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Molecular characterization of induced mutation of jewawut (*Setaria italica* ssp. *italica*) from Buru Island, Indonesia using SRAP

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Abstract. *Yulita KS, Ridwan. 2018. Molecular characterization of induced mutation of jewawut (Setaria italica ssp. italica) from Buru Island, Indonesia using SRAP. Biodiversitas 19: 1160-1168.* Jewawut (*Setaria italica* ssp. *italica*) or foxtail millet is minor crops domesticated and consumed only in few parts of Sulawesi, Maluku and East Nusa Tenggara, while in other region such as Java, it is known as food for bird despite the fact that jewawut is rich in fiber. The utilization of jewawut as an alternative crop was limited due to their small size of seeds as well low yield. An effort to improve the potency of jewawut as an alternative food is through genetic improvement by induced mutation. This present study was aimed to determinate mutant of jewawut from Buru Island irradiated by gamma rays as potential genotype to produce high grain yield. Two variants of jewawut were recorded from Buru Island, red and yellow variant each referring to the colour of their seeds. Samples were consisted of 26 red and 24 of yellow variant. Mutant was detected by molecular characterization using SRAP marker. UPGMA cluster analysis was also performed to ensure the position of mutant within the UPGMA dendrogram against the control samples. Mutant with the most distinct genyotypes for yellow variant are accession O1O2#10 treated in 100 Gy and O1E1#10 treated in 200 Gy gamma radiation, whereas in the red variant are accessions O2C4#13 treated in 50 Gy and O2E4#6 treated in 200 Gy gamma radiation. All the mutants have random agronomic performances relative to controls. Mutants with potential high grain yield are O2B2#14 treated in 25 Gy for the red variant, and this should be used as a candidate to develop superior genotype.

Keywords: Foxtail millet, gamma rays, jewawut, mutation, SRAP

INTRODUCTION

The term of millet is usually referring to a group of cereal crops that produce small seeds that include several annual grasses such as foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*), and fonio (*Digitaria* sp.) (Dwivedi et al. 2012). *Setaria italica* contains two subspecies *viridis* and *italica*. *Setaria italica* ssp. *viridis* or green millet was assumed to be the wild ancestor of ssp. *italia* for having nonwaxy endosperm (Nakayama et al. 1998; Kawase et al. 2005).

Jewawut (*Setaria italica* ssp *italica*) or foxtail millet is an important cereal in the region of southeastern Europa, South, Central and far East Asia. This species has been widely cultivated in Asian and Europe for thousands of years (Kawase et al. 2005); probably have been taken placed since about 8700 years BC (Lu et al. 2009). Foxtail millet is an annual grass with slender, erect, leafy stems with a height of 170 cm (our field data). The inflorescence consist of constricted panicle that often nods at the top and looks like a spike due to its short branches. The spikelets (one pistilled flower per spikelet) are packed, and with many firm bristles, the panicle looks like a foxtail, and hence the name refers (Lata et al. 2013). It is relatively short lived plants of only up to 15 weeks and several hundreds of seeds can be produced per inflorescence (Reddy et al. 2006; Doust et al. 2009). The seeds are small (~2 mm in diameter), sheathed in thin, delicate hull that can be separated without difficulty during threshing. The seeds usually germinate quickly and can be cultivated easily.

Jewawut was thought to be the earliest cereal cultivated in dry land of Southeast Asia (Lu et al. 2009). Records from herbarium collections in Herbarium Bogoriense Indonesia dated as early as 1900s suggested that jewawut distributed in Sulawesi, Java, southern Sumatra, Kalimantan, Lesser Sunda Islands, Maluku and Papua. In Indonesia, the main use of jewawut is for bird food, despite the fact that jewawut have high fiber content and low protein. Jewawut was planted in dry land habitat and are also known to have high tolerance to marginal environmental conditions, as well as have a good adaptability to low soil fertility (Li and Brutnell 2011; Jia et al 2013) and has the potential to produce C4 biofuel (Zhang et al. 2012).

Therefore it is important to develop jewawut as potential crops to be utilized as an alternative choice for staple food in Indonesia through genetic improvement by inducing mutation using radiation. Mutagenesis using radiation has developed rapidly since the discovery of high mutant frequency in *Drosophila melanogaster* by X-rays (Muller 1927). A large number of energy rays are frequently applied to produce mutants, such as X-, β-, and γ-rays, neutrons, and protons. Gamma rays is considered the most efficient tools to induce mutation in plants because it is easy operation, short cycle, and high mutation quantity (Naito et al. 2005; Sato et al. 2006; Eroglu et al. 2007; Selvi et al. 2007). Random mutations with gamma-ray irradiation have been widely used to obtain mutants with various objectives (Yulita et al. 2014; Martanti et al. 2016). This present study was aimed to assess genetic and phenotypic changes of two variants of jewawut from Buru islands after being treated in various dosages of gamma irradiation based on observation on some agronomic traits and SRAP profiles. Sequence-related amplified polymorphism (SRAP) is a PCR-based molecular marker targeting coding sequences in the genome and results in a moderate number of co-dominant markers (Li and Quiros 2001; Keyfi and Beiki, 2012; Zeng et al. 2012), thus suitable for mutant detections as well as assessment on genetic diversity (Song et al. 2010; Alghamdi et al. 2012). The main advantage in using SRAP marker is that it can analyze many loci in a single reaction, thus very practical but scientifically reliable markers. We targeted to obtain mutants that have agronomic traits related to high grain yield that can be utilized further to develop superior genotype.

MATERIALS AND METHODS

Samples

The jewawut accession used in this study collected from Buru Island, Maluku (Figure 1) in 2013 consisting of two variants, yellow (26 samples) and red (24 samples) (Table 1). The main difference of these variants was mostly on the colour of their grains whose the colour name is referring to . The seeds were sent to National Nuclear Province, IndonesiaEnergy Agency of Indonesia (Badan Tenaga Atom Nasional; Batan) at Serpong, Banten, Indonesia for irradiation treatment in March 2015. These seeds accessions were treated at seven dosages of 25, 50, 100, 200, 400 and 600 Gray, and control (0 Gray).

