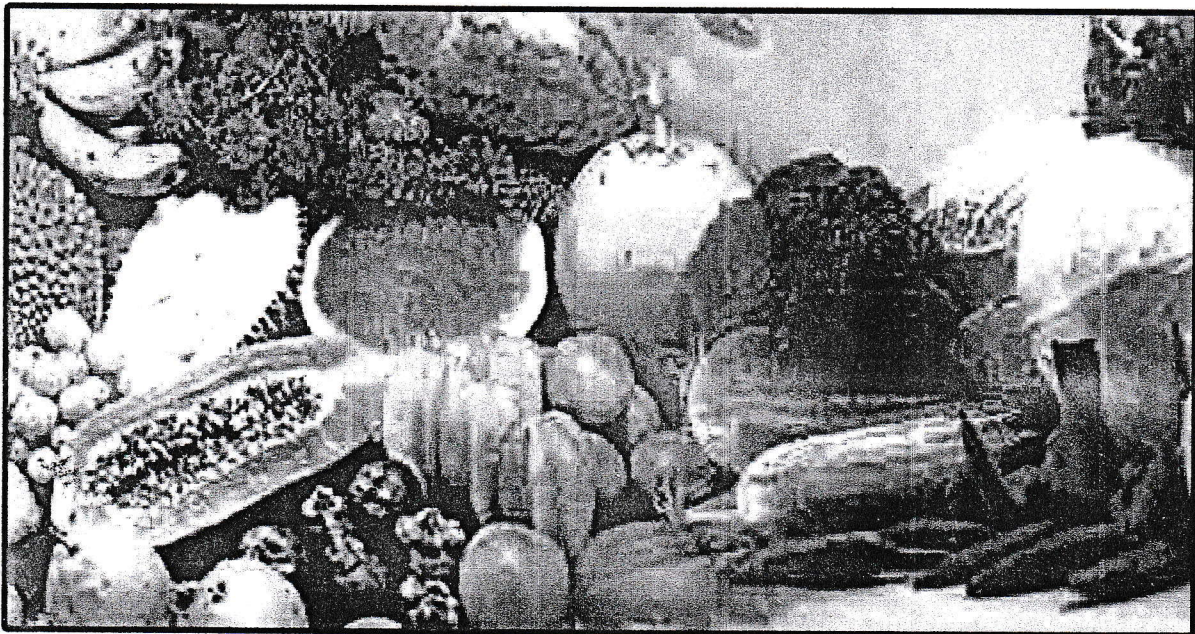


PROCEEDING

International Seminar

on Horticulture to Support Food Security 2010

June 22-23 ,2010
Bandar Lampung, INDONESIA



Editors:

Douglas Archbold
Michael Reed
Janet Paterson
Soesiladi Esti Widodo
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Organized By :



UK
UNIVERSITY OF
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TABLE OF CONTENTS

	Page
Preface	iii
Welcoming Address from the Organizing Committee	iv
Welcoming Address from Rector of Lampung University	v
Event Schedule	vi
Table of Contents	vii
KEYNOTE SPEAKER'S PAPER	
Increasing Food Security with Postharvest Research	KP-1
Douglas Archbold	
PLENARY SPEAKER'S PAPER	
Problems and Developing Aspects Relating to Harvest and Postharvest Handling of Tropical Fruits	KP-6
Soesiladi Esti Widodo	
SEMINAR PAPERS	
Group A: Horticultural Biology and Physiology	
1 Quality variation of Chilli fruit (<i>Capsicum annum</i>) due to the salt changes in the Saline Soil Solution	A-1
Wanti Mindari	
2 Adaptation Test of the Three Local Cultivars of North Maluku Tomato (<i>Lycopersicon esculentum</i>) on Saline Sand	A-7
Aisjah Rachmawaty Ryadin, Natal Basuki, Asrul Dedy Ali Hasan	
3 The Changes Content of Cytokinin and Gibberellin on Growth Stage and Age of Mangosteen Plant (<i>Garcinia Mangostana</i> L.).....	A-15
Ramdan Hidayat	
4 Accelerating the Growth of Mangosteen (<i>Garcinia mangostana</i> L.) at Agroforestry System in District of Kerinci, Jambi Province.....	A-23
Nerty Soverda	
5 Combining Wedelia trilobata and Inorganic-N Fertilizer for Pepper Growth and Yield.....	A-32
Nanik Setyowati, Uswatun Nurjanah, Melva M. Manurung	
6 Four Kinds Of Materials Litter Potentials As Substitution Material For Media Grows Of White Oyster Mushroom (<i>Pleurotus ostreatus</i>)	A-36
Widiwurjani	
7 Growth Analysis of Sweetcorn and Its Correlation to the Yield at Different Rate Application of Palm Oil Sludge Compost	A-41
Merakati Handajaningsih	

