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Research Article

Microsatellite Marker for Genetic Variation Analysis in Local Chili Pepper (*Capsicum frutescens* L.) Induced by Ethyl Methane Sulfonate (EMS)

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ABSTRACT

Mutation using Ethyl Methane Sulfonate (EMS) is a simple and quick method to produce genetic variation in chili pepper. In this study, a total of 3 genotypes of local chili pepper (*Capsicum frutescens* L.), i.e. Genotype 2 (G2), Genotype 7 (G7), and Genotype 11 (G11) were induced by EMS with concentrations of 0% (K0), 0.01% (K1), 0.02% (K2), and 0.04% (K3). Genetic variation analysis in mutant was performed based on 3 microsatellite markers CA 19, CA 27, CA 62. Those molecular markers successfully detected the genetic variation in chili pepper mutant based on the number and size of microsatellite alleles variation. The 3 genotypes of chili pepper mutant produced a total of 15 alleles with the average Polymorphism Information Content (PIC) value of 0.82. Compared to the control plant, genetic variations in genome level were observed in local chili pepper. Furthermore, the treatment of EMS with concentration of 0.04% produced the most notable genetic variation in 3 genotypes of local chili pepper.

Keywords: Red yeast rice, foam cell, hypertriglyceridemia, AST-ALT

Introduction

Chili pepper (*Capsicum frutescens* L.) is the member of genus *Capsicum*, which belongs to Solanaceae family consists of 35 species, among which 5 species have been cultivated and 30 species remain wild [1]. *Capsicum* species that have been cultivated and widely distributed in the world are *C. annuum* L., *C. frutescens* L., *C. baccatum* L., *C. chinense* Jacq. and *C. pubescens* Ruiz et Pav. [1,2,3]. Two species of Capsicum that have been cultivated and widely used in Indonesia are *C. annuum* L. and *C. frutescens* L. [2,4]. Chili pepper fruit is a source of metabolite compounds such as carotenoid, ascorbic acid, tocopherol, flavonoids, and capsaicinoid [5].

Metabolite compounds in chili pepper, especially capsaicinoid has a high economical value utilized as analgesia, anticancer, anti-inflammatory, antioxidant, and anti-obesity [6, 7, 8, 9]. In other utilization, phytochemical study on *C. annuum* spp. microcarpum L. showed the uses of capsaicin as an ingredient in biopesticides [10].

Based on its high beneficial, chili pepper became one of the horticultural commodities that highly cultivated by Indonesian people [11]. This condition demands high quality of varieties to fulfill farmer needs for seed resource. The one effort in crop improvement and plant breeding program is increasing the genetic variation using mutation [12,13]. Mutation is a simple method to produce genetic, morphology and agronomic variation in crop [12, 14, 15]. The other studies, mutation was induced by chemical mutagen such as Hydrazine (HZ) and Ethyl Methane Sulfonate (EMS) in the *Solanum lycopersicon* L. var. Arka vikas (Sel-22) showed wide range of phenotypic variability. The different of EMS treatments were produced more variability than using HZ [13].

EMS is a monofunctional of ethylating agent produced change on nucleotide base such as transitions, insertions or deletions, as well as extensive intragenic deletions, the clear-cut evidence that

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Table 1. Microsatellite markers

Marker	Motif	Forward and reverse primer (5'- 3')
CA 19	$(TC)_{12}$	F: CCGCAATGGCAGTATGATCT R: CGGCTCTATCTACAACGGTG
CA 27	$(CA)_{12}(CT)_{17}$ ATCG$(CT)_9$	F: GCAGAGGACCAGTTAGCATA R: TGTTCTGAGTCCACGATGCT
CA 62	$(AG)_{22}$	F: CGCATATAGGCAGATCAAAT R: GGTCAGACTACGACTCTCTCA

EMS is able to break chromosomes [13, 16]. Mutation using EMS is a simple and quick method to produce genetic variation, produce inherited quantitative and qualitative traits by random point mutation [12, 16, 17].

In this study, the presence of mutation was observed based on variation in the genome. Genetic variation in the genome level can be detected using Simple Sequence Repeat (SSR) or microsatellite marker [18,19]. Microsatellite marker has been developed in chili pepper. Microsatellite markers CA 19, CA 27, and CA 62 have been designed based on the flanking region from repetitive region of *C. annuum* L. These markers are transferable and polymorphic when tested on *C. frutescens* L. and *C. chinense* Jacq. Therefore, these markers are suitable for identification of genetic, diversity, and phylogenetic studies [1].

