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Growth clusterring of Gamma-irradiated *Artemisia annua* shoot culture by Ward method

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Abstract *Artemisia annua* is a medicinal plant for antimalaria that has been used for hundred years ago. Many techniques have been applied to increase production of artemisinin including irradiation of Gamma rays in order to obtain mutant plants. Mutation is also attempted to increase genetic variability. The cluster of shoots culture of *A. annua* after Gamma irradiation treatment based on their growth on MS medium was determined. Shoot tips of 82 clones after irradiation with 0 (control treatment), 5, 10, 20, 30 and 40 Gy of Gamma ray were cultured on MS medium for 6 weeks. The results showed that ward method on growth of *in vitro* shoots from 82 clones at the fourth week resulted into 5 clusters. Cluster 3 was the best for growth of *A. annua* shoot culture containing 14 clones e.i. 5.3.a , 5.4.a, 5.19.a1, 10.10.a*, 10.13.a, 10.37.a, 20.1.a, 20.6.a, 20.16.a 30.12.a, 30.15.a, 30.18.a, 30.28.a, 30.38.a. This cluster consisted of 3 clones came from irradiation 5 Gy, 3 clones from 10 Gy, 3 clones from 20 Gy, and 5 clones from 30 Gy, respectively. The research finding was beneficial for selection of superior clones for the genetic improvement.

Keywords: *Artemisia annua* L., Gamma-irradiated shoot, Ward method

Introduction

Artemisia annua is a medicinal plant species useful as antimalaria drug, tradisionally used since a long time ago. Artemisinin, the main chemical substance found in the leaves of *A. annua,* is not only effective to cure malaria, but also potential as anticancer, anti parasite such as for schistosomiasis, as well as antiviral such as for hepatitis B (Efferth, 2007). This plant is originally from subtropic area, but it has been distributed and cultivated in many tropical countries such a Malaysia, Brazil, Vietnam, Madagaskar, and Sub Sahara in Afrika (Laughlin, 2002), also in Indonesia (Gusmaini and Nurhayati, 2007; Rahman *et al.,* 2014). Level of artemisinin in *A. annua* leaf is low at 0.02-1.1% from the dry weight basis (Kindermans *et al.,* 2007; Rombauts *et al.,* 2015). So

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that for commercial purposes is not effective. To overcome these problems, some research are required to improve plant genetically to produce varieties with superior properties that have higher biomass and artemisinin content, making it more economical for a large scale production purposes.

Several plant genetic improvement efforts can be carried out conventionally such as by introduction, hybridization, selection, mutation or by application of biotechnology. Induction of mutations can be done with mutagenic substances to plant organs such as seeds, stem cuttings, pollen, roots, rhizome, and so on (Lestari *et al.,* 2010). Mutation can also be induced by irradiation with Gamma in order to increase genetic variation of plant species. Afterwards, selection must be done to obtain mutants with targetted trait (AlSafadi *et al.,* 2000; Pardal, 2014). The more genetic variation obtained, the higher oppotunities to find out genotypes having best quality. In vitro irradiation with Gamma ray have been conducted in many species such a *Phalaenopsis amabilis* L. (Suwarno *et al.*, 2013), *Colocasia esculenta* (Martin *et al.,* 2015), *Tacca leontopetaoloides* (Hapsari *et al.,* 2015), *Sorghum bicolor* L. Moench (Lestari *et al.,* 2015).

Induction of mutations with Gamma-ray irradiation in *A. annua* has also been performed *in vitro* by using shoot bud explants (Lestari *et al.,* 2010) and ex vitro using seeds (Raymond *et al.,* 2015). The resulting mutants have variations in their genotypes and have broad agronomic properties such as in leaf morphology, leaf color, stem color, stem diameter, stem length, plant height, biomass, length of petal, flower shape and flower formation, and its artemisinin content. Variations in plant populations suggest potential genotypes that are expected to have better properties (Syamsudin *et al.,* 1997). Clustering of specific characteristics is needed to make sellection of plant population easier.