8	The Role of Coconut Water in Horticultural Plant Tissue Culture	A-46
	Jeany Polii Mandang	
9	Energy Input-Output Analysis for Watermelon Production	A-53
	Agus Haryanto, Dwi Cahyani, Fadil Murda Kusuma, Arif Dwi Santoso	
10	Developing Hydroponic technology at Medium Altitude, without pesticide for medium and small agribusiness Case:tomato cuvar Recento.....	A-60
	Dedy Widayat, Aos M Akas and Nursuhud	
11	Effects Of Goat Manure On Growth, Yield, And Economic Impacts Of Vegetable Intercrops In Young Coffee Plantation.....	A-66
	Agus Karyanto, Sugiatno, and Rusdi Evizal	
12	The Response of Cocoa Seedlings due to Application of Trichoderma spp Grown on Different Media.....	A-75
	Sriwati R, Chamzurni T, Ardiansyah	
13	The Effect of Nitrogen Sources and Types of Medium Subculture on <i>Brassolaeliocat-tleya</i> (Blc.) Amy Wakasugi Shoots Growth.....	A-81
	Yayat Rochayat, Anne Nuraini and Mirna Oktavani	
14	Effects of Benzyladenine on in vitro shoot multiplication of Banana (<i>Musa paradisiaca</i> Linn) cv. Ambon Kuning and Tanduk.....	A-88
	Dwi Hapsoro, Mochamad Ivan Alisan, Titiek Ismaryati, and Yusnita	
5	In Vitro Propagation of Anthurium plowmanii cv. Wave of Love and Plantlet Acclimatization.....	A-95
	Yusnita, Sismanto, and Dwi Hapsoro	
6	Ethylene Used in The Breaking of Potato Tuber Dormancy (<i>Solanum tuberosum</i> L) Variety of Atlantic and Superjohn.....	A-101
	Johannes E. X. Rogi, Selvie Tumbelaka, and Shubzan Andi Mahmud	
	Habitat Mapping and Raflesia Condition in Bengkulu	A-104
	Yulian Idris	
	Insect Diversity on The Ecosystem of Citrus (<i>Citrus</i> spp.) Plantation In East Java	A-111
	Indriya Radiyanto and Ketut Sri Marhaeni J	
	In Vitro Seed Germination, Seedling Growth and Acclimatization of Dendrobium hybrids (<i>Orchidaceae</i>)	A-116
	Sri Ramadiana, Ronald Bunga Mayang, Dwi Hapsoro, and Yusnita	
	Yield Tests of Some yard Long Bean Genotype on Two Environment	A-123
	Nyimas Sa'diyah, Tjipto Roso Basoeki, Eko Suprihanto, Ricky Aris Tiawan, and Setyo Dwi Utomo	
	Responsen of Protocorm Like Bodies Hybrid Dendrobium Orchid on Various Kind Types and Concentration of Cytokinin and Auxin on Murashige and Skoog (MS) Medium	A-130
	Anne Nuraini, Wieny Heriliya R., Erni Suminar, and Eva Marlina	
	Effect of Vermin Compost and Bokashi on Nutrient Content of Mustard Green and Lettuce	A-136
	Yacobus Sunaryo	

