January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

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Performance of Introduced Lines Based on Morphological Markers for Diversity Enrichment of Indonesian Chili Pepper (*Capsicum annuum* L.) Varieties

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Abstract. The success and sustainability of chili pepper breeding depends upon diversity in genetic diversity resources. The genetic diversity can be increased through the introduction of material from other countries. The present study aimed to measure the contribution of introduced lines to enrich diversity of IVEGRI's breeding program based on morphological characterization. The experiment was carried out at IVEGRI research station from July to December 2020, and arranged with two replications in a randomized block design. The treatments involved 60 genetic materials, 30 of which were derived from Indonesia and other 30 lines were introduced from World Vegetable Center. Morphological traits were measured following the guidelines for testing the distinctness, uniformity, and stability from Indonesian Plant Variety Protection Center, Ministry of Agriculture. Genetic diversity analysis was conducted using the Euclidean dissimilarity coefficient. The results showed an increase of 88.2% in genetic distance after introduction. The unique morphological characters, such as shortened internodes, hairiness of nodes, leaf shapes, secondary color of corolla, fruit color before maturity, fruit color at maturity, and fruit attitude, were observed from the introduced chili pepper lines reflecting that those could be beneficial for future improvement of chili pepper breeding program.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) consisted of a wide genetic diversity. Bosland and Votava [1] reported three primary gene pools among the domesticated species and their wild progenitors, including the *C. annuum* complex (*C. annuum*, *C. frutescens*, *C. chinense*, *C. chacoense*, and *C. galapagoense*), the *C. baccatum* complex (*C. baccatum*, *C. praetermissum*, and *C. tovarii*), and the *C. pubescens* complex (*C. pubescens*, *C. eximium*, and *C. cardenasii*). Hybridization to expand genetic diversity can still occur within one or between species complexes using standard pollination or with biotechnological approaches, such as protoplast fusion [2] and embryo rescue [3], when prezygotic barriers to fertilization exist.

Genetic diversity is important for the success of chili pepper breeding programs [4]. Since Indonesia is not the "center of origin" for chili pepper [5], the process for increasing the genetic diversity of chili pepper in Indonesia is still ongoing through introduction, hybridization, and mutation. Introduction is an activity frequently used to increase the genetic diversity of chili pepper. One of the sources for chili crop genetic material introduction is the World Vegetable Center. Bosland and Votava [1] stated that this institution is very active in collecting chili accessions across the world and it has been used widely in increasing the genetic diversity of chili in Indonesia, and reportedly predominates focuses on resistance to plant pests and diseases [6, 7]. In addition to increasing the genetic

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diversity and the character for pests and diseases resistance, introduction increasing the chance of identifying heterotic effects due to hybridizations between lines with large genetic distances. Several studies have reported the chance of acquiring heterosis by using parental lines with a broad genetic distance.

Several studies estimated the genetic diversity between individual or population by using morphological marker [8–10], which is still relevant to be done in Indonesia, especially in Indonesian Vegetable Research Institute (IVEGRI). It is also still in accordance with the regulation for varietal registration, in which new variety at least must have one stable morphological character that differentiates from the established variety. Morphological estimation of genetic diversity can be carried out at the initial stage of plant breeding activities, which can then be complemented by genotyping using molecular markers.

The first step for the effective utilization of introduced genetic material from World Vegetable Center is the by characterizing of morphological characters and measuring the increase in genetic diversity achieved after obtaining enrichment through introductory activities. The genetic diversity measure before enrichment through introduction (only IVEGRI's genetic material) referred to before condition, while the genetic diversity measured from the breeding material and the introduction referred to the after condition. The study compared the conditions of genetic diversity before and after introductory activities using morphological markers. In addition, we identified a number of unique characters that can be used as special traits. By doing so, the introduced lines can be used as donors for special characters to support the acceleration of registration in chili.

MATERIALS AND METHODS

Research Location and Time

The research has was conducted in Lembang Experimental Station, IVEGRI from July to December 2020.

Genetic Materials

The research treatment involved 60 genetic materials (30 introduction lines and 30 Indonesian accessions/working collections/varieties) (Table 1).

TABLE 1. Identity of 60 genetic materials of chili pepper used in this study.