The advantages of microsatellite marker are highly variable, abundant and equally distributed in the genome, co-dominant, and has multiallelic types of variation [18, 20]. Moreover, microsatellite marker uses a simple of detection method using Polymerase Chain Reaction (PCR) [18]. The objective of this study was to analyzed genetic variation in local chili pepper caused by EMS treatments based on microsatellite markers CA 19, CA 27, and CA 62. The results of this study are expected to support crop improvement and plant breeding program in chili pepper.

Material and Methods

Plant material

Seeds of local chili pepper (*C. frutescens* L.) genotype 2 (G2) and genotype 11 (G11) from Malang, East Java and chili pepper genotype 7 (G7) from Lombok, NTB were subjected to Ethyl Methane Sulfonate (EMS) treatment with concentrations of 0% (K0) (control), 0.01% (K1), 0.02% (K2), and 0.04% (K3).

EMS induced mutagenesis

Seeds were placed in flask and soaked in distilled water overnight (approximately 15 hours). Furthermore, the water was decanted and seeds were soaked with different concentrations of EMS (0.01%, 0.02%, and 0.04%) for 6 hours at room temperature. Seeds were soaked with 1% natrium thiosulfate for 5 minutes. Then, the seeds were rinsed under running water for 15 minutes and dried in room temperature [21]. Seeds were planted in pots consist of a mixed of soil and organic fertilizer.

Genomic DNA isolation and amplification

Young and fresh leaves of mutant and control plants were used for genomic DNA isolation by slightly modified CTAB method [27]. Amplification was conducted using PCR technique using 3 microsatellite markers from *C. annum* (CA) i.e. CA 19, CA 27, and CA 62 (20 pmol/µL) [1] (Table 1). The PCR reaction consisted of initial denaturation at 94°C for 2 minutes, 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 51°C, 52°C, 53°C for 1 minute (CA 62, CA 27, and CA 19), extension at 72°C for 1 minute and final extension at 72°C for 5 minutes [1].

The amplification result was visualized using electrophoresis with 8% polyacrylamide gel (30% polyacrylamide, 5× pH 8 TBE, sterile aquades, 10% APS and TEMED) at 50 V for 4 hours. The gel staining used EtBr dye (50 mL TBE 1× and 10 µl EtBr). The polyacrylamide gel was visualized using UV transilluminator.

Data analysis

The amplification product was called as allele, the variation of allele was determined based on the variation of the number and size of alleles (bp). Alleles were scored to produce binary data format (1 for allele's presence and 0 for allele's absence)

[15, 18, 20, 22]. The level of polymorphism of the microsatellite was determined by the number and frequency of the alleles [23]. The value of PIC was determined by the formula of PIC = 1 - ∑fi2, (fi) which is the frequency value of alleles [18, 22, 20, 23]. Binary data format was used to determine the genetic similarity for construction of dendrogram, which will be used to assess genetic relationship. The dendrogram was developed using UPGMA method based on the Jaccard coefficient in Paleontological Statistics Software (PAST 2.17) [18, 22].

Results and Discussion

The amplification using microsatellite marker CA 19 produced varies number of alleles from 1 to 8 alleles (Figure 1). Variation of alleles were detected in chili pepper genotype 2 induced by EMS 0.04% (G2K3), chili pepper genotype 7 was induced by EMS 0.02% (G7K2), and chili pepper genotype 11 was induced by EMS 0.01%, 0.02%, and 0.04% (G11K1, G11K2, and G11K3).

The amplification using microsatellite marker CA 27 produced the similar number of alleles among the genotype. There were 2 alleles in one locus (Figure 2). Chili pepper genotype 11 was induced by EMS 0.01% (G11K1) produced smaller size of allele compared with control and other mutants.

The amplification using microsatellite marker CA 62 produced varies number of alleles from 1 to 5 alleles (Figure 3). Variation of alleles were detected in chili pepper genotype 7 induced by EMS 0.04% (G7K3) and chili pepper genotype 11 induced by EMS 0.01%, 0.02% and 0.04% (G11K1, G11K2, and G11K3).