23	Isolation of Plant Growth Promoting Rhizobacteria (PGPR) from Various Plant Rhizospheres	A-141
	M. A. Syamsul Arif	
24	Respiration of Packaged Fresh Oyster (Tiram) Mushroom (<i>Pleurotus ostreatus</i>).....	A-149
	Gede Arda, B. Rahardjo	
25	Flower development and Induction of Haploid Population from Anther Culture	A-156
	A Husni, M Kosmiatin, and A. Purwito	
26	Dose Effect Of Compound Fertilizer Npk Ratios On Growth Red Betel (Piper Crocatum Ruiz And Pav.) With Two Types Of Planting Media	A-164
	Rugayah	
✓ 27	Introgression Of CMV Tolerance Genes To Hybrid Parent Of Hot Pepper: Employing Morphological And Rapid Marker To Identify Recurrent Parent Characteristics In BC2 Population	A-174
	Catur Herison, Sri Wina:sih, Merakati Handayaningsih, and Rustikawati	
28	Improvement of Cayenne Chili-Pepper of Landrace Germplasms through selection for a Reduction of Abortive Flowers	A-181
	Saiful Hikam and Paul Timotiwu	
29	Genetic analysis of Maize Quantitative Traits On Ultisol Under Low Input	A-188
	Suprpto and M. Taufik	
30	Propagation of Gladiol (<i>Gladiolus hibrida</i>) by Using Benzil Adenin (BA).....	A-197
	Tri Dewi Andala Sari, Fitri Juwita Susanti	
31	Model Simulation of "Sawah-Kolam" System for Rainwater Harvesting to Support Rainfed Paddy Production in Metro City Lampung	A-201
	Sugeng Triyono, Oktafri, and Bustomi Rosadi	
32	Growth and Development of Protocorm Like Bodies Hybrid Dendrobium Orchids on MS Medium with Cytokinin and Auxin Combination	A-210
	Wieny H. Rizky, Anne Nuraini, Erni Suminar, and Karlina Syahrudin	
33	Evaluation of Mung Bean Genotypes for Resistance to Field and Storage Deterioration	A-217
	Marwanto	
Group B: Horticultural Postharvest Handling and Processing Technology		
34	Model of Technology Valuation System (A Case of Evaporative Cooling System for Horticulture Products).....	B-1
	Budi Dharmawan, Ropiudin	
35	Effect of Some Types of Banana Sago Flour and Substitution with Chocolate Powder to Taste Lompong Sago Produced	B-8
	Zuraida Zuki, Diana Silvi, Mutia Elfira	
36	The Storage of Gnetum Seeds by Mixing with Dry Sand and Burried in Soil	B-15
	Tamrin, Sandi Asmara, Henny Nurpa Anggraini	
37	Characterization of the Drying Process of Melinjo Seed.....	B-20
	Sarono, Yatim R. Widodo	

INTROGRESSION OF CMV TOLERANCE GENES TO HYBRID PARENTS OF HOT PEPPER: EMPLOYING MORPHOLOGICAL AND RAPD MARKER TO IDENTIFY RECURRENT PARENT CHARACTERISTICS IN BC2 POPULATION

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ABSTRACT

Superior and CMV tolerant hybrid cultivar can only be developed by crossing a pair of high heterobeltiosis parents and both of which are CMV tolerance parents. Gene introgression have to be accomplished if the tolerance is not exist in the parents. This research was objected to employ DNA markers on identifying reccurent parent characteristics in BC2 generations to cut down the backcrossing cycle in CMV tolerance gene introgression. This research employed five BC2 populations generated from crosses of hybrid parents PBC1354 and PBC378 with tolerant parents of C1024, C1042 and C1043. The BC2 populations were selected for their CMV tolerance before and were characterized morphologically and molecularly. The results showed that genotype A24, A25, A29 of BC2A ([378/[378/(378/1042)]-11]); B12, B2, B4 of BC2B ([378/[378/(378/C1024)]-6]), C16, C33, C4 of BC2C ([378/[378/(378/C1043)]-13]) population were tolerance individuals and resemble to recurrent hybrid parent PBC378. While individuals D11, D33, D38 of BC2D ([1354/[1354/(1354/C1043)]-18]); and individuals E12, E20, E31 of BC2E ([1354/[1354/(1354/C1024)]-4]) population were CMV tolerance and resemble to recurrent hybrid parent PBC1354. Employing of both morphological and RAPD marker were efective to identify the recurrent parent characteristics in early backcross generation.

