	3	1

Number in parenthesis indicates the base pairs of generated unique AFLP fragment.

Table 3. The morphological characteristics of common bean variety Hwachia and its NaN_3-induced mutants.

Accession	Growth habit	Vexillum petel color	Wing petel color	Pod ground color	Pod secondary color	Seed ground color	Seed secondary color	Seed shape
Hwachia	D[a]	Pink	Pink	Yellow	Light red	Brown	Red	Elliptic
SA-01	D	Pink	Pink	Yellow	Light red	Cream	Red	Elliptic
SA-02	D	Pink	White	Yellow	Light red	Brown	Red	Elliptic
SA-03	D	Pink	White	Yellow	Light red	Cream	Red	Elliptic
SA-04	D	Pink	Pink	Yellow	Light red	Cream	Red	Elliptic
SA-05	I	Pink	Light pink	Green	Red	Brown	Red	Short elliptic
SA-06	D	Pink	Pink	Brown	Light red	Cream	Red	Elliptic
SA-07	I	Red	Red	Green	Purple	Brown	Dark-brown	Short elliptic
SA-08	I	Red	Red	Green	Purple	Brown	Dark-brown	Short elliptic
SA-09	D	Red	Light red	Yellow	Purple	Cream	Black	Elliptic
SA-10	I	Pink	Pink	Yellow	Light red	Brown	Red	Elliptic
SA-11	D	Light pink	White	Red	None	Reddish-brown	Red	Elliptic
SA-12	D	Red	Light red	Yellow	Purple	Cream	Black	Elliptic
SA-13	D	Pink	Light pink	Yellow	Light red	Cream	Red	Elliptic
SA-14	D	Pink	Light pink	Yellow	Light red	Cream	Red	Elliptic
SA-15	D	Pink	Pink	Light red	None	Cream	Red	Elliptic
SA-16	D	Pink	White	Light pink	Light red	Cream	Red	Elliptic
SA-17	D	Light pink	Light pink	Yellow	Light red	Cream	Red	Elliptic
SA-18	D	Pink	Pink	Light pink	Light red	Cream	Red	Elliptic
SA-19	D	Pink	White	Yellow	Light red	Brown	Red	Elliptic
SA-20	D	Pink	Pink	Yellow	Light red	Brown	Red	Elliptic
SA-21	D	Pink	Pink	Yellow	Light red	Brown	Red	Elliptic
SA-22	D	Pink	Light pink	Yellow	Light red	Cream	Red	Elliptic
SA-24	SI	Pink	Pink	Yellow	Red	Cream	Red	Short elliptic
SA-25	D	Pink	White	Yellow	Light red	Cream	Red	elliptic
SA-26	D	Pink	Light pink	Yellow	Red	Brown	Red	Short elliptic
SA-27	D	Pink	White	Yellow	Light red	Cream	Red	Elliptic
SA-28	D	Pink	White	Yellow	Light red	Cream	Red	Elliptic
SA-29	D	Pink	White	Yellow	Light red	Cream	Red	Elliptic
SA-30	I	Red	Light red	Green	Purple	Brown	Dark-brown	Short elliptic
SA-31	I	Red	Red	Green	Purple	Brown	Dark-brown	Short elliptic
SA-32	D	Red	Light red	Yellow	Purple	Cream	Black	Elliptic
SA-33	D	Pink	White	Yellow	Light red	Cream	Red	Elliptic
SA-34	D	Pink	White	Red	None	Reddish-brown	Red	Elliptic
SA-35	D	Pink	Light pink	Yellow	Light red	Cream	Red	Kidney

[a] D, determinate; SI, semi indeterminate; I, indeterminate.

Data analyses

The percentage of polymorphism, polymorphism information content (PIC) and Jacard'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 un-weighted pair group method of arithmetic averages (UPGMA) and a dendrogram was generated with the help of NTSYS-PC software version 1.80 (Exeter Software, New York, USA) (Rohlf 1998). AFLP data were also subjected to principal coordinates analysis (Kovach 1998).

Morpho-agronomic data were numerically coded to the following categorical traits for statistical analysis:

(1) growth habit (1: determinate, 2: semi determinate, 3: indeterminate);
(2) vexillum petel color (1: red, 2: pink, 3: light pink);
(3) wing petel color (1: white, 2: light pink, 3: pink, 4: light red, 5: red);
(4) pod ground color (1: green, 2: yellow, 3: light pink, 4: light red, 5: red);
(5) pod secondary color (1: none, 2: light red, 3: red);
(6) seed ground color (1: cream, 2: brown, 3: reddish brown);
(7) seed secondary color (1: dark brown, 2: red, 3: black);
(8) seed shape (1: short elliptic, 2: elliptic, 3: kidney);
(9) vegetative growth duration (1: < 38 days, 2: 38-45 days, 3: > 45 days);
(10) total growth duration (1: < 87 days, 2: 87-94 days, 3: > 94 days);
(11) plant height (1:< 70 cm, 2: 70-92 cm, 3: > 93 cm);
(12) pod width (1: < 1.19 cm, 2: 1.19-1.34 cm, 3: > 1.35 cm);
(13) pod length (1: < 12.1 cm, 2: 12.1-14.3 cm, 3: > 14.4 cm);
(14) number of pods per plant (1: < 16, 2: 16-20, 3: > 21);
(15) number of seeds per pod (1: < 3.10, 2: 3.10-4.00, 3: > 4.01);
(16) seed weight (1: < 0.39, 2: 0.39-0.51, 3: > 0.52);
(17) seed yield (1: < 1387 kg ha^{-1}, 2: 1387-1852 kg ha^{-1}, 3: >1853 kg ha^{-1}).

Clustering and principal coordinates analyses on the morpho-agronomic data were calculated by software version 1.80 (Exeter Software, New York, USA). The standardized data matrix of the morpho-agronomic data was used to generate dissimilarity indices based on Euclidean distances. The correspondence between the matrix of Euclidian distances and the matrix of AFLP based similarities was examined with the Mantel test (Mantel 1967).

RESULTS

In the present study, across all 35 common bean accessions that had received PCR-amplification through eight pre-selected primer pairs, a total of 482 fragments were observed; of which 402 were polymorphic (Table 1). The polymorphic banding patterns of these primer pairs averaged from 56.52% (13 polymorphic fragments/23 total fragments) for *E-AAC/M-CAA* to 97.50% (78 polymorphic fragments/80 total fragments) for *E-ACG/M-CAT*. The average percentage of polymorphic fragments, across eight primer pairs, was 80.40%. Six of the eight primer pairs generated greater than 80% polymorphism. The calculated polymorphism information content (PIC) values for the eight primer pairs ranged from 0.1803 (*E-AAC/M-CAA*) to 0.1990 (*E-AGC/M-CTC*). The average PIC value for the amplification products, across eight primer pairs, was 0.1981.

PCR amplification with some of the selected primer pairs revealed that, in some cases, a highly specific fragment was present in comparison to other mutants. For example, two fragments (311 and 125 bp) and three fragments (240, 113 and 74 bp) were present in mutant SA-05 and SA-30, respectively. However, those fragments were absent in Hwachia and other mutants when the extracted genomic DNA was amplified with AFLP primer pair *M-CAT/E-ACG*, (Table 2). On the other hand, only one unique fragment was detectable in mutants SA-09 and SA-31, but each was of a different size when amplified with primer pair M-CAT/E-ACG (Table 2). Some unique AFLP fragments were also found in other mutants when the extracted genomic DNA was amplified with primer pair *M-CAT/E-ACC*, *M-CAG/E-ACT*, *M-CAA/E-ACT*, *M-CTC/E-ACC*

and *M-CTC/E-AGC*, and each was of a different size (Table 2).

The generated DNA fragments were used further for genetic diversity study. The calculated similarity coefficients ranged from 0.469 to 0.839, with an average of 0.673 (data not presented). As shown in Fig. 1, only six combinations (1.01%) out of a total of 595 combinations showed similarity greater than 0.80. The majority (77.48%) of similarity coefficients (461 combinations) were in the range of 0.60-0.80. A total of 128 combinations (about 22%) had similarity less than 0.60.

A dendrogram showing the clustering pattern, based on AFLP markers for all the common bean accessions, was constructed (Fig. 2A). It consisted of two major clusters. Cluster I contained four mutants including SA-07, SA-08, SA-30 and SA-31. Wild type variety Hwachia and other 30 mutants were placed in cluster II, which indicated that mutants SA-07, SA-08, SA-30 and SA-31 were more distinct from Hwachia and other mutants. Additionally, six mutants, namely SA-01, SA-05, SA-09, SA-24, SA-34 and SA-35, clustered in Cluster I, and they were also distinct to some extent as they diverged from Hwachia (Fig. 2A). The principal coordinate method based on the molecular similarity also distinguished mutants SA-05, SA-07, SA-08, SA-24, SA-30 and SA-31 from wild type Hwachia and other 28 mutants (Fig. 3A). The first and second coordinates accounted for 16.01% and 6.06%, respectively, of the total variation.

The morphological characteristics of 35 common bean accessions were shown in Table 3. Wild type variety Hwachia and the majority of the mutants had determinate growth habits. Nevertheless, mutants SA-05, SA-07, SA-08, SA-30 and SA-31 showed indeterminate growth habits, while mutant SA-23 showed semi-indeterminate growth habits. Differentiation among Hwachia and mutants also occurred in flower color (both vexillum petel and wing petel colors), pod color, seed coat color and seed shape, indicating the occurrence of genetic diversity among the evaluated mutants (Table 3).