Variation of alleles indicate the genetic variation in the genome level. The greater discrepancy between the number of alleles per locus may indicate the better of genetic variability [24]. Chili peppers G2, G7, and G11 produced variation of alleles in the mode of increasing and decreasing the number of alleles per locus compared with control. The presence of allele variation may be due to point mutation in the repeated region of microsatellite markers CA 19 with motif $(TC)_{12}$, CA 27 with motif $(CA)_{12}(CT)_{17}ATCG(CT)_9$, and CA 62 with motif $(AG)_{22}$. This finding is in accordance with the theory that variation of allele occurred due to the difference in the number of tandem repeat sequence on microsatellite marker region

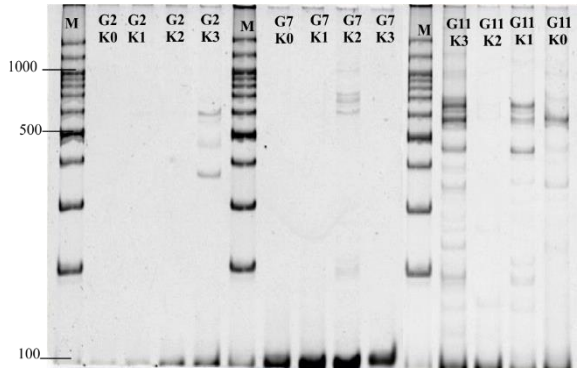

Figure 1. Polymorphism resulted by microsatellite marker CA 19 in chili pepper genotypes 2, 7, and 11 (G2, G7, and G11) were induced by EMS 0%, 0.01%, 0.02%, and 0.04% (K0, K1, K2, and K3). Note: M (Marker): Ladder DNA 100 bp

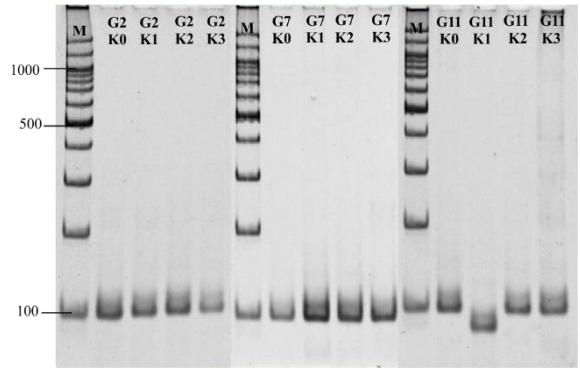

Figure 2. Polymorphism resulted by microsatellite marker CA 27 in chili pepper genotypes 2, 7, and 11 (G2, G7, and G11) were induced by EMS 0%, 0.01%, 0.02%, and 0.04% (K0, K1, K2, and K3). Note: M (Marker): Ladder DNA 100 bp

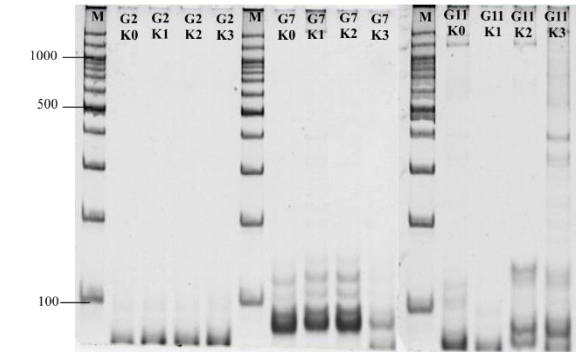

Figure 3. Polymorphism resulted by microsatellite marker CA 62 in chili pepper genotypes 2, 7, and 11 (G2, G7, and G11) were induced by EMS 0%, 0.01%, 0.02%, and 0.04% (K0, K1, K2, and K3). Note: M (Marker): Ladder DNA 100 bp

[23]. Microsatellite region are conserve, there is not easily influenced by environmental conditions [18].

Molecular markers have different qualities based on their ability to show polymorphism. Microsatellite markers CA 19, CA 27, and CA 62 exhibit highly polymorphic qualities indicated by PIC value in the range of greater than 0.50. The average PIC value of microsatellite markers CA 19, CA 27, and CA 62 were 0.82. Polymorphic Information Content (PIC) was determined by the number of alleles and the frequency of those alleles [23]. Microsatellite marker is categorized to have high discriminatory capability when the number of alleles expressed was very high and that allele found in more than one locus. The PIC values are in the range from 0 to 1 (0 for monomorphic, 1 for very high discriminative) [23].

Chili peppers G2, G7, and G11 were distributed into 3 main group (Figure 4). Each genotype basically was grouped into similar cluster. However, some genotypes were clustered into different genotype group, i.e. chili peppers genotype 11 induced by EMS 0.01% and 0.04% (G11K1 and G11K3), and chili pepper genotype 7 induced by EMS 0.04% (G7K3) were clustered into the G2 cluster. This indicates big genetic differences experienced by G7 and G11.