Keywords : Capsicum, CMV tolerance, gene introgression, RAPD

INTRODUCTION

Hot pepper (*Capsicum annuum* L.) is one most important vegetable crop in Indonesia. However, the national production has not yet been able to fulfill the increasing demand annually. For instance, in the year of 2006, Indonesian government had to import hot pepper product as much as 11,885.5 ton (BPS, 2007). The main constraint of national hot pepper production are genetically low production potential of vareties planted by famers and CMV infection in the field. Among 45 different viruses detected from hot pepper fields, CMV is the most widespread and has potential to cause heavily yield losses (Duriat, 1996). Although quantitative yield loss due to CMV is not well documented, this virus tends to cause total yield loss in the field. Infection during early growth stage decreased 81.4% and 82.3% of total number of fruit and fruit weight per plant respectively (Sari *et al.*, 1997). In Korea, a field survey on paprika producing region showed that CMV is the most dominant virus in the field, followed by PepMoV, PMMoV, and TSWV (Mun *et al.*, 2008; Ryu *et al.*, 2009).

The most efficient and promising control measure against CMV and to increase pepper production is cultivation of high yielding hybrid cultivars tolerance to CMV. Such hybrids can merely be developed by crosses of pairs of parents with high heterobeltiosis potential and both of which tolerance to CMV. Introgression of tolerance into hybrid parents is compulsory if the trait does not exist in both parents. The conventional method to do gene introgression is a backcross breeding.

The final objective of the backcross selection is to find individuals identical to the recurrent parent with an additional characteristic, CMV tolerance. To identify the recurrent parental characteristics in a segregating backcross population is the most important and difficult step during the backcross selection. The identification process have to be carried out accurately, otherwise this will lead to an ineffective selection. Therefore, efforts to employ as many traits as possible will be helpful to do the selection in an early backcross population.

Two groups among the traits are morphology and DNA markers. Morphological traits such as plant height, number of dichotomous branch, leaf length, leaf width, fruit length, fruit diameter, total number of fruit, and total fruit weight per plant are commonly used in morphological characterization of hot pepper (IPGR, AVRDC and CATIE, 1995). DNA markers have been used to assist selection in breeding program on biotic stress (Klein-Lankhorst *et al.*, 1991; Wechter *et al.*, 1995; Saidi dan Warade, 2008). DNA markers were employed to assist introgression of high protein content into a superior wheat cultivars by mean of backcross breeding (Davies *et al.*, 2006).

The objective of this study was to employ morphological traits and RAPD markers to identify recurrent parental characteristics in early backcross populations during introgression of CMV tolerance gene into hybrid parents by means of backcross breeding.

MATERIALS AND METHODS

The study was conducted in the greenhouse of Dept. of Agronomy, Faculty of Agriculture, Univ. Bengkulu, and RAPD analysis was carried out at RGCI Laboratory of Bogor Agricultural University in the period of June to October 2009.

Development of MAS of Recurrent Parent

A MA of recurrent parent is a set of DNA markers used to identify the similarity of BC individuals to their recurrent parent. Genetic materials used in this study were the recurrent parents PBC378 and PBC1354. Chemicals used were DNA isolation and amplification kit REExtract-N-Amp Plant PCR Kits XNAP (Sigma-Aldrich USA), 58 random primers of OPERONS, and electrophoresis chemicals. Principal apparatus employed were Eppendorf micro pippete (0.5-2,5 µl, 10-100 µl, 100-1000 µl), eppendorf tips, tubes, waterbath, high sonic centrifuge Sorvall RC-55 Dupont, DNA drier, DNA Thermal Cycler PE Gene Amp PCR system 2400, electrophoresis set, UV transiluminator and digital camera.