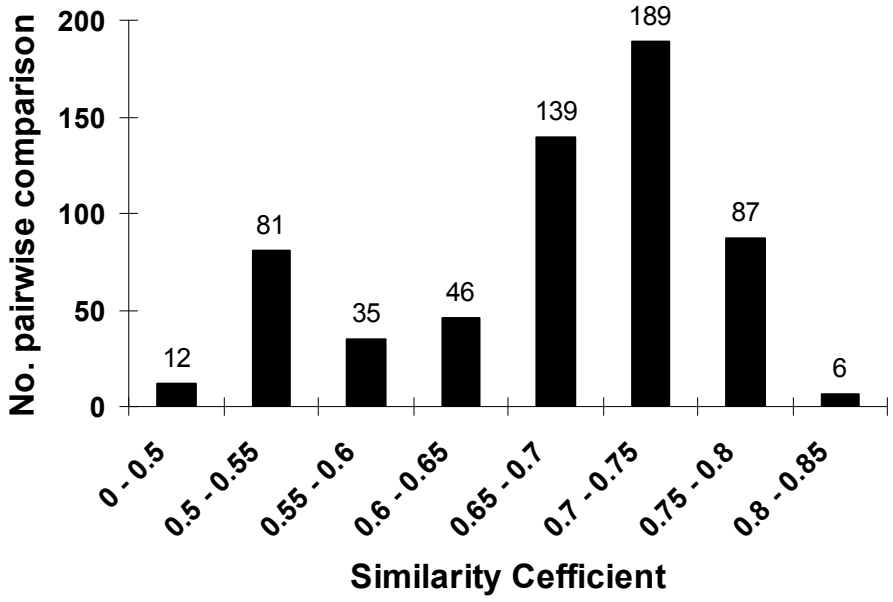

Fig. 1. The distribution of 595 pair-wise Jaccard's coefficients among variety Hwachia and its' 34 NaN_3-induced mutants.