Genetic similarity indicates relationship closeness among the genotype. High genetic similarity may increase the possibility of relationship closeness the genotype [18]. In contrast, small genetic similarity may decrease the possibility of relationship closeness the genotype [18]. Chili pepper genotype 11 showed sensitive response to EMS treatments. Different concentrations of EMS give different amount of genetic changes. The concentration of EMS 0.04% gives the greater effect to the DNA genome. These based on the fact that compared to the other mutants, chili peppers G2, G7, and G11 were induced by EMS 0.04% (G2K3, G7K3, and G11K3) had lower similarity coefficient compared to the control plants, which make them separated from the control plants in the dendrogram (Figure 4).

EMS is an alkylating agent commonly employed in plant genetic [25]. Alkylating agent is the chemical compound producing variation, miscoding deviant, or lethal, and destroy non coding region [25]. DNA repairing mechanism has required in all living plant tissue to reduce the toxic effects of the accumulation of DNA damage. Mutagenic and repairing process will be able to create genetic variation or genetic diversity [25].

Table 2. Description and PIC value for the microsatellite markers examined in 3 genotypes of local chili pepper

Marker	Size of allele	Number of alleles	Allele frequency	PIC
CA 19	100-800	8	0.18	0.97
CA 27	80-110	2	0.66	0.56
CA 62	70-380	5	0.29	0.92
Mean		5	0.38	0.82

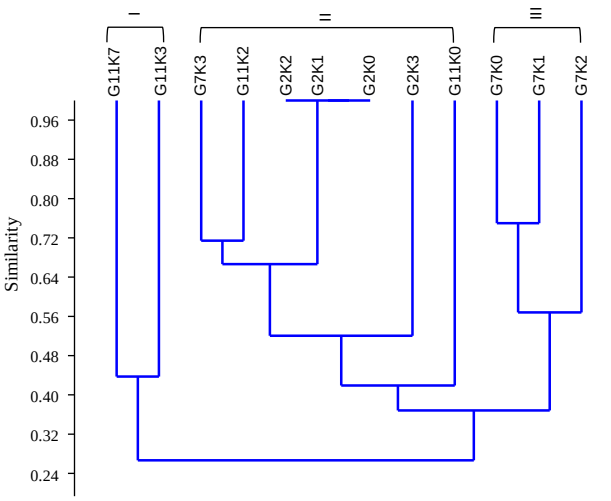

Figure 4. Dendrogram showed the clustering of local chili pepper. Note: Chili pepper genotypes 2, 7, and 11 (G2, G7, and G11) were induced by EMS 0%, 0.01%, 0.02%, and 0.04% (K0, K1, K2, and K3). The major clustered are marked on the right side of the dendrogram (I, II, and III)

Conclusion

Genetic variations based on microsatellite markers CA 19, CA 27, and CA 62 were demonstrated by the presence of different alleles variation on chili peppers G2, G7, and G11. The different genotype of chili pepper showed different response against of EMS treatments. The EMS concentration of 0.04% produced the most notable genetic variations on 3 genotypes of local chili pepper.