Some leaf disks 0.5 cm in diameter were placed in a 2 ml plastics tube containing 100 µl of XNAP SIGMA KIT extraction solution and incubated at 95°C for 10 minutes. Dilution solution of 100 µl was then added in to the tube and shaken gently to maximize the extraction process. The solution was then removed into new tubes and added with 200 µl of aqua bidestilata and 100 µl CIA (cloroform:isoamyl alcohol, 24:1), and centrifuged at 10000 rpm, 4°C, for 10 minutes to separate extracted DNA from other leaf tissues. The liquid phase was then pipetted carefully and put into new tubes, and the DNA was precipitated with 1 ml of 95% alcohol, and incubated at 4°C for at least 30 minutes. Presipitant of DNA genom was centrifuged at 10,000 rpm, 4°C, for 10 minutes to separate DNA pellets from their solution. DNA pellets were then dried in a vacuum drier. The final step, the DNA pellets were then diluted with 100 µl sterilized ion free water.

DNA amplification was performed with random primers able to amplify hot pepper DNA genom following the RAPD tehnikue of William *et al.* (1990). The RAPD markers were generated through polymerase chain reaction (PCR) using PE 2400 gene Amp-DNA thermal cycler. Electrophoresis was run on agarose gel (0.8 w/v) in TAE buffer and gel staining was done by shoaking the gel in etidium bromide solution (0.5 mg/l) for 20 seconds. DNA bands were visualized by UV transiluminator and documented by Nikon D1000 digital camera.

Selection of BC2 Population

DNA amplification products of the recurrent parents were than used as marker assisted selection. DNA fingerprint of recurrent parents along with their morphological trait were then used to identify similarities of BC2 individuals to their recurrent parents. Genetic materials selected in this study were 5 populations of BC2 generated from a cross between parental hybrids and CMV

tolerance donor, namely BC2A= PBC378/[PBC378/ (PBC378/C1024)]-11, BC2B= PBC378/[PBC378/(PBC378/C1042)]-6, BC2C= PBC378/ [PBC378/(PBC378/C1043)]-13; BC2D= PBC1354/[PBC1354/(PBC1354/C1043)]-18; dan BC2E= PBC1354/[PBC1354/(PBC1354/C1024)]-4. DNA genom of five most CMV tolerance individuals of each BC2 population were isolated and amplified by random primers which previously were used to characterize their recurrent parents. Those individuals were also characterized morphologically. Based on morphological traits and RAPD markers they were analyzed their similarity to their recurrent parents by *Cluster Analysis*. Three most identical individuals to their recurrent parent were selected to generate further BC generation.

RESULTS AND DISCUSSION

Development of MAS of Recurrent Parent

Primer selection was done to determine primers used to characterize recurrent parents. Amplification of DNA genome with 58 random primer of OPERONS OPA, OPC, OPE, OPF, OPH and OPM showed that most of them were able to amplify DNA genome of hot pepper. However, less than 10 primers produced more than 4 bands, and the best 5 primers were then used in further study. They were OPE20 (AACGGTGACC), OPE7(AGATGCAGCC), OPE15 (ACGCACAACC), OPH5 (AGTCGTCCCC), and OPH13 (GACGCCACAC) (Figure 1).