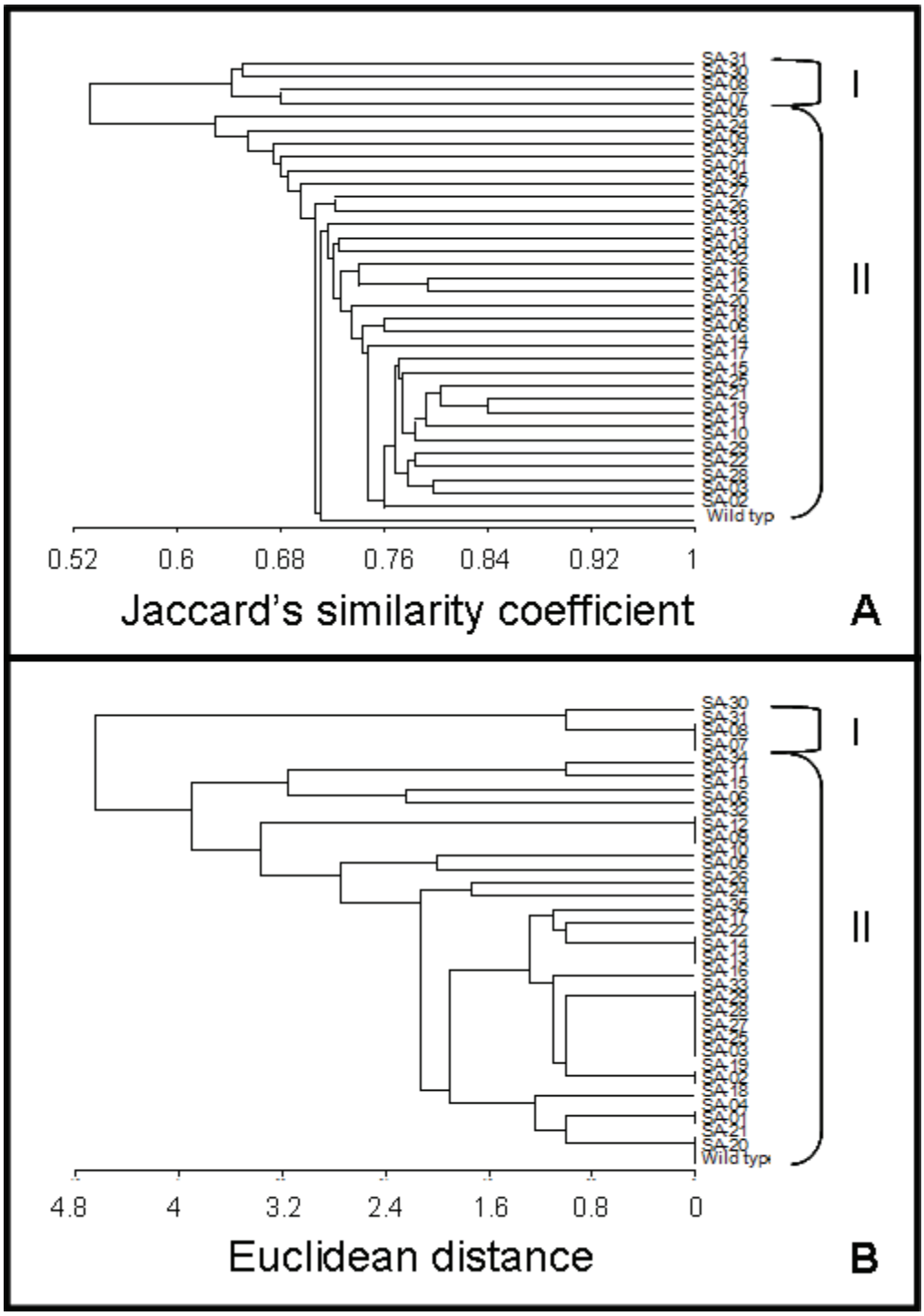

Fig. 2. Dendrograms showing variations among variety Hwachia and its' 34 NaN_3-induced mutants based on the calculated Jaccard coefficients using AFLP fragments obtained by 8 primer pairs (A) and the calculated Euclidian distances using 17 morpho-agronomic characteristics (B).