Agronomic experiment

The induced mutation seeds were germinated in a tray in an experimental house in March 2015 until they were forming sprouts. The seedlings were then grown in experimental field in Cibinong Science Centre, Bogor, West Java, Indonesia using Randomized Block Design with 2 factors (the variants) and irradiation dosages (0, 25, 50, 100, 200, 400,dan 600 Gy), so that there were 14 combinations of treatments. These combinations were replicated 4 times (4 blocks). The planting was carried out a month later (in April 2016). Each planting hole was filled with 0.25 kg of green fertilizer. The plants were fertilized twice with NPK with a ratio of 15: 15: 15, the first fertilizer was given at the first planting and the second was 21 days after planting with a dosage of 400 kg/Ha (1,6 gram/plant). Sixty percent of these were given at the first fertilizing and the remaining 40% was at the second fertilizing. The harvesting was carried out on 58 days after planting.

Measurement of agronomic traits was recorded on plant height, leaf size, leaf colour (colour standard following IRRI), number of tillers and weight of panicles. Regression analyses were performed to assess the correlation between each agronomic trait and the irradiation dosages.

Molecular characterization

Total genomic DNA was isolated from collected DNA material using Genomic DNA Mini Kit (Plant) from GeneAid. DNA genotyping of foxtail millet was generated using SRAP set of primers (Li and Quiros 2001). Five combinations of SRAP primers were used to amplify genomic DNA (Table 2). A total volume of 15 µL PCR reactions consisted of 1x PCR master mix (Promega), ~10 ng DNA template, 2 µM of each primer. The optimum condition for PCR amplification was as followed: 94^{o}C for 5 min, 30 amplification cycles containing 94^{o}C for 1 min, 50^{o}C for 45 second and 72^{o}C for 2 min. The reaction was terminated by extension at 72^{o}C for 5 min. Amplicons were

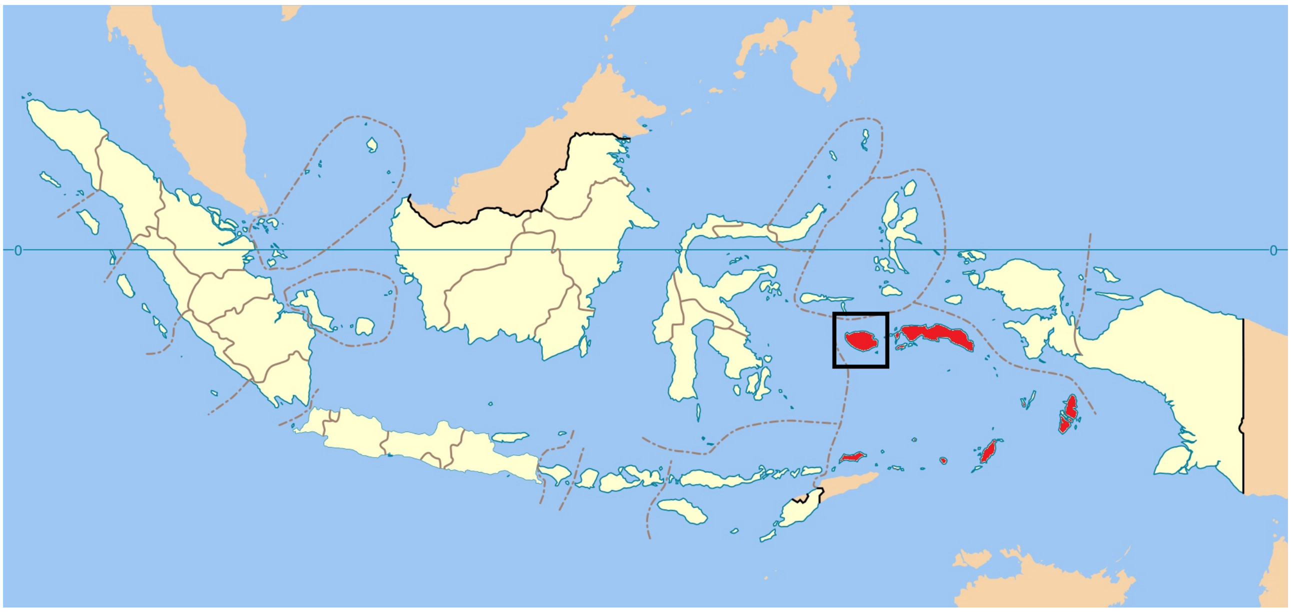

Figure 1. Location of Buru Island, Maluku in the Indonesian Archipelago

visualized using 2% agarose gel run electroporecally then stained in GelRed (Biotium) before photographed using gel documentation system (Atto Bioinstrument). Observations of electrophoretic gel photos showed cleared observable bands to score. Present band was scored 1 and absent band was scored as 0. The data matrix was compiled in Excel to perform cluster analysis using UPGMA (Unweighted Pair Group Method with Arithmetic Average, Sneath and Sokal 1973). Cluster analysis was calculated using Nei and Li coefficient of distance. UPGMA was performed using MVSP (Multi Variate Statistical Package, Kovach 2007).

RESULTS AND DISCUSSION

Mutations with gamma ray irradiation treatment with varying dosages starting at 25 to 600 Gy were performed on yellow and red variant. There were changes in genetic properties for the genotypes that were exposed to irradiation even at the lowest dose (in this study was 25 Gy). However, to identify the most likely mutants, the genotypes were expected to be the most distinct from the control, thus when the grouping analysis is performed, the mutants should be separated distantly to the control samples, regardless their position at the dendrogram. Mutant with the most distinct genotype observed in different dosage in both variants, is. 50 and 200 Gy in red and 100 and 200 Gy in yellow. This may indicate that the most efficient dosage for mutation was ranged between 50-100 Gy. Mutants exposed in 200 Gy even though may have distinct genotype from the controls but the agronomic performances were decreased (Figure 3 and 5).