Ward method has been applied for clustering analysis of plant population in order to grouping clones having more similarity in growth or productivity variables. Using Euclidean Distance similarity or dissimilarity will be determined so that cluster will be formed. Several genotypes having high similarity on the same variables will stay in the same claster (Cornish, 2007). This method has been applied for *Colocasia esculenta* (Martin *et al.,* 2015), *Tacca leontopetaoloides* (Hapsari *et al*., 2015) using *in vitro* shoot culture after irradiation with Gamma ray. In *Cicer arietinum* (Zali *et al.,* 2011), *Triticum aestivum* (Khodadadi *et al.*, 2011; Aharizad *et al.,* 2012; Fahim, 2014), *Oriza sativa* (Maji and Shaibu, 2012), clustering was also done by Ward method in order to do identification, evaluation and selection of genetic variation. This study was aimed to determine growth clusterring of Gamma-irradiated

Artemisia annua shoot culture by Ward Method. This finding is beneficial for selection of superior clones for the genetic improvement.

Materials and methods

This research used Gamma-irradiated shoots culture of *A. annua* which is presented in Table 1. Eighty one clones of irradiated shoots and one clone of control treatment were selected then grown on MS (Murashige Skoog) medium containing no plant growth regulators. Medium was suplemented with 30 g/l sucrose, solidified with 8 g/l agar. Medium was sterilized using autoclave at 120 ^{o}C, 1 atm for 15 min. Shoot tips of each clones were grown on the medium culture, then they were incubated in a culture room at 26-28 ^{o}C for 5 weeks. Experiments were done by Group Randomized Design. Each clone consisted of 4 explants per culture vessel, and 3 replicates. After 6 weeks of subture, growth was determined by recording height of shoots, number of leaves, number of lateral shoots, length of the 4th leaf blades, length of the 4th leaf petioles and number of roots.

Table 1. Clones of irradiated-shoots cultures of *A. annua* after treatments

No	Dose of irradiation (Gy)	Number of clones
1	5	19
2	10	20
3	20	17
4	30	22
5	40	3
6	Control	1
	Total	82

Data were analysis using hierarcy cluster by Ward method, followed by Euclidean distance to determine degree of similarity or dissimilarity. Cluster analysis was performed by Minitab ver. 16 software. Cluster with best growth characteristics was determined by Analysis of varian on each cluster formed, followed by Duncan's Multiple Range Test (DMRT) using IBM SPSS ver. 22 program.

Results

The purpose of mutation induction with Gamma ray irradiation is to increase the genetic diversity of plants. To facilitate selection, the acquired mutant candidate needs to be grouped by growth. Cluster analysis is one of the statistical techniques for grouping to individual or object into groups with different characteristics between groups. Therefore, cluster analysis can be used in A. annua shoot culture clones to classify clusters based on their growth. The results showed that the parameters of plant height, the number of leaves, the number of roots, the number of lateral buds, the length of the 4th leaf blade and the length of the 4th petiole was varied (Table 2). The shoot height ranged from 2.2-10.9 cm, the number of leaves ranged from 7-17, the number of lateral shoots ranged from 0-7 buds, the length of 4th leaf blade ranged from 0.3 to 1.5, and the number of roots between 0-16.7, respectively.

Table 2. Growth of irradiated-shoots of *A. annua* after 6 weeks grown on MS medium