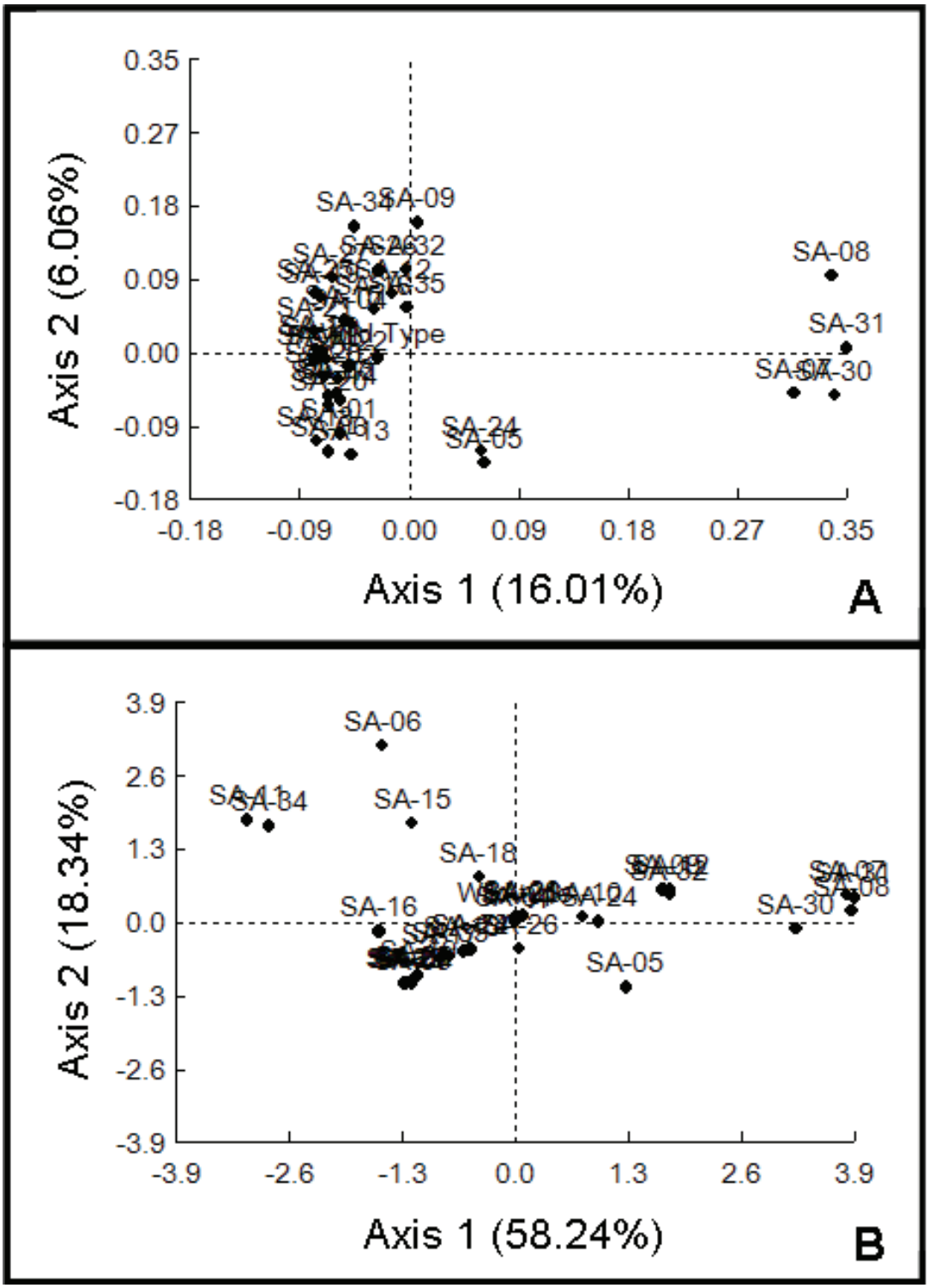

Fig. 3. Principal coordinate plot showing dispersions of variety Hwachia and its' 34 NaN_3-induced mutants based on AFLP fragments obtained by 8 primer pairs (A) and 17 morpho-agronomic characteristics (B).

Nine agronomic characteristics, which were quantitative, of tested common bean mutants were shown in Table 4. When compared with wild type variety Hwachia, all the mutants displayed a wide variation in most of the agronomic characteristics studied. Five mutants, SA-05, SA-07, SA-08, SA-30 and SA-31, with indeterminate growth habits had longer vegetative growth period (47 to 48 days) than the mutants with determinate (31 to 36 days) and semi-determinate (38 days) growth habits. The mutants with indeterminate (SA-05, SA-07, SA-08,

Table 4. The agronomic characteristics (data was expressed as average of three replications) of common bean variety Hwachia and its NaN_3-induced mutants.

Accession	Vegetative growth duration (days)	Total growth duration (days)	Plant height (cm)	Pod width (cm)	Pod length (cm)	No. pods plant^{-1}	No. seeds pod^{-1}	Seed weight (g)	Yield (kg ha^{-1})
Hwachia	31(1)	87(2)	50.0(1)	1.29(2)	13.5(2)	22(3)	3.66(2)	0.44(2)	2294(3)
SA-01	34(1)	82(1)	57.0(1)	1.32(2)	13.2(2)	17(2)	3.55(2)	0.51(2)	1873(3)
SA-02	36(1)	80(1)	52.4(1)	1.32(2)	12.0(1)	17(2)	2.84(1)	0.54(3)	1598(2)
SA-03	34(1)	80(1)	53.0(1)	1.25(2)	12.9(2)	14(1)	3.23(2)	0.51(2)	1408(2)
SA-04	34(1)	85(1)	51.6(1)	1.36(3)	12.6(2)	18(2)	3.24(2)	0.52(3)	1834(2)
SA-05	48(3)	98(3)	75.0(2)	1.23(2)	11.8(1)	20(2)	3.10(2)	0.42(2)	1609(2)
SA-06	34(1)	84(1)	47.2(1)	1.18(1)	13.1(2)	16(2)	3.22(2)	0.48(2)	1516(2)
SA-07	47(3)	101(3)	93.0(3)	1.02(1)	9.8(1)	23(3)	4.08(3)	0.29(1)	1687(2)
SA-08	47(3)	98(3)	74.4(2)	1.05(1)	10.4(1)	22(3)	4.04(3)	0.31(1)	1660(2)
SA-09	31(1)	81(1)	56.4(1)	1.09(1)	15.0(3)	21(3)	3.93(2)	0.46(2)	2318(3)
SA-10	36(1)	80(1)	78.8(2)	1.10(1)	13.5(2)	12(1)	3.00(1)	0.52(3)	1172(1)
SA-11	32(1)	82(1)	50.6(1)	1.20(2)	11.6(1)	19(2)	2.22(1)	0.42(2)	1035(1)
SA-12	31(1)	83(1)	58.4(1)	1.09(1)	14.0(2)	13(1)	3.83(2)	0.42(2)	1308(1)
SA-13	36(1)	84(1)	52.8(1)	1.15(1)	13.6(2)	16(2)	3.23(2)	0.51(2)	1608(2)
SA-14	31(1)	83(1)	54.0(1)	1.08(1)	13.4(2)	22(3)	3.43(2)	0.50(2)	2305(3)
SA-15	31(1)	80(1)	51.0(1)	1.05(1)	12.1(2)	25(3)	3.28(2)	0.46(2)	2203(3)
SA-16	36(1)	84(1)	66.4(1)	1.50(3)	16.9(3)	15(1)	4.89(3)	0.49(2)	2102(3)
SA-17	35(1)	83(1)	54.8(1)	1.15(1)	13.9(2)	10(1)	3.35(2)	0.48(2)	971(1)
SA-18	31(1)	81(1)	54.0(1)	1.20(2)	13.2(2)	14(1)	3.23(2)	0.47(2)	1252(1)
SA-19	34(1)	80(1)	47.6(1)	1.36(3)	13.4(2)	15(1)	2.54(1)	0.47(2)	1051(1)
SA-20	36(1)	82(1)	49.6(1)	1.26(2)	12.0(1)	17(2)	2.55(1)	0.48(2)	1246(1)
SA-21	36(1)	82(1)	49.0(1)	1.35(3)	11.4(1)	15(1)	2.42(1)	0.49(2)	1104(1)
SA-22	34(1)	83(1)	53.8(1)	1.19(2)	13.0(2)	17(2)	3.26(2)	0.48(2)	1549(2)
SA-24	38(2)	93(2)	74.0(2)	1.38(3)	12.9(2)	17(2)	3.26(2)	0.50(2)	1743(2)
SA-25	31(1)	78(1)	59.6(1)	1.19(2)	12.9(2)	19(2)	2.53(1)	0.63(3)	1862(3)
SA-26	36(1)	87(2)	52.4(1)	1.36(3)	11.1(1)	13(1)	2.18(1)	0.53(3)	920(1)
SA-27	32(1)	82(1)	53.0(1)	1.31(2)	11.6(1)	14(1)	2.20(1)	0.52(3)	990(1)
SA-28	31(1)	80(1)	55.2(1)	1.14(1)	12.0(1)	13(1)	3.04(1)	0.47(2)	1127(1)
SA-29	33(1)	79(1)	53.4(1)	1.15(1)	11.9(1)	16(2)	2.98(1)	0.45(2)	1271(1)
SA-30	47(3)	92(2)	112.8(3)	0.98(1)	10.2(1)	18(2)	4.07(3)	0.26(1)	1153(1)
SA-31	47(3)	92(2)	103.6(3)	1.03(1)	10.6(1)	15(1)	4.19(3)	0.26(1)	933(1)
SA-32	30(1)	82(1)	59.6(1)	1.05(1)	14.5(3)	16(2)	3.82(2)	0.43(2)	1595(2)
SA-33	30(1)	79(1)	56.0(1)	1.23(2)	13.8(2)	16(2)	3.48(2)	0.56(3)	1959(3)
SA-34	32(1)	86(1)	52.0(1)	1.22(2)	12.4(2)	17(2)	2.61(1)	0.44(2)	1175(1)
SA-35	36(1)	80(1)	99.4(3)	1.09(1)	16.5(3)	11(1)	4.68(3)	0.42(2)	1317(1)
$LSD_{0.05}$	2	7	11.2	0.07	1.1	5	0.32	0.04	480