No.	Variety	Description	Source	No.	Variety	Description	Source
1	AVPP 9905	Introduced line	World Vegetable Center	31	Tanjung-1	Released in year 2001	IVEGRI
2	VI 037603 (PBC 370)	Introduced line	World Vegetable Center	32	Tanjung-2	Released in year 2001	IVEGRI
3	VI 037599 (PBC 143)	Introduced line	World Vegetable Center	33	Branang	Released in year 2010	IVEGRI
4	AVPP 1105	Introduced line	World Vegetable Center	34	Ciko	Released in year 2011	IVEGRI
5	AVPP 0022	Introduced line	World Vegetable Center	35	Lingga	Released in year 2011	IVEGRI
6	VI 047030 (PBC 972)	Introduced line	World Vegetable Center	36	Inata Agrihorti	Released in year 2001	IVEGRI
7	VI 041283 (PBC 145)	Introduced line	World Vegetable Center	37	Carvi Agrihorti	Released in year 2018	IVEGRI
8	VI 037449 (PBC 148)	Introduced line	World Vegetable Center	38	Hot Beauty	Released in year 2006	IVEGRI
9	VI 012260 (PBC 316)	Introduced line	World Vegetable Center	39	Mia	Released in year 2008	Enterprise
10	VI 037606 (PBC 370)	Introduced line	World Vegetable Center	40	Pertiwi	Released in year 2017	Enterprise
11	VI 037607 (PBC 411)	Introduced line	World Vegetable Center	41	Pilar	Released in year 2011	Enterprise
12	VI 012249 (PBC 148)	Introduced line	World Vegetable Center	42	Lembang-1	Released in year 2001	IVEGRI
13	VI 037522 (PBC 495)	Introduced line	World Vegetable Center	43	Kencana	Released in year 2011	IVEGRI
14	VI 044287 (PBC 507)	Introduced line	World Vegetable Center	44	Pancanaka Agrihorti	Released in year 2019	IVEGRI
15	VI 037556 (PBC 535)	Introduced line	World Vegetable Center	45	Kastilo	Released in year 2010	Enterprise
16	VI 046886 (PBC 564)	Introduced line	World Vegetable Center	46	TM-999	Released in year 2005	Enterprise
17	VI 037558 (PBC 596)	Introduced line	World Vegetable Center	47	LV-8451	Accession	IVEGRI
18	VI 039356 (PBC 535)	Introduced line	World Vegetable Center	48	LV-8469	Accession	IVEGRI
19	AVPP 1806	Introduced line	World Vegetable Center	49	WC-OP-IVEGRI 1	Working collection	IVEGRI
20	AVPP 1811	Introduced line	World Vegetable Center	50	WC-OP-IVEGRI 2	Working collection	IVEGRI
21	AVPP 1721	Introduced line	World Vegetable Center	51	WC-OP-IVEGRI 3	Working collection	IVEGRI
22	AVPP 0715	Introduced line	World Vegetable Center	52	WC-OP-IVEGRI 4	Working collection	IVEGRI
23	AVPP 1127	Introduced line	World Vegetable Center	53	WC-OP-IVEGRI 5	Working collection	IVEGRI

No.	Variety	Description	Source	No.	Variety	Description	Source
24	AVPP 1130	Introduced line	World Vegetable Center	54	WC-OP-IVEGRI 6	Working collection	IVEGRI
25	VI 044930 (PBC 1582)	Introduced line	World Vegetable Center	55	LV-8449	Accession	IVEGRI
26	AVPP 1128	Introduced line	World Vegetable Center	56	Serambi	Released in year 2011	Enterprise
27	AVPP 1131	Introduced line	World Vegetable Center	57	WC-OP-IVEGRI 7	Working collection	IVEGRI
28	VI 037538 (PBC 456)	Introduced line	World Vegetable Center	58	WC-OP-IVEGRI 8	Working collection	IVEGRI
29	VI 041282 (PBC 144)	Introduced line	World Vegetable Center	59	WC-F1-IVEGRI 2	Working collection	IVEGRI
30	AVPP 0209	Introduced line	World Vegetable Center	60	WC-F1-IVEGRI 3	Working collection	IVEGRI

Research Implementation

Each entry was planted in an experimental plot measuring $6m^2$ containing 20 plants. Experimental design was a randomized block design with two replications. The chili cultivation technique followed the best management practices according to the IVEGRI's recommendation. Chili pepper seeds were sown and transplanted 1 month after sowing. Natural fertilizer using chicken manure (30 t/ha) was basally applied at planting, while chemical fertilizer using NPK 16:16:16 (1 t/ha) was applied as side dressing, 21 days after planting (DAP), and continued every ten day until 100 DAP. The weeds were removed once in month. The control of plants pest and diseases using pesticides was given at interval of once a week.