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References

1. Carvalho SLC, Ragassi CF, Oliveira LB et al. (2015) Transferability of microsatellite markers of *Capsicum annuum* L. to *C. frutestescens* L. and *C. chinense* Jacq. Genetics and Molecular Research 14 (3): 7937 – 7946. doi: 10.4238/2015.july.17.1.
2. Musfiroh I, Mutakin T, Angelina, Muchtaridi M (2013) Capsaicin level of various capsicum fruits. International Journal of Pharmacy and Pharmaceutical Sciences 5 (1): 248 – 251.
3. Keyhaninejad N, Curry J, Romero J, O'Connell MA (2014) Fruit specific variability in capsaicinoid accumulation and transcription of structural and regulatory genes in capsicum fruit. Plant Science 215 – 216: 59 – 68. doi: 10.1016/j.plantsci.2013.10.013.
4. Djarwaningsih T (2005) *Capsicum* spp. (Chilli): origin, distribution, and its economical value. Biodiversitas 6 (4): 292 – 296. doi: 10.13057/biodiv/d060417.
5. Wahyuni Y, Ballester AR, Sudarnowati E et al. (2011) Metabolite biodiversity in Pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and amplifications for breeding. Phytochemistry 72 (11 – 12): 1358 – 1370. doi: 10.1016/j.phytochem.2011.03.016.
6. Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (Capsicum annuum L.). Journal Agriculture Food Chemistry 53 (5): 1750 – 1756. doi: 10.1021/jf035331k.
7. Mori A, Lehman S, O'Kelly J et al. (2006) Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Research 66 (6): 3222 – 3229. doi: 10.1158/0008-5472.can-05-0087.
8. Aza-Gonzales C, Nunez-Palenius HG, Ochoa-Alejo N (2011) Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Reports 30 (5): 695 – 706. doi: 10.1007/s00299-010-0968-8.
9. Dima C, Coman G, Cotarlet M, Alexe P, Dim S (2013) Antioxidant and antibacterial properties of capsaicin microemulsions. Food Technology 37 (1): 39 – 49.
10. Gudeva LK, Mitrev S, Maksimova V, Spasov D (2013) Content of capsaicin extracted from hot pepper (*Capsicum annuum* spp. microcarpum L.) and its use as an ecopesticide. Hemijska Industrija 67 (4): 671-675. doi: 10.2298/hemind120921110k.
11. Habibi M, Manggabarani AM, Sulasmi ES, Listyorini D (2013) AT3 gene (Acyltransferase) isolation from *Capsicum frutescens* L. cv. Cakra Hijau. Journal of Tropical Life Science 3 (2): 83-86. doi: 10.11594/jtls.03.02.02.
12. Arisha MH, Shah AYM, Gong Z et al. (2015) Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (*Capsicum annuum* L.). Frontiers in Plant Science 6: 399. doi: 10.3389/fpls.2015.00399.
13. Laskar RA, Chaudhary C, Khan S, Chandra A (2016) Induction of mutagenized tomato populations for investigation on agronomic traits and mutant phenotyping. Journal of The Saudi Society of Agricultural Sciences 17 (1): 51 – 60. doi: 10.1016/j.jssas.2016.01.002.
14. Devi AS, Mullainathan L (2011) Physical and chemical mutagenesis for improvement of chili (*Capsicum annuum* L.). World Applied Sciences Journal 15 (1): 108 – 113.
15. Dhakshanamoorthy D, Selvaraj R, Chidambaram A (2014) Utility RAPD marker for genetic diversity analysis in gamma rays and Ethyl Methane Sulphonate (EMS) treated Jatropha curcas plants. Comptes Rendus Biologies 388 (2): 75 – 82. doi: 10.1016/j.crvi.2014.12.002.
16. Sega GA (1984) A review of the genetic effects of Ethyl Methane Sulfonate. Mutation Research 134: 113 – 142.
17. Mullainathan TAL (2015) Effect of gamma rays and EMS on phytochemical constituents in Chili (*Capsicum annuum* L.var-K1 in M2 generation). International Journal of Pharmacy and Pharmaceutical Research 4 (3): 92 – 101.
18. Efendi R, Sunarti S, Musa Y et al. (2015) Selection of homozygosity and genetic diversity of Maize Inbred using Simple Sequence Repeats (SSRs) marker. International Journal of Current Research in Biosciences and Plant Biology 2 (3): 19 – 28.
19. Zhang XM, Zhang ZH, Gu XZ et al. (2016) Genetic diversity of pepper (*Capsicum* spp.) germpalsm resources in China reflects selection for cultivar types and spatial distribution. Journal of Integrative Agriculture 15 (9): 1991 – 2001. doi: 10.1016/S2095-3119(16)61364-3.
20. Kwon YS, Lee JM, Yi GB et al. (2005) Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. Molecules and Cells 3 (19): 428 – 435.
21. Talebi AB, Talebi AB, Shahrokhifar B (2012) Ethyl Methane Sulphonate (EMS) induced mutagenesis in Malaysian rice (cv.MR219) for lethal dose determination. American Journal of Plant Sciences 3 (12): 1661 – 1665. doi: 10.4236/ajps.2012.312202.
22. Rocha EA, Paiva LV, Carvalho HH, Guimaraes CT (2010) Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. Crop Breeding and Applied Biotechnology 10 (3): 204 – 210. doi: 10.1590/S1984-70332010000300004.
23. Smith JSC, Chin ECL, Shu H et al. (1997) An evaluation of the utility of SSR loci as molecular markers in Maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theoretical and Applied Genetics 95 (1 – 2): 163-173. doi: 10.1007/s001220050544.
24. Carvalho N, Canela FM, Leitie PHS et al. (2017) Analysis of genetic variability of commercial melon cultivars using SSR molecular markers. Genetic and Molecular Research 16 (3): 1 – 8. doi: 10.4238/gmr16039739.

25. Britt AB (1996) DNA damage and repair in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47: 75-100. doi: 10.1146/annurev.arplant.47.1.75.
26. Shahriar MH, Robin AHK, Begum SN, Haque A (2014) Diversity analysis of some selected rice genotypes through SSR based molecular markers. Journal Bangladesh Agricultural University 12 (2): 307 – 311. doi: 10.3329/jbau.v12i2.28689.
27. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19 (1): 11 – 15.