Cluster analysis that was performed on yellow variant showed that all the accessions have 80% similarity, thus only 20% differences among the accessions. This indicated that gamma irradiation resulted in to 20% variations in the genomic coding regions genetic implied by SRAP profiles. Five accessions of control were located at the terminal dendrogam, while the furthest accessions from the controls are three samples O1A2#19 (Control), O1D2#10 (14) treated in 100 Gy and O1E1#10 (17) treated in 200 Gy located at the basal dendrogram (Figure 2). The detection of accession O1A2#19 as a mutant allegedly due to technical faults of collection / planting, given the small size of the seed so that technical errors are easily to occur. We therefore excluded this accession from this discussion.

The mutant O1D2#10 (14) have a comparatively lower plant size i.e. 142 cm compared to average of 148 cm, and was the smallest plant size among the accessions irradiated in 100 Gy dosages (Table 3). The mutant has an average leaf sheaths, number of tillers, and panicle size. However, the mutants have smaller leaf size, panicle size and less panicle weigh compared to the average sizes. The second mutant (O1E1#10) have plant height just above the average size, i.e., 153 cm compared to average size of 148 cm and larger leaf size 108 cm compared to average size of 88 cm). Other agronomic traits were in average size but have a slightly heavier panicle size of 15 g (average size was 11 g).

The highest (168 cm) plant was observed on O1D4#8 (100 Gy) and the shortest (110 cm) was O1G2#1 (600 Gy). The highest number of leaf sheaths (14) recorded on O1E1#10 (200 Gy) and the fewest (5 sheets) was O1B4#19 (25 Gy), one of the controls (O1A3) had also 6 leaf sheets. The largest leaf (124 cm2) was occurred in O1B2#3 (25 Gy) and the smallest leaf (65.38 cm2) was accounted for O1G3#20 (600 Gy) and O1F2#4 (400 Gy). There was no different on leaf colors on all accessions. The number of tillers were mostly 2 except for O1B3#6, O1B4#19 (both 25 Gy), O1C2#9 (50 Gy), O1D3#17 (100 Gy) and O1E3#11 (200 Gy). The longest panicle (37 cm) was observed on O1F4#1 (400 Gy), while the shortest (20 cm) was found in O1G3#20 (600 Gy). The heaviest panicle (21.50 g) was recorded on O1A3 control and_O1C4#19 50 Gy (19.20 g) while the lightest 4 g was found in O1E2#3 200 Gy.

Table 1. List of Jewawut samples of yellow and red variant irradiated in gamma rays

Yellow variant			Red variant		
No	Accession code	Dosage	No	Accession code	Dosage
1	O1A1#3	0	1	O2A1#2	0
2	O1A2#19	0	2	O2A2#19	0
3	O1A3	0	3	O2A3#3	0
4	O1A4#3	0	4	O2A4#6	0
5	O1B1#4	25	5	O2B1#4	25
6	O1B2#3	25	6	O2B2#20	25
7	O1B3#6	25	7	O2B3#14	25
8	O1B4#19	25	8	O2B4#12	25
9	O1C1#9	50	9	O2C1#13	50
10	O1C2#9	50	10	O2C2#20	50
11	O1C3#18	50	11	O2C3#1	50
12	O1C4#19	50	12	O2C4#13	50
13	O1D1#16	100	13	O2D1#2	100
14	O1D2#10	100	14	O2D2#19	100
15	O1D3#17	100	15	O2D3#8	100
16	O1D4#8	100	16	O2D4#3	100
17	O1E1#10	200	17	O2E1#9	200
18	O1E2#3	200	18	O2E2#9	200
19	O1E3#11	200	19	O2E3#12	200
20	O1E4#14	200	20	O2E4#16	200
21	O1F1#3	400	21	O2F2#4	400
22	O1F2#4	400	22	O2F2#9	400
23	O1F3#1	400	23	O2G1#3	600
24	O1F4#1	400	24	O2G4#1	600
25	O1G2#1	600			
26	O1G3#20	600			

Table 2. List of SRAP primers used in this study

Primer name	DNA sequence
me 1F	TGAGTCCAAACCCGATA
me 2F	TGAGTCCAAACCGGAGC
me 3F	TGAGTCCAAACCGGAAT
me 4F	TGAGTCCAAACCGGACC
me 5F	TGAGTCCAAACCGCAAC
em 1R	GACTGCGTACGAATTAAT
em 2R	GACTGCGTACGAATTTGC
em 3R	GACTGCGTACGAATTGAC
em 4R	GACTGCGTACGAATTTGA
em 5R	GACTGCGCACGAATTGCA

Among the agronomic traits, the weight of panicle seems to be the most important traits because it is related to grain yield. Heavier panicles present in good conditions with no symptom of pathogenic attack means higher yield obtained. Accession O1A3#19 (Control) have the heaviest panicle of 21.3 g followed by mutant O1C4#19 (19.2 g) and O1B2#3 (18.2 g). The results of regression analysis between irradiation dosage and weight of panicle suggested that weight of panicles tend to decrease when the irradiation dosage was increased in the yellow variant, as also recorded for other agronomic traits such as plant height, number of leaves, and length of panicles (Figure 3).