No	Clone	Height of shoots (cm)	Number of leaves	Number of lateral shoots	Length of the 4th leaf blades	Length of the 4th leaf petioles	Number of roots
A	**5 Gy**						
1	5.1.a	3.9±0.38	12.8±0.85	0.8±0.48	0.7±0.10	0.6±0.05	1.5±0.65
2	5.2.a1.1	7.2±0.00	13.5±0.65	0.5±0.50	0.8±0.09	0.8±0.08	8.3±2.06
3	5.3.a	8.9±0.32	14.5±0.65	2.8±1.31	0.8±0.14	0.7±0.10	12.8±0.85
4	5.4.a	8.2±0.80	16.0±1.83	0.0±0.00	0.7±0.17	0.7±0.11	12.8±2.21
5	5.5.a1	3.8±0.19	10.8±0.25	0.0±0.00	0.5±0.03	0.8±0.03	5.0±1.96
6	5.9.a1	5.8±0.41	13.0±0.82	0.0±0.00	0.6±0.07	0.8±0.10	8.3±1.65
7	5.10.a	5.8±0.46	14.8±1.02	3.2±1.39	0.4±0.02	0.7±0.07	5.0±1.07
8	5.14.a	4.9±0.55	11.8±1.18	4.5±2.33	0.8±0.00	0.8±0.05	11.0±2.42
9	5.15.a1	4.8±0.32	11.3±0.48	0.0±0.00	0.5±0.06	0.5±0.09	7.3±1.79
10	5.17.a	5.2±0.68	11.5±0.87	0.0±0.00	0.5±0.041	0.7±0.05	7.8±2.87
11	5.18.a	4.3±0.43	14.5±1.04	2.5±1.85	0.8±0.06	0.8±0.03	7.5±0.65
12	5.19.a1	5.1±0.23	14.8±1.65	1.8±1.44	0.6±0.03	0.8±0.07	12.5±1.19
13	5.20.a1 *	2.2±0.09	9.5±0.65	0.0±0.00	0.5±0.05	0.6±0.08	1.8±0.85
14	5.22.a	4.8±0.72	13.8±1.16	0.2±0.20	0.5±0.07	0.6±0.09	8.2±2.48
15	5.23.a	9.0±0.45	14.4±0.25	4.4±1.83	1.0±0.07	0.5±0.23	7.6±1.29
16	5.30.a	7.7±0.66	14.5±0.96	5.8±1.80	0.8±0.08	0.7±0.09	9.3±1.80
17	5.32.a	8.1±1.16	13.3±0.48	3.0±1.29	1.0±0.18	0.9±0.09	8.3±1.04
18	5.34.a	7.3±1.26	14.3±2.19	4.0±2.68	0.7±0.06	0.5±0.09	10.7±3.84
19	5.36.a	7.0±1.165	13.0±0.70	1.0±1.00	0.4±0.03	0.7±0.03	6.5±1.76
B	**10 Gy**						
20	10.1.a1	5.1±0.27	14.0±0.45	0.0±0.00	0.6±0.05	0.6±0.05	9.2±1.71
21	10.2.a	4.4±0.64	11.7±0.88	0±0.00	0.7±0.18	0.4±0.17	0.0±0.00
22	10.3.a1	7.1±0.23	11.8±0.48	1.3±0.75	0.9±0.10	0.8±0.12	7.5±0.29

Table 2 (Con.)