Research Parameters

Observations were made on 53 morphological characters based upon Guidelines for the Conduct of Test for Distinctness, Uniformity, and Stability [11] (Table 2).

TABLE 2. Research parameter based upon Guidelines for the Conduct of Test for Distinctness, Uniformity, and Stability [11].

No.	Character	Category
1	Anthocyanin coloration of hypocotyl	1 = absent, 9 = present
2	Plant habit	1 = upright, 2 = semi upright, 3 = prostrate
3	Length of stem	3 = short, 5 = medium, 7 = long
4	Shortened internode	1 = absent, 9 = present
5	Number of internodes between the first flower and shortened internodes	1 = none, 2 = one to three, 3 = more than three
6	Length of internode	3 = short, 5 = medium, 7 = long, 9 = very long
7	Anthocyanin coloration of nodes	1 = absent, 9 = present
8	Intensity of anthocyanin coloration of nodes	1 = very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
9	Hairiness of nodes	1 = very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
10	Plant height	1 = very short, 3 = short, 5 = medium, 7 = tall, 9 = very tall
11	Leaf length of blade	1 = very short, 3 = short, 5 = medium, 7 = long, 9 = very long
12	Leaf width of blade	1 = very narrow, 3 = narrow, 5 = medium, 7 = broad, 9 = very broad
13	Leaf intensity of green color	1 = very light, 3 = light, 5 = medium, 7 = dark, 9 = very dark
14	Leaf anthocyanin coloration	1 = absent, 9 = present
15	Leaf shape	1 = lanceolate, 2 = ovate, 3 = broad ellipse
16	Leaf undulation of margin	1 = very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
17	Leaf blistering	1 = very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
18	Leaf profile in cross section	1 = strongly concave, 3 = moderately concave, 5 = flat, 7 = moderately convex, 9 = strongly convex
19	Leaf glossiness	1 = very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
20	Flower peduncle attitude	1 = erect, 2 = semi-dropping, 3 = dropping
21	Stigma exertion	1 = below, 2 = same level, 3 = above
22	Anthocyanin coloration in anther	1 = absent, 9 = present
23	Anthocyanin coloration in filament	1 = absent, 9 = present

No.	Character	Category
24	Secondary color of corolla	1 = absent, 2 = at margin, 3 = at base, 4 = at margin and base
25	Fruit color before maturity	1 = greenish white, 2 = yellow, 3 = green, 4 = purple
26	Fruit intensity of color before maturity	1 = very light, 3 = light, 5 = medium, 7 = dark, 9 = very dark
27	Anthocyanin coloration	1 = absent, 9 = present
28	Fruit attitude	1 = erect, 2 = horizontal, 3 = drooping
29	Fruit length	1 = very short, 3 = short, 5 = medium, 7 = long, 9 = very long
30	Fruit diameter	1 = very narrow, 3 = narrow, 5 = medium, 7 = broad, 9 = very broad
31	Fruit ratio length/diameter	1 = very small, 3 = small, 5 = medium, 7 = large, 9 = very large
32	Fruit shape in longitudinal section	1 = oblate, 2 = circular, 3 = chordate, 4 = square, 5 = rectangular, 6 = trapezoidal, 7 = moderately triangular, 8 = narrowly triangular, 9 = horn shaped, 10 = linear
33	Fruit twisting	1 = absent, 9 = present
34	Fruit shape in cross section (at level of placenta)	1 = elliptic, 2 = angular, 3 = circular
35	Fruit sinuation of pericarp at basal part	1 = absent or very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
36	Fruit sinuation of pericarp excluding basal part	1 = absent/very weak, 3 = weak, 5 = medium, 7 = strong, 9 = very strong
37	Fruit texture of surface	1 = smooth or very slightly wrinkled, 2 = wrinkled, 3 = strongly wrinkled
38	Fruit color at maturity	1 = yellow, 2 = orange, 3 = red, 4 = brown, 5 = green
39	Fruit intensity of color at maturity	3 = light, 5 = medium, 7 = dark
40	Glossiness	3 = weak, 5 = medium, 7 = strong
41	Fruit stalk cavity	1 = absent, 9 = present
42	Fruit depth of stalk cavity	1 = very shallow, 3 = shallow, 5 = medium, 7 = deep, 9 = very deep
43	Fruit shape of apex	1 = very acute, 2 = acute, 3 = rounded, 4 = moderately depressed, 5 = very depressed
44	Fruit of interloculary grooves	1 = absent/very shallow, 3 = shallow, 5 = medium, 7 = deep
45	Fruit number of locules	1 = predominantly two, 2 = equally two and three, 3 = predominantly three, 4 = equally three and four, 5 = predominantly four and more
46	Fruit thickness of flesh	1 = very thin, 3 = thin, 5 = medium, 7 = thick, 9 = very thick
47	Stalk length	1 = very short; 3 = short; 5 = medium; 7 = long; 9 = very long
48	Stalk thickness	1 = very thin, 3 = thin, 5 = medium, 7 = thick, 9 = very thick
49	Calyx aspect	1 = non enveloping, 2 = enveloping
50	Fruit capsaicin in placenta	1 = absent, 9 = present
51	Fruit intensity of capsaicin	1 = weak, 2 = medium, 3 = strong
52	Time of beginning of flowering	3 = early, 5 = medium, 7 = late
53	Time of maturity	1 = very early, 3 = early, 5 = medium, 7 = late, 9 = very late