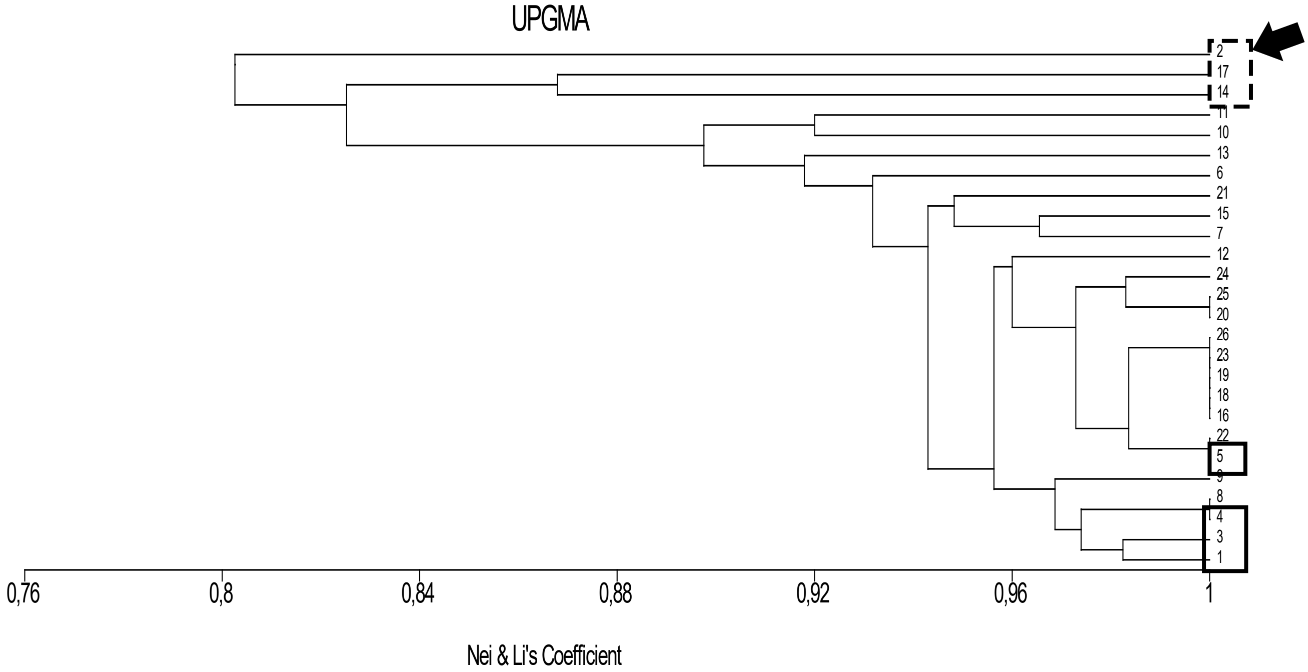

Figure 2. UPGMA dendrogram of 26 accessions of jewawut yellow variant based on Nei&Li’s coefficient of similarity

Table 3. List of agronomic traits of yellow variant of Jewawut from Buru Island irradiated by gamma rays in various dosages

Accession code	Radiation dosage (Gray, Gy)	Plant height (cm)	Number of leaves (sheets)	Leaf size (cm$^{2)}$	Leaf colour	Number of tillers	Length of panicle (cm)	Weight of panicle (g)
O1A1#3	0	130	8	76.15	2	2	28	6.30
O1A2#19	0	156	10	80.46	2	2	28	9.80
O1A3	0	150	6	108.75	2	2	27	21.30
O1A4#3	0	152	8	86.92	2	2	36	17
O1B1#4	25	140	10	89.07	2	2	25	7.30
O1B2#3	25	166	10	126.41	2	2	33	18.20
O1B3#6	25	163	7	65.38	2	1	32	13
O1B4#19	25	155	6	91.23	2	1	28	14.70
O1C1#9	50	135	8	76.15	2	2	29	8.90
O1C2#9	50	163	10	101.28	2	1	32	14.50
O1C3#18	50	167	7	76.15	2	2	28	14.80
O1C4#19	50	152	8	96.25	2	2	29	19.20
O1D1#16	100	151	12	95.54	2	2	29	4.50
O1D2#10	***100***	***142***	***8***	***65.38***	***2***	***1***	***30***	***9***
O1D3#17	100	160	7	76.15	2	2	29	18
O1D4#8	100	168	8	118.87	2	2	32	18.30
O1E1#10	***200***	***153***	***14***	***108.82***	***2***	***2***	***28***	***15.40***
O1E2#3	200	140	8	80.46	2	2	32	4
O1E3#11	200	157	7	76.15	2	1	32	12.35
O1E4#14	200	150	8	101.28	2	2	31	10.60
O1F1#3	400	138	10	88.71	2	2	30	3.80
O1F2#4	400	150	8	65.38	2	2	32	11.30
O1F3#1	400	140	6	118.87	2	2	33	9.40
O1F4#1	400	153	8	101.99	2	2	37	5.20
O1G2#1	600	110	8	76.15	2	2	24	6.30
O1G3#20	600	126	8	65.38	2	2	20	4.70
Average		148.7±13.73	8.38±1.83	88.97±17.79	2±0	1.81±0.40	29.77±3.58	11.46±5.33

Note: Underhypened type letters refer to control accessions; bold italic typed letters refer to the most distant mutants relative to controls

Meanwhile, the results of the grouping analysis performed on the red variant showed that all accessions formed a group with 67% similarity, thus suggested that gamma irradiation resulted in maximum of 33% variation of genetic contents reflected by SRAP profiles (Figure 4). This value was a little higher than that of yellow variant (~20%). Four control accessions were located at the half position to the tip of terminal dendrogram. The accession that were located at the most basal position were accession 12 (O2C4#13) treated in 50 Gy and accession 21 (O2E4#16) treated in 200 Gy. These were mutants with the most distinct genotype for the red variant.

Mutant O2C4#13 have the tallest form (167 cm) compared to average size of 147 cm. Other agronomic features showed average sizes (number of leaf sheaths and tillers) but smaller leaf size (80 cm in compared to average 96 cm). Length and weight of panicles were larger than the average size (Table 4). The second mutant O2E4#16 mostly have average size on most of its agronomic feature except for smaller plant size and lower panicle size (both length and weight) (Table 3).

