JOURNAL OF TROPICAL LIFE SCIENCE
2019, Vol. 9, No. 3, 223 – 228
http://dx.doi.org/10.11594/jtls.09.03.02

Research Article

Genetic Variation Analysis of EMS-Induced Chili Pepper (*Capsicum frutescens* L.) Mutants Using SSR Markers

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Article history:
Submission April 2018
Revised September 2018
Accepted September 2018

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ABSTRACT

Mutation induction with chemical mutagen ethyl methane sulfonate (EMS) capable of producing genetic variation in plants. Various concentration of EMS (0%; 0.01%; 0.02%; 0.04%) were applied to Indonesian local chili pepper Genotypes 2, 7, and 11. Genetic variation among three genotype of chili pepper was assessed using three SSR primers namely CA26, CA52 and CA96. A total of 18 alleles were identified for the three SSR loci with an average Polymorphic Information Content (PIC) value of 0.829. Three genotype of chili pepper had different sensitivity to EMS mutation induction. Genotype 11 plants showed higher sensitivity to EMS treatment than Genotypes 2 and 7. Dendrogram constructed based on Jaccard's similarity coefficient was divided chili pepper mutants and control plants into three main clusters. Similar genotype either control or mutants plant, especially Genotypes 2 and 7 were grouped into similar cluster. However, large genome changes in Genotype 11 caused mutant plants G11K1, G11K2, G11K3 had low genetic similarity to their control plant, so the mutants were separated in a different group from the control plant. This study revealed that EMS mutation induction capable of increasing genetic variation in chili pepper plants based on SSR molecular marker analysis.

Keywords: Genetic variation, EMS, SSR, chili pepper, mutant

Introduction

Chili pepper (*Capsicum frutescens* L.) is a member of Solanaceae family that has commercial value because of the combination of flavor, color, and taste. Chili fruit is source of vitamin A, C, E, carbohydrate, protein, fat, minerals, carotenoid, oleoresin, phenol and capsaicinoid [1, 2]. The presence of capsaicinoid causes the spicy sensation of chili pepper fruit [3]. Capsaicinoid is widely used in the food sector as a spice, in cosmetic industry as additives in a series of hair loss prevention shampoo, in the pharmaceutical field as analgesic, anti-cancer, anti-inflammatory and anti-obesity [4, 5]. These benefits cause chili pepper to become one of the important horticultural crops in Indonesia.

Chili breeding constraints in Indonesia are abiotic stress and biotic stress that can reduce chili productivity. One effort to improve the quality and quantity of crop productivity is by increasing genetic variation, followed by selection to assemble new cultivars [6]. One way to increase genetic variation can be done by mutation induction using chemical mutagen ethyl methanesulfonate (EMS) [7]. EMS is widely used in plant breeding because it has high mutation rates, low lethality and easy to apply [8]. In this research, mutation induction using EMS to three genotypes of Indonesian local chili pepper plant Genotypes 2, 7 and 11 were conducted. EMS causes random point mutation in the plant genome [7,9], so analysis at the molecular level needs to be done.

A simple sequence repeat (SSR) molecular marker is very suitable for analysis at the genome level [10]. Simple sequence repeat has several characters i.e. co-dominant, reproducible, easily

How to cite:
Dwinanti EF, Mastuti R, Arumningtyas EL (2019) Genetic Variation Analysis of EMS-Induced Chili Pepper (*Capsicum frutescens* L.) Mutants Using SSR Markers. Journal of Tropical Life Science 9 (3): 223 – 228. doi: 10.11594/jtls.09.03.02

distinguishable alleles, high degree of polymorphism and easily detected through PCR technique [11, 12].

Microsatellite specific primer development requires relative high cost and time-consuming process to obtain DNA sequence information [10]. However, several studies have shown the similarity of DNA sequences located in the repetitive regions between different species, indicating that microsatellite primers can be transferred between species in the same genus [13, 14]. This is beneficial because can reduce the cost and longtime of the research [15]. Transferability of microsatellite primer from one species to another has been successfully carried out on chili plants, which was transferability of microsatellite primer from C. annum to *C. frutescens* with 19 polymorphic primers [14]. In the current research, three microsatellite primers were chosen from that research based on PIC and high heterozygosity value namely CA26, CA52, and CA96. The main objective of this research was to evaluate genetic variation of EMS-induced chili pepper mutants using SSR molecular marker.