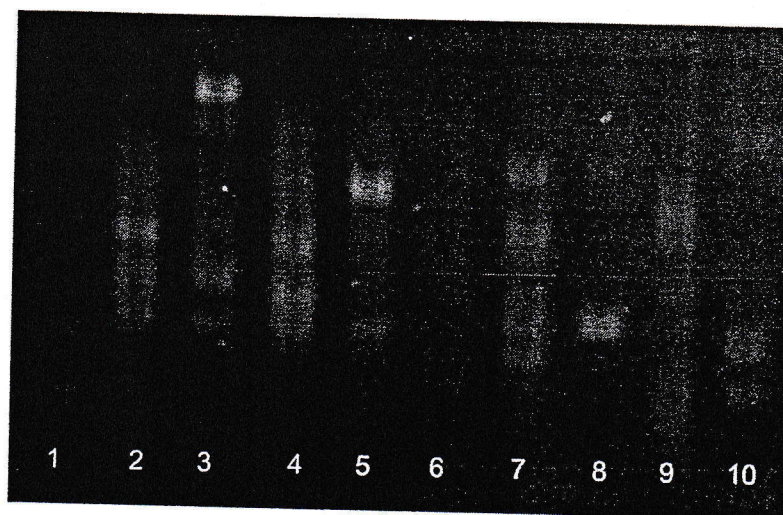


Figure 1. Example of visualisation during primer selection on recurrent parent PBC378: 1=OPE9, 2= OPE15, 3=OPE20, 4=OPH13, 5=OPH14, 6=OPH8, 7=OPE7, 8=OPE8, 9=OPH19, 10=OPH12

Based on the amplification of those five primers, DNA fingerprints of PBC1354 were characterized by the occurrence of band patterns at 1150, 650, and 300 bp when amplified with OPE20; at 1050, 800, 600, 500, 400, and 250 bp with OPE7, at 800, 700, 600, dan 350 bp with OPE15, at 700, 550, dan 300 bp with OPH5 and at 800 dan 600 bp with OPE13. DNA fingerprints of PBC378 were characterized by band patterns at 1150, 650, 600 and 300 bp when amplified with OPE20; at 800 and 500 bp with OPE7; at 800 dan 700 bp with OPE15, at 700, 500, dan 350 bp with OPH5; and at 800, 650 dan 400 bp OPH13 (Table 1).

Table 1. Characteristics of recurrent parents based on band pattern of DNA

Band pattern	PBC1354	PBC378
OPE20-1150	+	+
OPE20-650	+	+
OPE20-600	-	+
OPE20-550	+	-
OPE20-350	-	+
OPE20-300	+	-
OPE7-1050	+	-
OPE7-800	+	+
OPE7-600	+	-
OPE7-500	+	+
OPE7-400	+	-
OPE7-250	+	-
OPE15-800	+	+
OPE15-700	+	+
OPE15-600	+	-
OPE15-350	+	-
OPH5-700	+	+
OPH5-550	+	-
OPH5-500	-	+
OPH5-350	-	+
OPH5-300	+	-
OPH13-800	+	+
OPH13-650	-	+
OPH13-600	+	-
OPH13-400	-	+

+ presence, - absence

Fingerprints of both recurrent parents had dissimilarity level of 46% meaning that both of them were genetically not closely related. The similarity level of 54% was due to both of them belong to the same species, *Capsicum annum*.

Selection of BC2 Population

Morphologically, based on plant height, total number of dycotomous branch, leaf length and width, total number of fruit, total fruit weight, fruit length and diameter, each of the BC2 population was segregated. This indicated that the recurrent and donor parents for each of BC population were morphologically different. The range of calculated coefficient variability on those variable were variably within each of selected BC2 individuals. The range coefficient variability of BC2A, BC2B, BC2C, BC2D, and BC2E were 9.47-34.67, 5.61-57.69, 6.91-36.39, 7.51-51.64, and 8.58-33.71 in percentage, respectively.

Similarly, the DNA amplification product (Figure 2) also showed variability between individuals within each BC2 population which indicated that between recurrent and donor parent were genetically different. The calculated coefficient variability based on RAPD marker ranging higher than those of morphological traits. The range coefficient variability of BC2A, BC2B, BC2C, BC2D, and BC2E were 76.25-161.02, 67.27-147.96, 95.93-195.00, 76.25-226.78 and 80.90-147.96 in percentage, respectively. The segregation in each of BC2 population also indicated that eventhough

they were previously selected for their CMV tolerance, there were still segregation among the selected individuals.