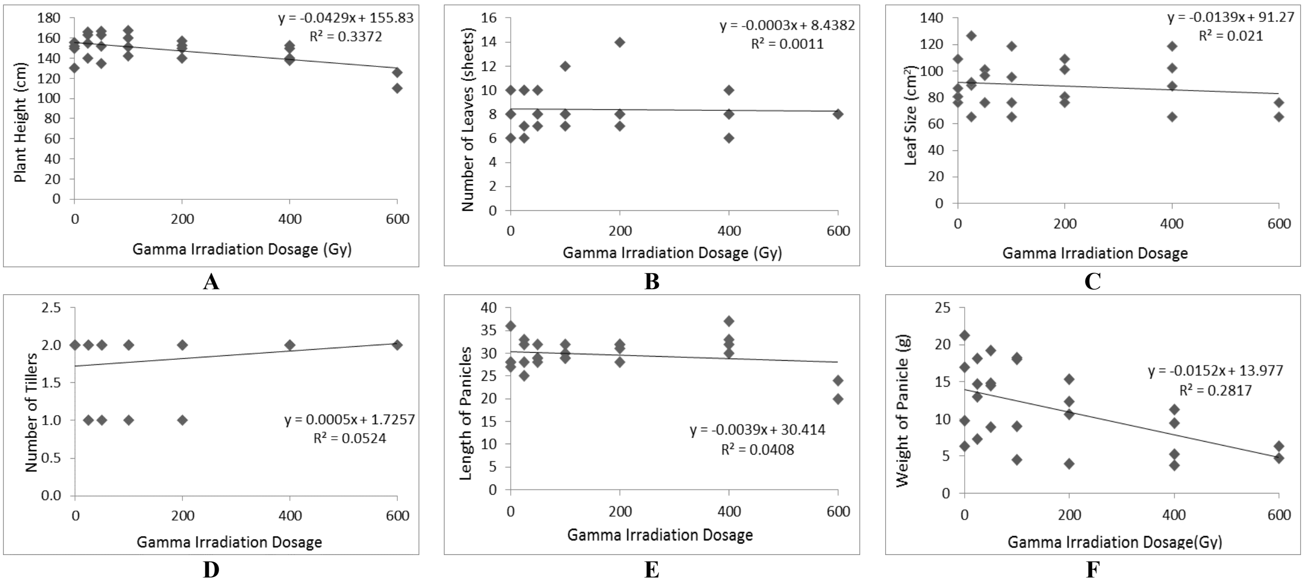

Figure 3. Correlation between gamma irradiation dosages and agronomic traits on yellow variant of foxmillet. A. Plant height, B. Number of leaves, C. Leaf size, D. Number of tillers, E. Length of panicles, F. Weight of panicle

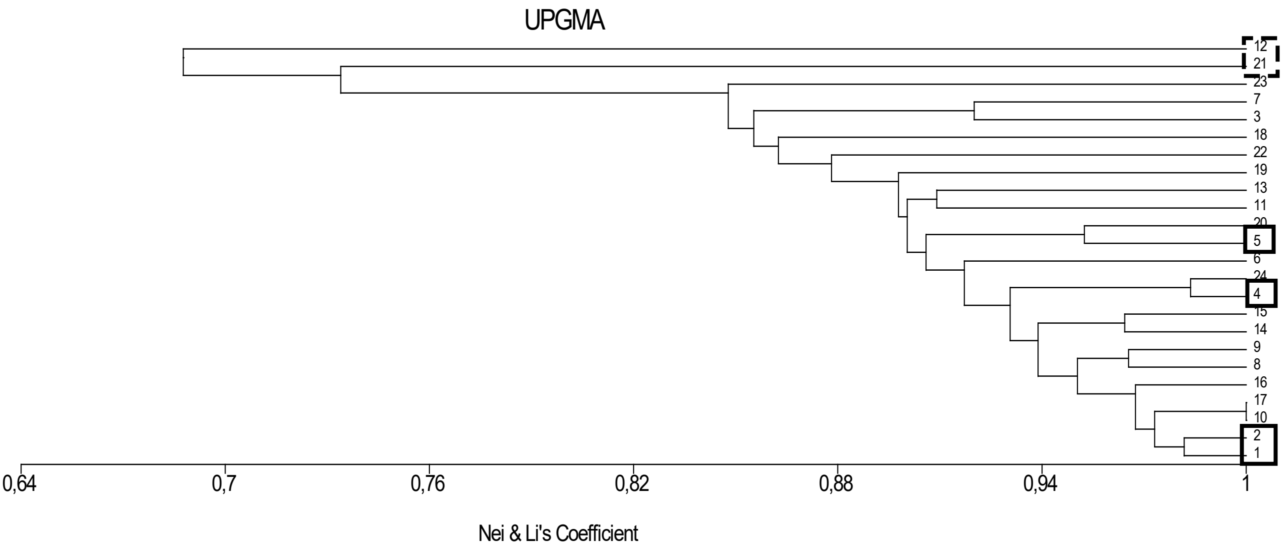

Figure 4. UPGMA dendrogram of 26 accessions of jewawut yellow variant based on Nei & Li's coefficient of similarity

Mutant O2C4#13 (treated in 50 Gy) have the tallest form (167 cm) and the shortest (110 cm) was O2G4#1 (600 Gy) (Table 4). The highest number of leaf sheaths (12) recorded on O2C1#13 (50 Gy) and the fewest (4 sheets) was O2G4#1 (600 Gy). The largest leaf (147.24 cm2) was occurred in O2B3#14 (25 Gy) and the smallest leaf (65,38 cm2) was accounted for O2G4#1 (600 Gy). There was no different on leaf colours on all accessions. The number of tillers were mostly 2 except for O2C1#13 (50 Gy) that had only one tiller. The longest panicle (33 cm) was observed on O1F2#4 (400 Gy), while the shortest (20 cm) was found in O2G4#1 (600 Gy). The heaviest panicle (23.60 g) was recorded on O2B3#14 (25 Gy) while the lightest 3.90 g was found in O2F2#9 (400 Gy).