Number in parenthesis indicates the numerically coded class converted for clustering and principal coordinates analysis.

SA-30 and SA-31) or semi-indeterminate (SA-24) growth habits also had longer total growth duration period (92 to 101 days) than the mutants with determinate growth habits (78 to 87 days) (Table 4). Significant variations in plant height, ranged from 47.2 to 112.8 cm, were also observed among the evaluated mutants (Table 4). The mutants with indeterminate or semi-indeterminate growth habits were generally taller (74.0-112.8 cm) than the mutants with determinate growth habits (47.2-66.4 cm). SA-10 and SA-35 were the only two mutants with determinate growth habits that had a plant height of 78.9 and 99.4 cm, respectively, which was comparable to the plant height of mutants with indeterminate or semi-indeterminate growth habits (Table 4).

The yield and yield components also differed among the tested mutants (Table 4). Seeds from mutants SA-08, SA-08, SA-30 and SA-31 weighed lighter (0.26-0.31 mg seed^{-1}) than the seeds from wild type Hwachia (0.44 mg seed^{-1}) (Table 4). As a result, these four mutants produced lower yield (933-1687 kg ha^{-1}) compared to Hwachia (2294 kg ha^{-1}) (Table 4). It should be pointed out that four mutants (SA-09, SA-14, SA-15 and SA-16) produced yield comparable to Hwachia, even though the majority of induced mutants yielded less than Hwachia (Table 4).

The UPGMA dendrogram based on Euclidean distances between morpho-agronomic characteristics was shown in Fig. 2B. Two major clusters were also observed, one consisting of mutants SA-07, SA-08, SA-30 and SA-31 (cluster I), and the other consisting of wild type variety Hwachia and 28 other mutants (cluster II). In the principal coordinate analysis (Fig. 3B), the first and second coordinates accounted for 58.24% and 18.34%, respectively, of the total variation. Again, SA-07, SA-08, SA-30 and SA-31 were dispersed in the right part of graph.

Comparison between relations depicted by the AFLP and the morpho-agronomic dendrograms showed partial agreements between the topologies of both dendrograms. Mantel test indicated a significant correlation (r = –0.713, P < 0.01) between both dendrograms. The negative sign was the result of comparing Euclidian distance and Jaccard similarity values.