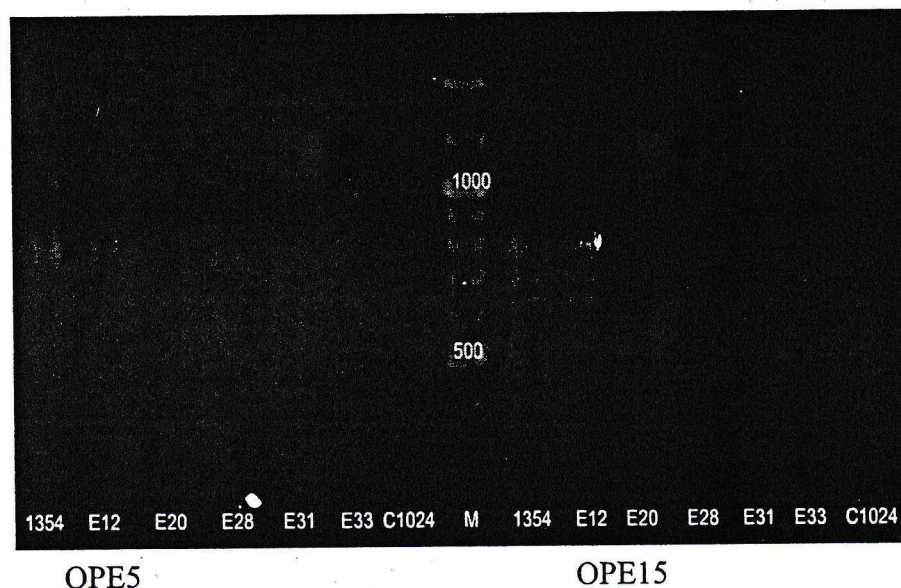


Figure 2. Example of DNA band pattern form RAPD analysis with primer OPE5 and OPE15

Employing all variable measured, selection was performed based on similarity level to the recurrent parents. On BC2A population, all selected individuals had similarity level of 97.57% to their recurrent parent PBC378. Therefore, each of the individuals previously selected for CMV tolerance could be used similarly to generate further BC generation. On BC2B population, B12 was most identical individual to PBC378 with similarity level almost 100%, and followed by B2 and B4 with similarity level of 99%. So that those three individuals were best for further generation. The last alternative individuals were B37 and B49 with similarity level of 97.08 to their recurrent parent. Individuals of BC2C population almost identical among them with similarity level were more than 99%. All of them formed one group which was 98.25% similar to PBC378. Therefore, each of them was suitable to develop further BC generation. Genotype D11 and D33 of BC2D were most identical to their recurrent parent PBC1354 with their similarity level about 98% and each of them, therefore, was suitable to develop further generation. The next alternative was either D3 or D38 which the similarity level to their recurrent parent was about 93%. Genotype E12, E31, and E20 of BC2E population were most identical to their recurrent parent, PBC1354 with similarity level of about 99%. Therefore, they were the best genotype to be used to develop further generation. The rest genotype, E28 and E33, merely 96.27% similar to their recurrent parent.

Comparing all BC2 population in this study, it was noticeably that there was different variation pattern between genotypes within each BC2 population although all of them were the selected genotype for CMV tolerance. Some genotypes were highly identical to their recurrent parent and the others were less similar to their recurrent. There seem to be depending on combination of the donor and recurrent parent used in the breeding program.

The results indicated that employing both morphological trait and RAPD marker were successful to identify genotypes highly identical (more than 99% similarity level) to their recurrent parent in the BC2 generation. This indicated that the use of morphological in combination to molecular trait was promising to shorten the backcross breeding program in introgressing CMV tolerance gene to a hybrid parent.