Observation on agronomic traits of the red variant suggested similar pattern as with the yellow variant in which agronomic performance decreased by the increase of radiation dosage except for the number of tiller (Figure 5). With regard to panicle size, all accession of red variant has random size and may not relate to the irradiation dosage. Mutant 02B2#14 (treated in 25 Gy) have the heaviest panicle (23.6 g) followed by control accession O2A4#6 (23.3 g), and mutant O2O3#8 (treated in 100 Gy) accounted for 20.3 g. This indicated that the best grain yield may be obtained from mutant 02B2#14, and that increasing irradiation dosage above 100 Gy provided genotypes with low grain yield as indicated from the results of regression analysis (Figure 5).

The mutations were generally carried out with aimed to improve genetic property, such as to obtain mutants with high yields and mutants capable of adapting to global climate change. Mutations need to be made for minor food commodities that have long been cultivated for allegedly having eroded genetic diversity. Gamma rays are often used for mutation induction as an effort to enrich genetic diversity and genetic improvement. This is because gamma rays have deep penetration ability into plant tissue. Although the mutants to be generated from induction of these mutations are random, the technique is expected to alter one or more particular characters while retaining most of the original characters (Wardiyati et al. 2002). However, almost all agronomic parameter observed for the mutants were reduced following the increase of γ-radiation using dosage used in this study except for leaf colour (Table 3 and 4). Jia and Lee (2008) reported that all agronomic parameters of red mutants of *Fagopyrum dibotrys* were reduced significantly by γ-radiation using dosage of 5-20 Gy. The low dosage of γ-radiation has also affected secondary metabolite of *Lithospermum erythrorhizon* (Chung et al. 2006). This may caused by slow cell division, lower hormone synthesis, abnormal nutrimental transportation, and metabolic disorders by apical meristem damage under γ-radiation (Celso and Maria 1992; Okamoto and Tatara 1995).

Table 4. List of agronomic traits of red variant of Jewawut from Buru Island irradiated by gamma rays in various dosages

Accession code	Radiation dosage (Gray, Gy)	Plant height (cm)	Number of leaves (sheets)	Leaf size ($cm^{2)}$)	Leaf colour	Number of Tillers	Length of panicle (cm)	Weight of panicle (g)
O2A1#2	0	132	8	76.14	2	2	22	10.40
O2A2#19	0	138	8	101.28	2	2	23	13.50
O2A3#3	0	164	7	88.71	2	2	29	19.90
O2A4#6	0	166	8	109.90	2	2	27	23.30
O2B1#4	25	134	6	86.92	2	2	23	12.40
O2B2#20	25	150	10	86.92	2	2	27	10.90
O2B3#14	25	166	6	147.24	2	2	30	23.60
O2B4#12	25	165	8	96.25	2	2	29	12
O2C1#13	50	141	12	96.25	2	1	30	16.70
O2C2#20	50	156	8	101.28	2	2	27	16.60
O2C3#1	50	159	8	118.87	2	2	30	17.30
O2C4#13	***50***	***167***	***8***	***80.46***	***2***	***2***	***31***	***17.90***
O2D1#2	100	128	6	76.15	2	2	25	12
O2D2#19	100	140	8	96.25	2	2	24	11.20
O2D3#8	100	158	7	101.28	2	2	31	20.30
O2D4#3	100	160	7	115.64	2	2	31	18.70
O2E1#9	200	145	8	104.15	2	2	30	7.40
O2E2#9	200	143	8	76.15	2	2	29	11.90
O2E3#12	200	150	6	76.15	2	2	35	11.30
O2E4#16	***200***	***135***	***7***	***101.28***	***2***	***2***	***26***	***14.50***
O2F2#4	400	153	10	93.17	2	2	33	4.20
O2F2#9	400	130	8	113.85	2	2	26	3.90
O2G4#1	600	110	4	65.38	2	2	20	4.50
Average		147.39 ± 15.14	7.65±1.61	96.07	2	1.96	27.74	13.67

Note: Underhypened type letters refer to control accessions; bold italic typed letters refer to the most distant mutants relative to controls