No	Clone	Height of shoots (cm)	Number of leaves	Number of lateral shoots	Length of the 4th leaf blades	Length of the 4th leaf petioles	Number of roots
23	10.7.a1	5.9±0.51	12.0±0.41	3.3±1.03	1.0±0.08	0.7±0.05	6.3±0.48
24	10.8.a	6.4±0.17	13.3±0.25	0.8±1.031	0.6±0.04	0.7±0.09	4.8±0.25
25	10.10.a*	6.6±0.52	13.2±0.58	0.6±0.60	0.6±0.05	0.7±0.10	12.8±1.46
26	10.11.a	5.0±0.10	12.8±0.85	0.8±0.25	0.9±0.06	0.3±0.18	0.0±0.00
27	10.12.a	7.0±1.05	12.0±0.58	3.3±1.85	0.9±0.19	0.7±0.03	7.8±0.75
28	10.13.a	9.2±0.24	12.0±0.45	1.8±0.92	0.6±0.04	0.7±0.07	11.6±9.68
29	10.16.a	7.3±0.32	12.6±0.68	2.2±1.11	0.7±0.10	0.6±0.06	10.8±0.51
30	10.18.a1	9.3±0.95	14.6±0.40	2.0±0.71	0.8±0.09	0.6±0.14	9.2±1.59
31	10.20.a	9.0±0.71	13.6±0.51	1.4±1.66	0.8±0.11	0.6±0.10	8.0±1.38
32	10.21.a	7.6±0.83	14.8±0.85	2.3±0.75	0.8±0.14	0.8±0.08	8.8±0.63
33	10.22.a	6.0±0.54	11.8±0.25	0.0±0.00	0.7±0.05	0.8±0.10	5.8±1.32
34	10.27.a	8.8±1.40	12.3±1.45	0.3±0.33	0.7±0.10	0.7±0.09	1.6±1.20
35	10.28.a1	7.5±0.63	12.8±0.82	0.3±0.25	0.6±0.04	0.8±0.12	10.0±1.92
36	10.30.a	6.6±0.28	13.0±0.58	0.7±0.67	0.9±0.10	0.6±0.00	11.0±2.08
37	10.37.a	8.3±0.70	13.8±1.03	0.3±0.25	0.7±0.06	0.2±0.08	14.5±1.55
38	10.40.a	6.3±0.38	12.0±0.25	0.0±0.00	0.8±0.06	0.6±0.09	4.3±0.85
39	10.41.a	5.7±0.38	12.6±0.51	0.4±0.25	0.5±0.02	0.6±0.04	9.0±0.95
C	**20 Gy**						
40	20.1.a	7.9±0.33	13.8±0.49	0.4±0.25	0.4±0.04	0.7±0.07	13.2±0.80
41	20.2.a1	6.1±0.43	13.8±0.20	4.2±0.37	0.4±0.04	0.4±0.06	13.0±0.89
42	20.3.a	5.8±0.19	12.8±0.86	0.4±0.40	0.3±0.04	0.5±0.05	10.4±0.75
43	20.4.a1	7.7±0.95	11.3±1.31	1.0±0.71	0.6±0.06	0.7±0.05	10.0±0.82
44	20.5.a	4.7±0.19	11.3±0.25	0.0±0.00	0.5±0.10	1.0±0.14	6.5±1.08
45	20.6.a	8.2±0.50	15.6±0.75	2.2±0.74	0.6±0.11	0.7±0.04	12.2±0.58
46	20.7.a1	6.5±0.27	11.8±0.37	0.2±0.20	0.4±0.07	0.6±0.11	10.4±1.50
47	20.8.a1	6.0±1.12	10.5±1.04	0.0±0.00	0.5±0.06	0.6±0.09	5.0±1.68
48	20.9.a	5.8±0.72	14.8±0.95	1.0±1.00	1.0±0.00	0.6±0.19	7.5±1.26
49	20.10.a1	6.7±0.35	16.0±0.32	0.6±0.40	0.3±0.04	0.6±0.02	9.4±1.63
50	20.12.a	5.9±0.18	11.8±0.25	0.7±0.25	1.5±0.28	0.1±0.00	6.8±0.85
51	20.14.a	6.8±0.58	12.8±0.85	0.3±0.25	0.6±0.09	0.6±0.08	8.8±1.32
52	20.16.a	7.4±0.23	14.3±0.25	2.0±1.08	0.7±0.08	0.8±0.05	13.3±1.80
53	20.17.a	5.2±1.39	13.5±1.55	0.0±0.00	0.5±0.07	0.6±0.11	4.5±1.32

Table 2 (Con.)