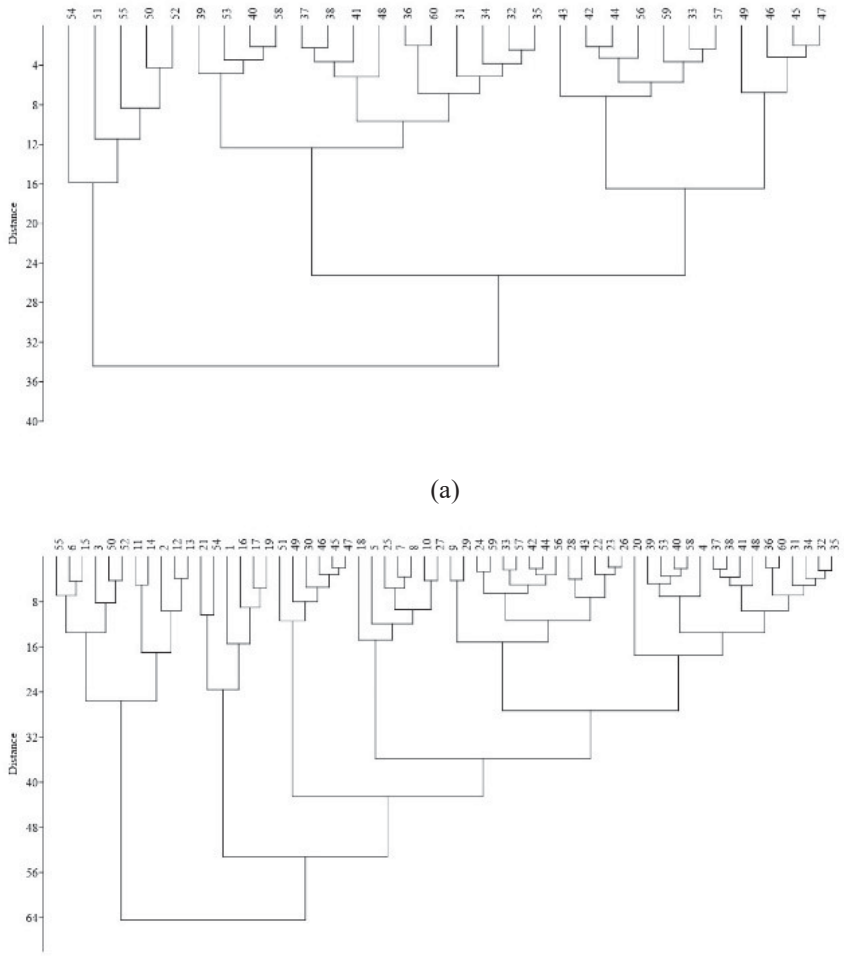
Data Analysis

The multivariate data analysis was carried out in stages using the Euclidean dissimilarity coefficient [9]. The first stage referred to the multivariate analysis of Indonesian genetic material (30 genotypes) and the second stage was the multivariate analysis of Indonesian genetic material and the genetic material introduced (60 genotypes). The percentage of diversity enrichment was calculated from the value of the farthest genetic distance after introduction subtracted before introduction, and then divided by farthest genetic distance before introduction.