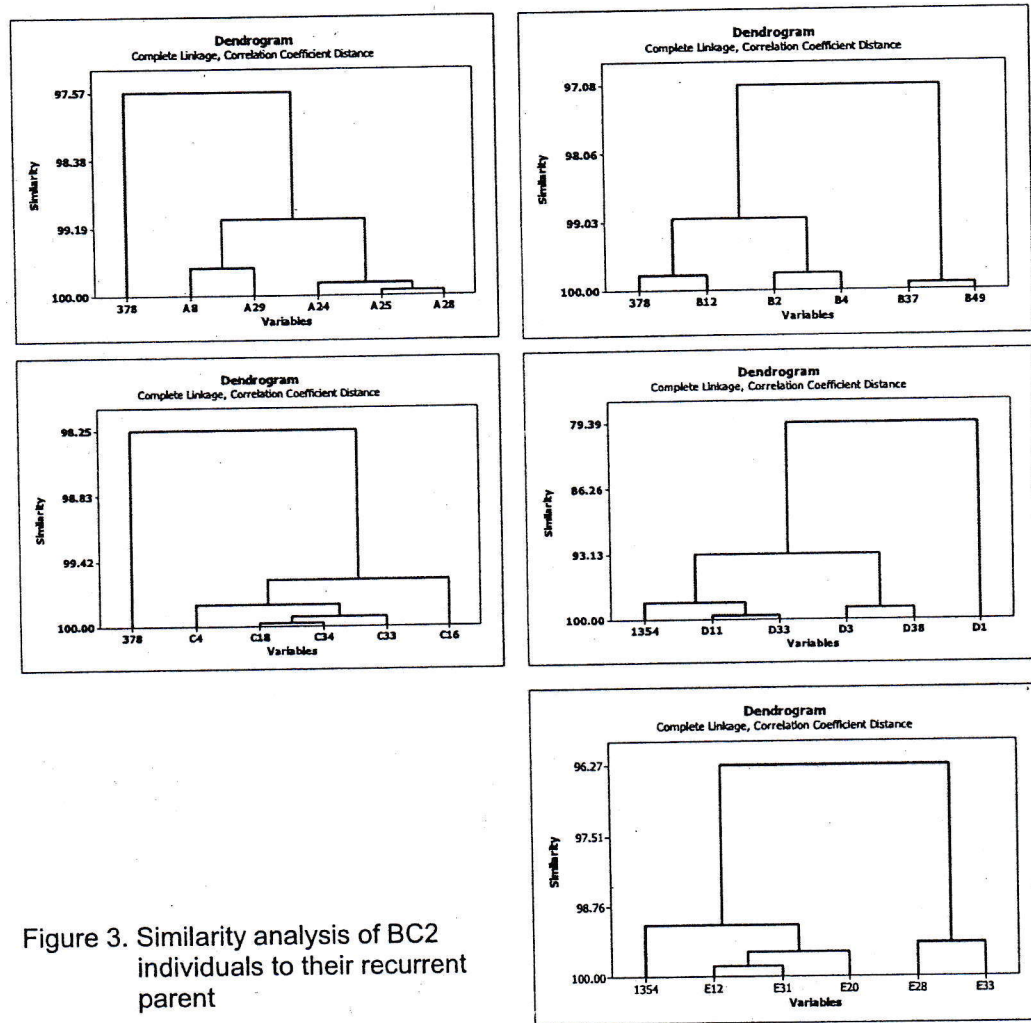


Figure 3. Similarity analysis of BC2 individuals to their recurrent parent

CONCLUSION

Genotype A24, A25, A29 of BC2A ([378/[378/(378/1042)]-11]); B12, B2, B4 of BC2B ([378/[378/(378/C1024)]-6]), C16, C33, C4 of BC2C ([378/[378/(378/C1043)]-13]) population were tolerance individuals and resemble to recurrent hybrid parent PBC378. While individuals D11, D33, D38 of BC2D ([1354/[1354/(1354/C1043)]-18]); and individuals E12, E20, E31 of BC2E ([1354/[1354/(1354/C1024)]-4]) population were CMV tolerance and resemble to recurrent hybrid parent PBC1354. Employing of both morphological and RAPD marker were effective to identify the recurrent parent characteristics in early backcross generation.

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