DISCUSSION

The molecular diversity of common bean plants has been the subject of a number of studies (Gómez *et al*. 2004, Durán *et al*. 2005, Rosales-Serna *et al*. 2005, Galvan *et al*. 2006). Kumar *et al*. (2008) and Svetleva *et al*. (2006) used AFLP technique, each with different primer pairs, to analyze the genetic diversity of common bean varieties and landraces collected from different locations, and found an average similarity coefficient of 0.524 and 0.913, respectively. However, there is no information available on genetic diversity study of common bean mutants evolved through chemical induction. In our study, a relatively large scale mutagenesis on the molecular level also occurred among the tested common bean mutants (Fig. 2A). Although all the mutants were derived from a single variety Hwachia, an average similarity coefficient of 0.673 was found, which was greater than that was reported by Kumar *et al*. (2008) but less than that was reported by Svetleva *et al*. (2006). These results clearly indicate that NaN_3-induced mutagenesis is an effective approach for obtaining greater genetic variation in common bean, even if the gene resource is donated from a single variety (Al-Qurainy and Khan 2009). This large scale mutagenesis found in our study would allow the common bean breeders to obtain useful alleles without the swarm of linked deleterious alleles present in exotic or wild germplasms, or from adapted inbred lines. Our results further showed that some of the selected AFLP primer pairs generated highly specific DNA fragments present only in some specific mutants (Table 2). These produced specific DNA fragments would be a useful tool in identifying and protecting these mutants from possible infringements in future.

Considerable variations among the evaluated common bean mutants also occurred in morphological characteristics, including growth habit, flower petel color, pod color, seed coat color and seed shape (Table 3), indicating the existence of genetic diversity among Hwachia and its mutants (Fig. 2B). Significant variations in the evaluated agronomic characteristics were also obtainable among Hwachia and its mutants (Table 4). These results confirm that NaN_3 treatment indeed generates many common bean mutants differing in morphological and agronomic traits (Fig. 3B). Moreover, the relations

among Hwachia and its mutants based on morpho-agronomic characteristics correlated with those based on AFLP data (r = –0.713**). This suggests that the two systems provide similar estimates of genetic relations among the tested materials. Some mutants, namely SA-05, SA-07, SA-08, SA-24, SA-30 and SA-31, which were relatively dissimilar from wild type Hwachia through AFLP analysis (Fig. 3A), also diversified from Hwachia using morpho-agronomic diagnosis (Fig. 3B). These results further confirm the effectiveness of using NaN_3-induced mutagenesis to generate useful alleles and subsequently broaden the gene pool of common bean.

Mutations are the ultimate source of genetic variability in organisms. The genetic variability caused by induced mutations is not essentially different from that caused by spontaneous mutations. During the past 70 years, more than 2200 varieties, which have been derived either as direct mutants or from their progenies have been released worldwide (Chopra 2005, Parry *et al*. 2009). Many induced mutants were released directly as new varieties; others were used as parents to derive new varieties. In the present study, mutants SA-09 and SA-14 can be released directly as new varieties. Moreover, several mutants deserve to be further explored for the possibility of differentiating promising mutants. Seeds of both mutants SA-09 (0.64 mg) and SA-33 (0.56 mg) weighed significantly heavier than those of Hwachia (0.44 mg) (Table 4). If this heavier-seed-weight characteristic of SA-09 or SA-33 can be introduced into Hwachia through backcrossing, an improved variety with greater yield could be available for common bean growers shortly. The similar approach can also be applied to SA-07 or SA-08, which produced seeds smaller in size compared to Hwachia (Table 4). On the other hand, SA-34, which had higher phenolic and anthocyanin content (Jeng *et al*. 2010) is inferior to Hwachia in the number of pods per plant and number of seeds per pod (Table 4). Thus, a yield improvement program would make modified SA-34, which is enriched with antioxidants, a promising and useful common bean variety in food and other applications.

In conclusion, the use of AFLP and morpho-agronomic markers has allowed a comprehensive study of the diversity and relationships of NaN_3-generated common bean mutants. The presence of molecular and morpho-agronomic diversities in wild type variety Hwachia and mutants, confirmed by cluster and principal coordinate analyses, indicates that there is room for selection and improvement in this variety, as well as for increasing the genetic diversity of the gene pool of common bean plants grown in Taiwan. Several mutants, such as SA-07, SA-08, SA-09, SA-14, are useful as a source of variation to derive new varieties.

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REFERENCES

Ahloowalia BS, M Maluszynski, K Nichterlein (2004) Global impact of mutation-derived varieties. **Euphytica** 135:187-204.

Al-Qurainy F, S Khan (2009) Mutagenic effects of sodium azide and its application in crop improvement. **World Appl Sci. J.** 6:1589-1601.