Material and Methods

Plant material

Chili pepper (*C. frutescens* L.) used in the current research were three genotypes of local Indonesian chili pepper, namely Genotype 2, 7, and 11. Genotype 2 and Genotype 11 from Malang East Java, while Genotype 7 from Lombok West Nusa Tenggara.

Seeds of chili pepper Genotype 2, 7 and 11 were presoaked in aquadest for 8 hours, then treated with EMS concentration of 0% (control); 0.01%; 0.02%; 0.04% for 6 hours. EMS solution was discarded, then chili pepper seeds were immersed in 1% sodium thiosulfate for 5 minutes. Seeds were then thoroughly washed under running water for 15 min, then dried at room temperature. The treated seeds were sown into polyethylene bags containing mixture of soil, compost, and husk. Maintenance of chili pepper plant was performed with regular daily watering and weekly fertilization.

SSR analysis

Young leaf of EMS-induced chili pepper mutants and control plants were used for DNA extraction using CTAB method [16] with minor modifications. Genomic DNA was amplified by PCR using three primer pairs (Table 1). DNA amplification was carried out in 20 µL volume containing 10 µL 2× PCR Master mix Solution (i-TaqTM), 1 µl primer forward and reverse (10 pmol), 1 µl template DNA and 7 µL de-ionized water. Amplification conditions were one cycle pre-denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C (CA26 and CA96), 54°C (CA52) for 1 minute, extension at 72°C for 1 minute, and one cycle final extension at 72°C for 7 minutes.

PCR products were separated on 8% polyacrylamide gel (30% polyacrylamide, TBE 5× pH 8, 10% APS, TEMED, distilled water). Electrophoresis was performed in 1× TBE buffer at 50 V for 3 hours. The gel was stained in mixture of 1x TBE and 10 µl ethidium bromide for 10 minutes, then rinsed with distilled water for 15 minutes. The gel was photographed under UV trans-illuminator attached to gel documentation system.

Data analysis

Fragments DNA were assigned as an allele of SSR loci. Alleles were scored based on binary format ("1" as the allele presence, "0" as the allele absence) [17, 18]. Binary data were used to calculated Polymorphic Information Content (PIC) value and constructed dendrogram. PIC value of each SSR primer was calculated using a formula: $PIC = 1 - \sum f_i^2$, where f_i is the i^{th} allele frequency [17, 19]. The dendrogram was constructed based on Jaccard's similarity coefficient to determine the genetic relationship among genotypes using PAST software version 2.17b. method based on the Jaccard coefficient in Paleontological Statistics Software (PAST 2.17) [18, 22].

Results and Discussion

Genetic variation of EMS-induced chili pepper mutants

Genetic variations of chili pepper genotype 2, 7 and 11 were detected with all three SSR primers based on variation of allele number and allele size. A total of 18 alleles has been identified at CA26, CA52, CA96 SSR loci among the three genotypes of EMS-induced chili pepper mutants and control plants. The number of alleles of each locus ranged from 1 to 9 alleles with an average of 6 alleles per locus (Figure 1).