Data analysis was also carried out on each character using the coefficient of variation (%). The diversity enrichment based on the coefficient of variation of each character was obtained from the reduction of the coefficient of diversity after introduction to the coefficient of diversity before introduction.

RESULTS AND DISCUSSIONS

Based on the results of cluster analysis using Euclidean, the farthest genetic distance of Indonesian genetic material (accessions, lines, and varieties) was 34%. Meanwhile, the farthest genetic distance of Indonesian genetic material plus introduced material was 64%, thus morphologically, there was an enrichment of genetic diversity by 88.2% (Fig. 1). These results indicate that the introduction activities have succeeded in adding or enriching the genetic diversity of IVEGRI's chili pepper based upon morphological marker analysis. The introduced lines, further, can be used directly as the varieties of introduced origin or indirectly through breeding activities as parent candidate crosses. Besides the morphological characters, the potential of these introduced lines as a source of resistance to pests and diseases of chilies needs to be explored through future evaluation activities [6].



(a)

(b)

FIGURE 1. Euclidean dissimilarity index of 53 chili pepper characters before (a) and after (b) introductory activity.

Plant Performance Characteristic

The increase of plant character diversity average after introduction was 20%, with a range of 1 to 91% (Fig. 2). The highest increasing rate was found in the shortened internode character obtained from the introduced line AVPP 1721. In addition, IVEGRI's breeding material received the additional hairiness of node character from the introduced line VI 037607 (PBC 411) (Fig. 3). The shortened internode character might be used to produce chilies with high yield and early maturity. The shortened internode character has been used in the breeding of maize plants

[12]. It has been observed, although not published, that the use internode length can be successful indicator of potential heterosis with short and long internode parental lines resulting in hybrid progeny with high levels of hybrid vigor. Several studies reported a positive correlation between the character of the hairiness of the node and the level of resistance to pests [13][14]. VI 037607 (PBC 411) line could be used as a donor parent for hairiness of node character in pest-resistant chili varieties breeding program especially to aphid.

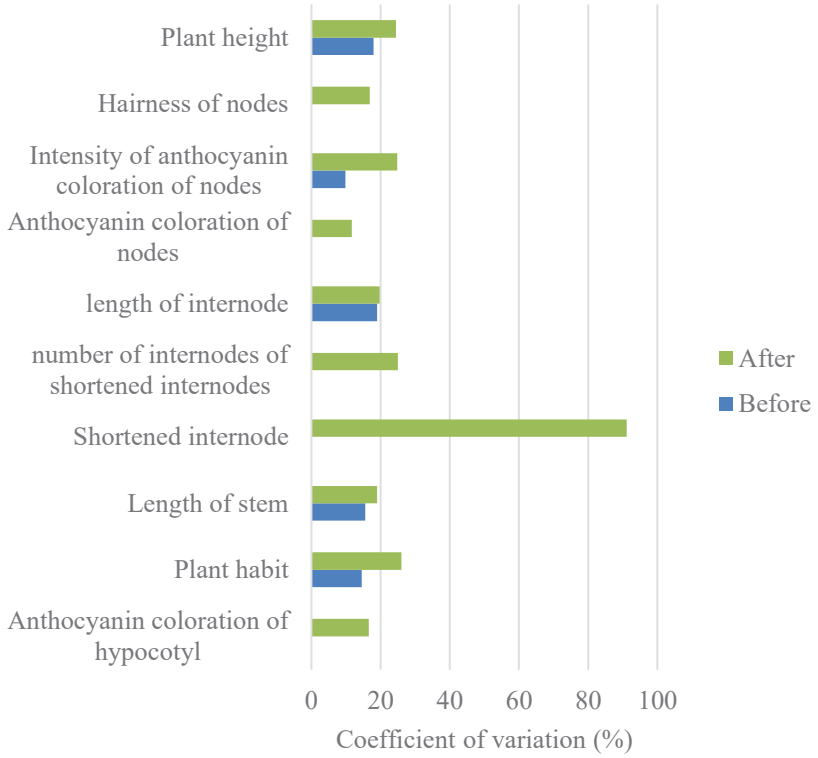


FIGURE 2. The variation of plant performance character before and after introduction activity.