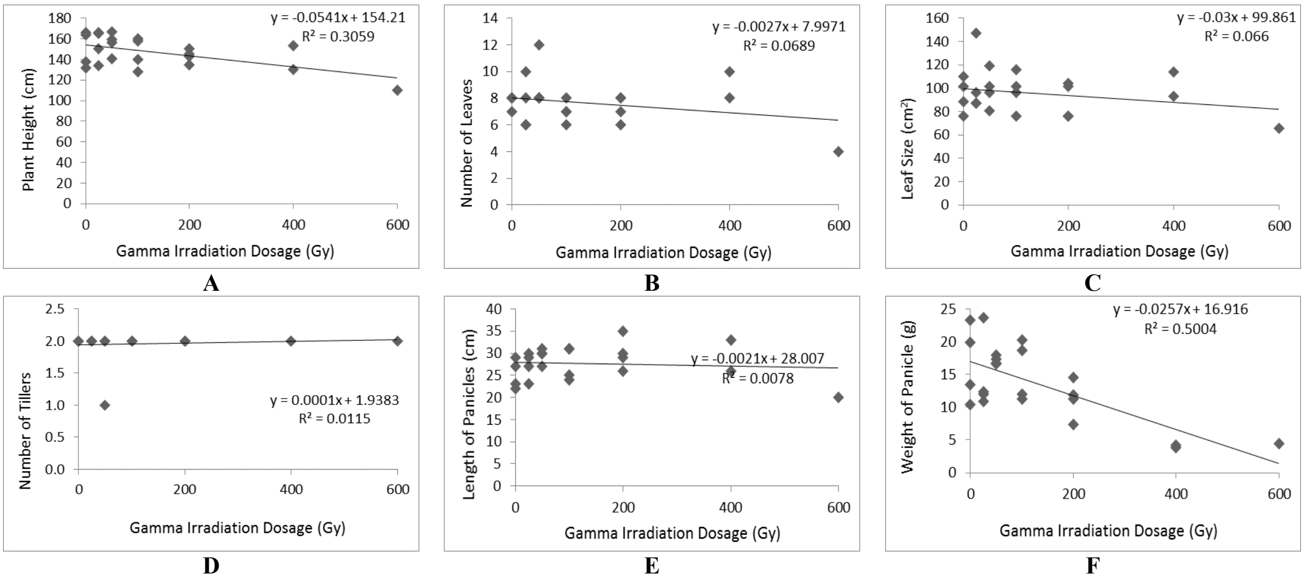

Figure 5. Correlation between gamma irradiation dosages and agronomic traits on red variant of foxmillet. A. Plant height, B. Number of leaves, C. Leaf size, D. Number of tillers, E. Length of panicles, F. Weight of panicle

One of the observed traits that have positive correlation with the increasing dosage of gamma radiation is the number of tiller. The number of tillers of all accession observed in this study are two, and there were no changed after treated in irradiation of gamma rays for most genotypes except for 3 genotypes of yellow variant (O1B3#6, O1B3#19 and O1C2#9) and 1 genotype of red variant (O2C1#13). These genotypes have one tiller under 25 and 50 Gy concentration of gamma rays (Table 3 and 4). Hence number of tiller may have been easily changed by induced mutation by gamma radiation even using low dosage. This may implied that number of tillers may have been one of the phenotypic traits that changed over the long period of domestication. Doust (2006) reported that the domesticated jewawut have reduced number of tillers (base of branch) and branching in the axil (armpits) along the tillers. He further considered that the domestication process may result in a reduction in plant architecture diversity and a reduction in the number of branches compared to its progenitor species.

Combination of SRAP primer to successfully produced PCR amplicons were of me1F+em2R, me2F+em3R, me4F+em2R, me4F+em5R, me5F+em1R, me5F+em5R (Figure 6); while for the red variant the SRAP pair were of me1F+em2R, me2F+em3R, me3F+em3R, me4F+em2R, me5F+em5R (Figure 7). Closer examination of each picture suggesting that there were only few differences on SRAP profiles between the controls and the mutants, even the same (Figure 6d). Several different profiles observed included the present and absent of particular bands compared to the controls.

Differences of primers ability in yielding polymorphisms are varied between species, mainly due to availability of complementary nucleotides within the genome, thus only six combination of SRAP primer could successfully yielded amplicons in the yellow variant and five combinations for the red variant. The irradiation gamma rays on Jewawut in this study only resulted in a considerably low genetic variation in the SRAP compared to those of ISSR and RAPD (Yulita et al. 2014). Their study on induced mutation of black potatoes using 25 and 35 Gy gamma rays resulted in genetic variation of around 60%. Polymorphism in SRAP has different nature from ISSR. SRAP were only contained coding regions (genes) that were more conserved for having slow mutation rate. While amplicons of ISSR contained both coding and non-coding regions, to which the non-coding regions were generally have faster mutation rates than coding regions. Hence, exposing the coding regions to gamma rays radiation were likely to produce lower level of variation compared to that of non-coding region, even under the same radiation dosage.

Examination of type of mutations on SRAP profiles can only be made by observing banding patterns. There are three types of mutations usually existed within the genome, insertion-deletion event, base substitution and inversion. Base substation and inversion can only be referred from the results of DNA sequencing. In our study, when comparing SRAP profiles of accessions of control and mutant, the mutants tend to have loss several bands than gaining bands and in some profiles did not show any difference on the profiles (Figure 6d). Loss of the bands may equal to deletions of nucleotides and gaining bands may equal to insertions. Morita et al (2009) have reported that induced mutation by gamma irradiation in rice resulted in almost 80% deletions, 12% of base substitution and 8% of inversion in several genes including waxy genes. It is therefore interesting to note that results from our study showed there were several additional fragments (Figure 7.A and 7.B). As have been stated previously that SRAP amplicons only included coding regions, it is therefore important to perform DNA sequencing on these additional fragments to determine the identity of these genes.