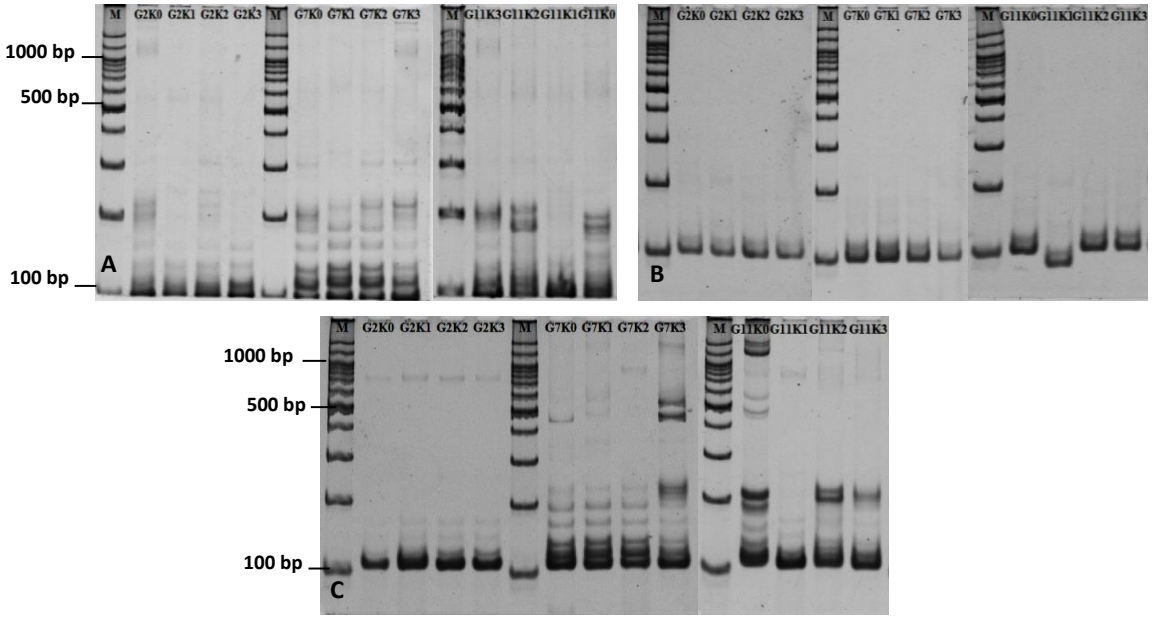

Figure 1. SSR profile of EMS-induced chili pepper mutants Genotypes 2, 7, 11 and control plants on 8% polyacrylamide gel. A. SSR CA26; B. SSR CA52; C. SSR CA96. M = DNA ladder 100 bp, G2 = Genotype 2, G7 = Genotype 7, G11 = Genotype 11, K0 = EMS 0% (control), K1 = EMS treatment 0.01%, K2 = EMS treatment 0.02%, K3 = treatment EMS 0.04%.

Table 1. Primer sequence, number of alleles and Polymorphism Information Content (PIC) value of SSR primers

Primer	Motif	Primer sequence (5'-3')	Number of alleles	PIC value
CA26	$(AG)_{23}$	F: CGCATATAGGCAGATCAAAT R: TGACTCAAATGCTCTCTGAA	7	0.792
CA52	$(GT)_{14}(AG)_{14}TAGC(GA)_{10}$	F: TAGCAGAGGACCAGTTAGCA R: ATGTTCTGAGTCCACGATGC	2	0.803
CA96	$(AG)_{23}$	F: CGCATATAGGCAGATCAAAT R: AATCTCTGTGGCTGACTCAA	9	0.893
Mean			6	0.829

Variation of the allele was detected in G2K1, G2K2, and G2K3 based on amplification results using SSR CA96. Genetic variation characterized by the presence of two new alleles (150 bp; 170 bp) on G2K1, three new alleles (130 bp; 150 bp;170 bp) on G2K2 and G2K3 compared to control plants that had only two alleles per locus (Figure 1C). In Genotype 7, the SSR CA96 amplification results were indicated genetic variation, especially in G7K3 (0.04% EMS) (Figure 1C).

Three genotypes of chili pepper plant showed different sensitivity to mutation induction with EMS. Genotype 11 plants showed a higher sensitivity to EMS treatment than Genotypes 2 and 7. Genetic variation was indicated by the presence of new allele 90 bp in G11K1 plant. Whereas in Genotype 2 and 7, not allele variation was detected based on amplification results with SSR CA52 (Figure 1B). In addition, the genomic alteration was indicated by allele loss of G11K2 and G11K3 plant based on amplification result with SSR CA26 and CA96. The more enormous genomic changes in Genotype 11 were shown by amplification results with SSR primers CA26 and CA96 characterized by loss of five alleles in G11K1 (0.01% EMS) (Figure 1A and 1C).