No	Clone	Height of shoots (cm)	Number of leaves	Number of lateral shoots	Length of the 4th leaf blades	Length of the 4th leaf petioles	Number of roots
54	20.24.a*	10.7±0.55	12.0±0.41	6.0±0.41	0.7±0.04	0.5±0.06	9.5±0.65
55	20,27.a1	5.9±0.56	7.0±0.55	0.4±0.25	0.3±0.04	0.6±0.04	8.4±1.50
56	20.28.a	7.0±0.49	11.4±0.98	0.4±0.25	0.3±0.02	0.5±0.03	6.2±1.66
D	**30 Gy**						
57	30.1.a	6.3±0.84	10.0±1.23	0.5±0.29	0.5±0.06	0.6±0.06	5.5±2.40
58	30.2.a1	6.6±0.42	13.5±0.87	2.8±1.31	0.5±0.03	0.6±0.05	5.5±2.60
59	30.5.a*	6.3±0.32	12.5±1.32	0.3±0.25	0.6±0.03	0.8±0.05	6.3±0.63
60	30.8.a	8.6±0.74	13.8±0.25	2.0±1.08	0.9±0.06	0.9±0.08	9.5±1.19
61	30.9.a1	7.0±0.72	14.5±0.25	1.8±0.75	0.6±0.10	0.7±0.16	10.0±2.42
62	30.11.a1	8.0±0.35	12.6±0.40	0.0±0.00	0.5±0.07	0.8±0.08	8.4±0.25
63	30.12.a	7.5±0.15	12.4±0.85	5.5±0.29	0.5±0.04	0.4±0.06	12.0±1.58
64	30.13.a1	7.9±0.75	12.8±1.25	2.8±1.70	0.5±0.09	0.7±0.06	13.0±1.03
65	30.14.a1	9.3±0.47	14.3±1.25	0.0±0.00	0.6±0.10	0.8±0.13	10.3±2.18
66	30.15.a	9.6±0.40	15.3±0.58	0±0.00	1.1±0.03	0.7±0.03	15.8±0.58
67	30.17.a1	7.3±0.41	12.0±0.65	1.0±0.00	0.9±0.05	0.8±0.11	7.0±2.14
68	30.18.a	6.7±0.07	9.5±0.58	2.6±0.68	0.7±0.13	0.1±0.00	12.8±0.71
69	30.21.a	7.8±0.39	13.3±0.25	6.8±1.11	0.4±0.05	0.3±0.03	10.0±1.03
70	30.22.a1	5.6±0.22	14.5±0.97	2.8±1.11	0.6±0.04	0.5±0.07	9.3±1.32
71	30.23.a1	4.1±0.50	13.7±0.63	3.5±1.44	0.5±0.03	0.5±0.05	8.0±0.96
72	30.24.a	10.9±0.41	15.8±0.65	1.3±0.63	0.6±0.05	0.5±0.09	10.8±0.87
73	30.25.a1	8.1±0.47	13.8±1.53	7.0±1.00	1.0±0.03	0.6±0.09	11.5±1.21
74	30.26.a1	6.8±0.88	11.5±1.15	0.0±0.00	1.0±0.10	0.9±0.12	3.5±2.19
75	30.27.a	9.0±0.45	17.0±0.75	0.0±0.00	0.8±0.12	0.7±0.03	9.7±1.08
76	30.28.a	6.9±0.67	14.0±1.08	0.5±0.50	0.8±0.13	0.9±0.05	16.7±0.96
77	30.38.a	8.1±0.69	10.8±0.68	1.0±0.45	0.5±0.23	0.8±0.07	12.0±0.51
78	30.41.a	5.8±0.52	11.8±0.67	4.0±2.31	0.8±0.07	0.5±0.13	7.5±1.00
E	**40 Gy**						
79	40.1.a	6.6±0.43	13.3±0.95	3.8±2.39	0.5±0.09	0.5±0.14	5.0±1.68
80	40.2.a	7.3±0.13	14.3±1.22	6.5±0.87	0.6±0.05	0.7±0.03	7.0±0.91
81	40.3.a*	7.4±0.41	14.3±0.48	4.3±0.25	0.8±0.10	0.3±0.05	7.0±1.08
82	**Control**	6.3±0.59	14.0±0.71	0.5±0.50	0.9±0.14	0.8±0.05	9.5±1.32

For the analysis of diversity, based on growth parameters, all *A. annua* shoot culture clones were determined by the group using SPSS software to obtain the ward linkage value. The results are presented in Table 3. Based on the ward coefficient data, it is known that at the 77th stage there is a considerable increase in coefficient value compared to the lower levels. The value was the basis for determining the number of clusters that can be formed from the 82 clones evaluated. The cluster number of determination was the number of clones that minus 77 (82-77) obtained by 5 clusters.

Table 3. Degree of coeficiency determined by Ward Linkage

Stage	**Cluster Combined**		**Coefficients**	**Stage Cluster First Appears**		**Next Stage**
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
61	5	24	178.039	48	47	67
62	1	13	189.740	36	0	77
63	30	32	201.662	55	42	74
64	8	18	213.743	57	43	74
65	7	11	226.864	46	32	75
66	15	54	241.695	51	0	71
67	5	34	257.822	61	0	72
68	2	6	274.568	56	44	76
69	3	66	293.880	59	50	73
70	9	55	317.546	58	0	72
71	15	16	341.311	66	60	75
72	5	9	376.361	67	70	77
73	3	25	414.905	69	54	78
74	8	30	457.248	64	63	76
75	7	15	509.295	65	71	79
76	2	8	**581.486**	68	74	78
77	1	5	**683.714**	62	72	80
78	2	3	798.577	76	73	79
79	2	7	1030.995	78	75	80
80	1	2	1624.425	77	79	0

The percentage of each dose of Gamma ray irradiation that contributes to each cluster member is shown in Table 4. Cluster 1 consisted of Gamma ray irradiated shoots resulting from irradiation of 5 and 10 Gy, clusters 2, 3 and 4 comprised of irradiated clones of 5.10, 20 and 30 Gy, while cluster 4 consisted of all 5-40 Gy of shoots of radiation, respectively.