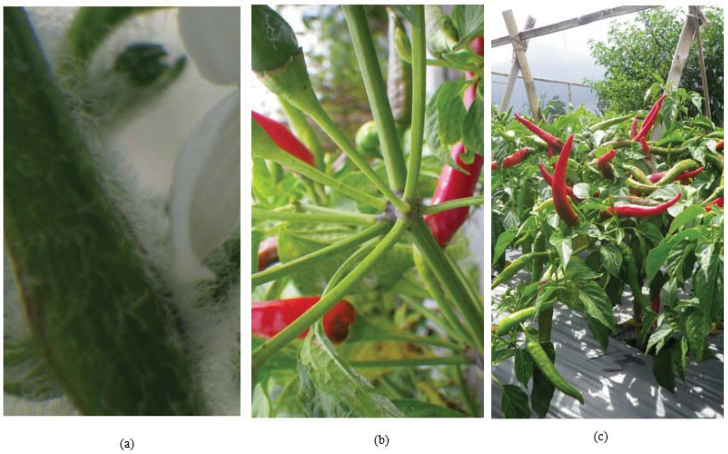


FIGURE 3. The performance of introduced lines. (a) Hairiness of node (VI 0377607/PBC 411). (b, c) Shortened internode (AVPP 1721).

Leaf and Flower Characteristics

The diversity of leaf and flower characteristics was lower than that of the plant performance characteristics, namely by 15%, with a range of 1 to 43% (Fig. 4). A high increase in diversity was found in the leaf shape and secondary color of corolla characters. The diversity of leaf shape characters of Indonesian material genetic was found narrower and dominated by lanceolate type. Through introduction, it was enriched by obtaining broad elliptic characters from the VI 037522 line (PBC 495) (Fig. 5), while the enrichment of purple flower characters was obtained from the introduced line VI 037603 (PBC 370) (Fig. 6). Several studies reported that purple flower could be used as a marker in producing hybrid lines and to study natural cross-pollination on chili pepper [15, 16].