In this research, mutation induction with EMS caused genomic change that was indicated by the presence of new alleles or loss of alleles in certain sizes compared to control plant. The magnitude of genomic change due to EMS treatment varies between plant genotypes. The disappearance of allele can be caused by DNA damage, modification of nucleotide, DNA fragment breakage and chro-

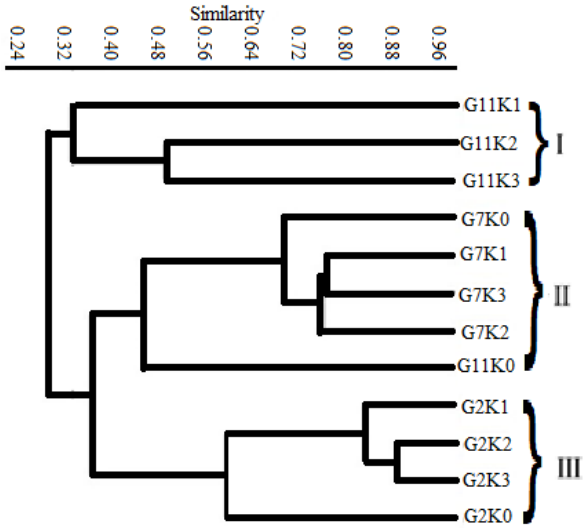

Figure 2. Dendrogram based on Jaccard's similarity coefficient was described genetic relationship of EMS-induced chili pepper (*C. frutescens* L.) mutants and control plants. G2 = Genotype 2, G7 = Genotype 7, G11 = Genotype 11, K0 = EMS 0% (control), K1 = EMS treatment 0.01%, K2 = EMS treatment 0.02%, K3 = treatment EMS 0.04%.

mosome rearrangement caused by EMS treatment. The appearance of new alleles can be caused by several nucleotide changes in the primer binding region of mutated plant [20].

The existence of alleles in the three genotypes of chili pepper on the three SSR loci was used to determine Polymorphic Information Content (PIC) value. PIC values of the three SSR loci ranged from 0.79 to 0.89 with an average of 0.829 (Table 1). PIC value provides an estimate to determine the strength of a molecular marker that is obtained not only the number of alleles at a locus but the relative allele frequency [21]. PIC value categorized into three types i.e. highly informative (PIC > 0.5), reasonably informative (0.25 > PIC > 0.5), and slightly informative (PIC < 0.25) [22]. The three SSR primer used in the current research were categorized in the highly informative and could be considered as a powerful marker.

Genetic relationship of EMS-induced chili pepper mutants and control plants

Genetic relationship between plant genotypes was presented by dendrogram based on Jaccards similarity coefficient. The dendrogram was divided into three main clusters of EMS-induced chili pepper mutants and control plants with similarity coefficients ranged from 0.34 to 0.90 (Figure 2).

Cluster I consisted of Genotype 11 mutant namely G11K1, G11K2, and G11K3 plants. Cluster II was divided into 2 sub-cluster: sub-cluster 1 namely G7K0, G7K1, G7K3, G7K2, and sub-cluster 2 only contained G11K0 plants. Cluster III consisted of G2K1, G2K2, G2K3, and G2K0 plants. The member of each cluster generally consisted of similar genotype. However, in every cluster, control plant was always separated from the mutants plant. This showed that EMS treatment caused notable genomic change in the three genotypes.

Greater deviation was showed by Genotype 11, G11K0 plant (control) was located in a different cluster from mutant plants. The G11K0 plant located in the same group with Genotype 7. The higher genetic similarity value between G11K0 and Genotype 7 plants showed high genotypic similarity. Low genetic similarity value in 11 mutant genotypes compared to control plants (G11K0) showed that EMS treatment caused a large genomic change in Genotype 11.

Genomic changes in plant induced mutation with EMS can be caused by mutagenesis mechanism which a G/C to A/T nucleotides change in the primer binding regions of SSR marker [23, 24, 25]. In addition, insertion or deletion of nucleotide in the DNA sequences of mutant plants inducing the lengthening or shortening repeat region of microsatellite marker [24, 26, 27]. Genetic relationship among three genotypes of chili pepper is helpful for designing future breeding program [28].

Conclusion

Genetic variation of EMS-induced chili pepper mutants based on SSR analysis was showed by variation of number and size alleles. EMS mutation induction caused genomic change which was indicated by the separation of control to the mutant plant. Three genotypes of chili pepper indicated different sensitivity to EMS treatment, genotype 11 was more sensitive compared to other genotypes. Genotype 11 mutant were located in a different cluster with the control plant. This suggests that mutation induction with EMS caused a large genomic change in mutant Genotype 11.

Acknowledgment

The authors are thankful to LPDP Thesis Scholarship, Ministry of Finance, Republic of Indonesia for providing funds of this research.

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