Aparicio-Fernández X, L Manzo-Bonilla, GF Loarca-Piña (2005) Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B1. **J. Food Sci.** 70: S73-S78.

Blair MW, T Porch, K Cichy, CH Galeano, P Lariguet, C Pankhurst, W Broughton (2007) Induced mutants in common bean (*Phaseolus vulgaris*), and their potential use in nutrition quality breeding and gene discovery. **Isr. J. Plant Sci.** 55:191-200.

Broughton WJ, G Hernandez, MW Blair, S Beebe, P Gepts, J Vanderleyden (2003) Beans (*Phaseolus* spp.)-model food legumes. **Plant Soil** 252:55-128.

Campion B, D Perrone, I Galasso, R Bollini (2009) Common bean (*Phaseolus vulgaris* L.) lines devoid of major lectin proteins. **Plant Breed.** 128:199-204.

Chiorato AF, SAM Carbonell, LL Benchimol, MB

Chiavegato, LA dos Santos Dias, CA Colombo (2007) Genetic diversity in common bean accessions evaluated by means of morpbo-agronomical and RAPD data. **Sci. Agri.** 64: 256-262.

Chopra VL (2005) Mutagenesis: Investigating the process and processing the outcome for crop improvement. **Curr. Sci.** 89:353-359.

Chuang SJ, CLChen, JJ Chen, WY Chou, JM Sung (2009) Detection of somaclonal variation in micro-propagated *Echinacea purpurea* using AFLP marker. **Sci. Horti.** 120:121-126.

Durán LA, MW Blair, MC Giraldo, R Macchiavelli, E Prophete, JC Nin, JS Beaver (2005) Morphological and molecular characterization of common bean landraces and cultivars from Caribbean. **Crop Sci.** 45:1320-1328.

Galvan MZ, MC Menéndez-Sevillano, AM De Ron, M Santalla, PA Balatti (2006) Genetic diversity among wild common beans from northwestern Argentina based on morpho agronomic and RAPD data. **Genet. Resour. Crop Evol.** 53:891-900.

Gómez OJ, MW Blair, BE Frankow-Lindberg, U Gullberg (2004) Molecular and phenotypic diversity of common bean landraces from Nicaragua. **Crop Sci.** 44:1412-1418.

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Jeng TL, YJ Shih, CC Lai, MT Wu, JM Sung (2010) Anti-oxidative characterization of NaN_3-induced common bean mutants. **Food Chem.** 119:1006-1011.

Kovach WL (1998) MVSP- A multivariate statistical package for windows. Version 3.0. Kovach Computing Services, Pentraeth, Wales, United Kingdom.

Kumar V, S. Sharma, S Kero, S Sharma, AK Sharma, M Kumar, KV Bhat (2008) Assessment of genetic diversity in common bean (*Phaseolus vulgaris* L.) germplasm using amplified fragment length polymorphism (AFLP). **Sci. Horti.** 116:138-143.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. **Cancer Res.** 27:209-220.

Metais I, C Aubry, B Hamon, R Jalouzot, D Peltier (2000) Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). **Theor. Appl. Genet.** 101: 1207-1214.

Parry MA, PJ Madgwick, C Bayon, K Tearall, A Hemandez-Lopez, M Baudo, M Rakszegi, W Hamada, A Al-Yassin, H Quabbou, M Labhilili, A Phillips (2009) Mutation discovery for crop improvement. **J. Exp. Bot.** 60:2817-2825.

Rohlf FJ (1998) NTSYS-pc Numerical taxonomy and multivate analysis system. Version 2.0. Exeter Publ., Setauket, NY, USA.

Roldán-Ruiz I, J Dendauw, E Van Bockstaele, A Depicker, M De Loose (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). **Mol. Breed.** 6:125-134.

Rosales-Serna R, S Hernández-Delgado, M González-Paz, JA Acosta-Gallegos, N Mayek-Pérez (2005) Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. **Crop Sci.** 45:1951-1957.

Schachl R, L De la Rosa (2001) Characterization of Phaseolus accessions. *In*: Handbook on Evaluation of *Phaseolus* germplasm. C De la Cuadra, AM De Ron, R Schachl (eds.) PHASELIEU-FAIR-PL97-3463. http://www.cesga.es/phaseliu/handbook_germplasm.pdf.

Singh SP (2001) Broadening the genetic base of common bean cultivars: a review. **Crop Sci.** 41: 1659-1675.

Svetleva D, G Pereira, J Carlier , L Cabrita, J Leitão, D Genchev (2006) Molecular characterization of *Phaseolus vulgaris* L. genotypes included in Bulgarian collection by ISSR and AFLP™ analyses. **Sci. Hort.** 109:198-206.

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