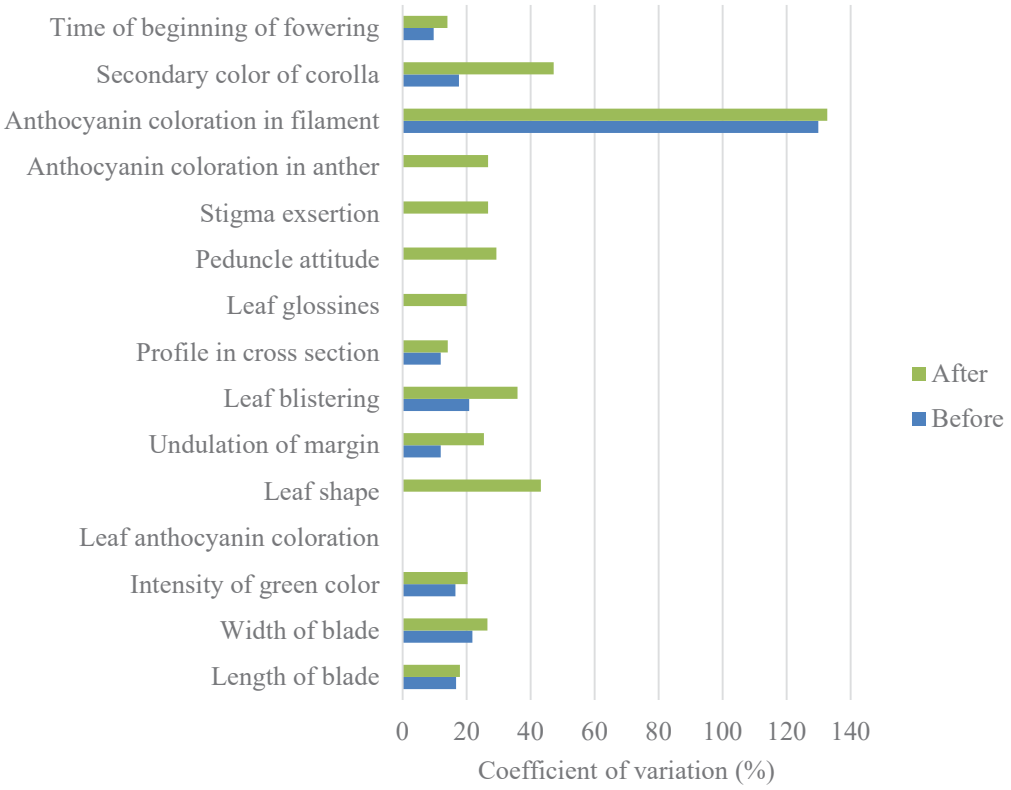


FIGURE 4. Coefficient of variation of leaf and flower characters before and after introduction activity.

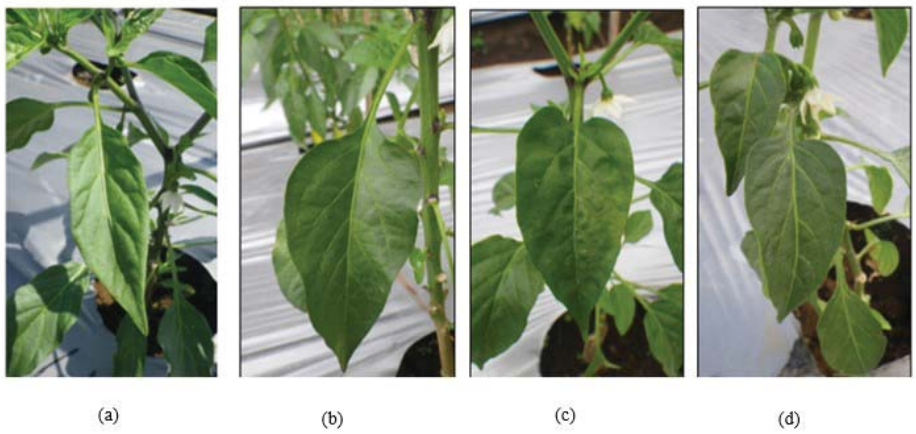


FIGURE 5. Leaf performance. (a) Indonesian varieties. (b–d) Introduced lines.

FIGURE 6. Flower appearances. (a) Indonesian varieties. (b–d) Introduced lines.