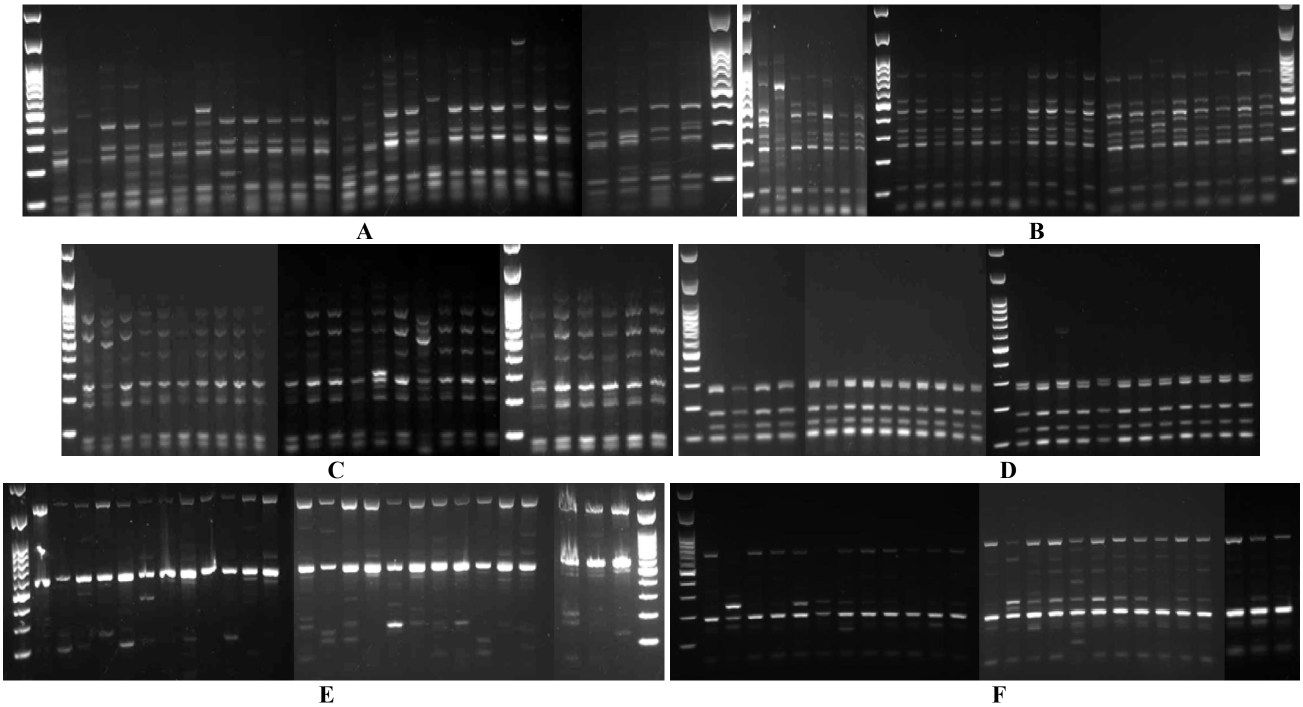

Figure 6. SRAP profiles of yellow variant of Jewawut visualized on 2% agarose gel electrophoresis using combinations of primer pairs me1F+em2R (A), me2F+em3R (B), me4F+em2R (C), me4F+em5R (D), me5F+em1R (E), me5F+em5R (F). The first lane from the left bands representing 100 bp DNA ladder, the second, third, fourth and fifth lanes are controls. The remaining lanes are the accessions

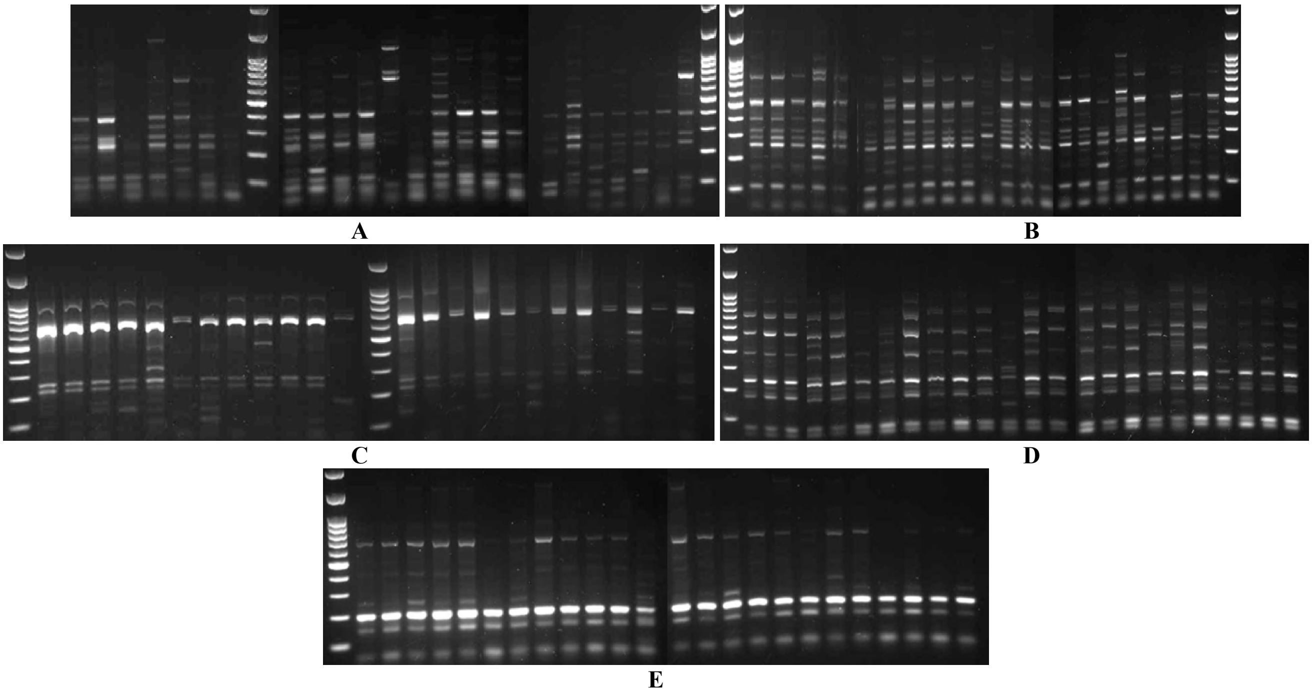

Figure 7. SRAP profiles of red variant of Jewawut visualized on 2% agarose gel electrophoresis using combinations of primer pairs me1F+em2R (A), me2F+em3R (B), me3F+em3R (C), me4F+em2R (D), me5F+em5R (E). **A.** The first four lanes are controls; the remaining lanes are accessions and 100 bp DNA ladder. **B-E.** The first lane from the left bands representing 100 bp DNA ladder, the second, third, fourth and fifth lanes are controls. The following lanes are the accessions

Mutation could not occur at the same rate in all individuals treated at the same dosage; even they are the same variant. The findings of this present study thus confirmed that irradiation of gamma rays can indeed enhanced mutation in broader sense, but individual plant will response differently to the mutation. We concluded that mutant with the most distinct genotypes for yellow variant were accession O1O2#10 treated in 100 Gy and O1E1#10 treated in 200 Gy gamma radiation, whereas in the red variant were accessions O2C4#13 treated in 50 Gy and O2E4#6 treated in 200 Gy gamma radiation. All the mutants have different yet random agronomic performances relative to controls. There was no mutant of yellow variant that have potential high grain yield. Mutant with potential high grain yield is O2B2#14 treated in 25 Gy for red variant, and this should be used as one of candidate to develop superior genotype.

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