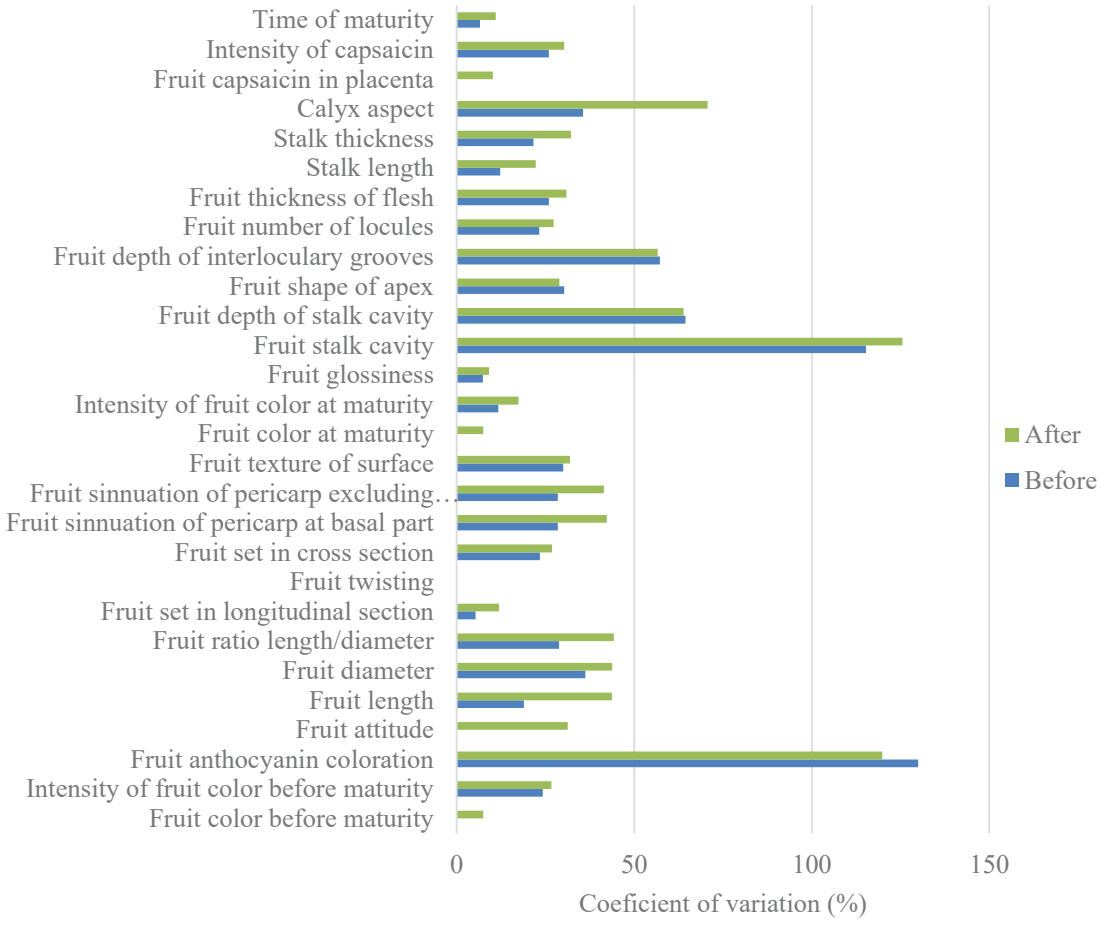


FIGURE 7. Coefficient of variation of fruit characters before and after introduction activity.

Fruit Characteristics

The average increase in the diversity of fruit characteristics was found to be the lowest compared to the characteristics of plants, leaves and fruit; at 8%, with a range of 2 to 35% (Fig. 7). The fruit had more characteristics

than plants, leaves and flowers. Fruit character enrichment was obtained from introduced lines AVPP 9905, VI 037603 (PBC 149), and VI 037599 (PBC 143) for fruit color before maturity and fruit color at maturity characters with orange fruit (AVPP 9905, VI 037558 [PBC 596], and VI 041282 [PBC 144]) (Fig. 8).

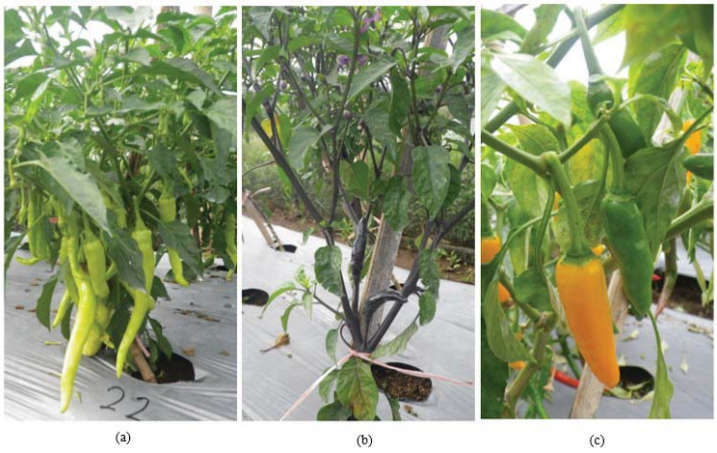


FIGURE 8. Introduced lines fruit performance at 90 DAP. (a) AVPP 9905. (b) VI 037603. (c) VI 037558.

Another character enriched was the fruit position where most of the introduced lines had erect fruit position characters, while the Indonesian lines generally had pendant fruit. Chilies with orange fruit were reported to had high nutritional value because they are rich in beta carotene [17–19]. Meanwhile, purple fruit also has high nutritional value because it is rich in anthocyanin as antioxidants [20, 21]. However, orange and purple chilies are less popular among Indonesian consumers, so it requires the promotion of the benefits of colored chilies fruit for increase uptake of these lines.

CONCLUSION

The introduction of the chili lines from World Vegetable Center increased the genetic distance of the Indonesian genetic materials. The enrichment of genetic material expands genetic diversity and can be utilized for the development of superior varieties of chili. Introduced lines contribute some useful characters as materials for assembling new superior varieties including shortened internodes, hairiness of nodes, leaf shapes, secondary color of corolla, fruit color before maturity, fruit color at maturity, and fruit attitude.

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