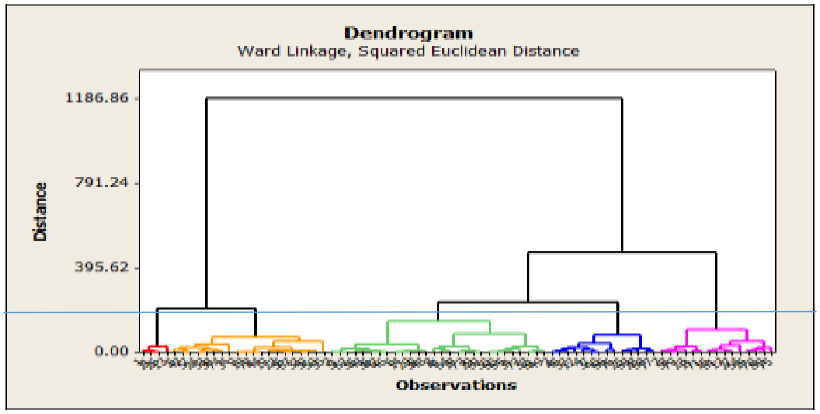

Figure 1. Dendogram of clustering of *A. annua* shoot clones

Table 4. Cluster of *A. annua* shoot clones and percentage of each irradiation doses in cluster

Cluster number	Number of clones	Clone member	Persentage (%) of irradiation dose (Gy)				
			5	10	20	30	40
1	4	5.1.a, 5.20.a1*, 10.2.a, 10.11.a	50.0	50.0	0	0	0
2	28	5.2.a1.1, 5.9.a1, 5.14.a, 5.22.a, 5.34.a, 10.1.a1, 10.16.a, 10.18.a1, 10.20.a, 10.21.a, 10.28.a1, 10.30.a, 10.41.a, 20.2.a1, 20.3.a, 20.4.a1, 20.7.a1, 20.9.a, 20.10.a1, 20.14.a, 30.8.a, 30.9.a1, 30.11.a1, 30.13.a1, 30.14.a1, 30.21.a, 30.24.a, 30.25.a1	17.8	28.6	25.0	28.6	0
3	14	5.3.a, 5.4.a, 5.19.a1, 10.10.a*, 10.13.a, 10.37.a, 20.1.a, 20.6.a, 20.16.a 30.12.a, 30.15.a, 30.18.a, 30.28.a, 30.38.a	21.4	21.4	21.4	35.8	0
4	20	5.5.a1, 5.15.a1, 5.17.a, 5.36.a, 10.3.a1, 10.8.a, 10.22.a, 10.27.a, 10.40.a, 20.5.a, 20.8.a1, 20.12.a, 20.17.a, 20,27.a1, 20.28.a, 30.1.a, 30.5.a*, 30.17.a1, 30.26.a1, 30.41.a	20.0	25.0	30.0	25.0	0
5	15	5.10.a, 5.18.a, 5.23.a, 5.30.a, 5.32.a, 10.7.a1, 10.12.a, 20.24.a*, 30.2.a1, 30.22.a1, 30.23.a1, 30.27.a, 40.1.a, 40.2.a, 40.3.a*	33.3	13.3	6.7	26.7	20.0

Cluster 1 had the smallest member (2 clones), cluster 2 had the largest member (all 5 clones). The distribution of all clones from each irradiated dose occupying each cluster is shown in Table 5. The 5 Gy irradiated clone occupies all clusters with the highest contribution in clusters 2 and 5, the lowest in cluster 1. Clone 10 Gy occupied all clusters with highest contribution in cluster 2, the lowest in clusters 1 and 5. Clones 20 and 30 Gy occupied the same 4 clusters (cluster 2-5) with the highest contribution to cluster 3, the lowest in cluster 5. Clone 40 Gy 100% occupied only one cluster (cluster 5).

Table 5. Clustering of *A. annua* shoot clones based of dose of irradiation

Irradiation Dose (Gy)	**Number of clones**	**Number of clones (%) in each cluster**				
		1	2	3	4	5
5	19	10.5	26.3	15.8	21.1	26.3
10	20	10.0	40.0	15.0	25.0	10.0
20	17	0	41.2	17.6	35.3	5.9
30	22	0	36.4	22.7	22.7	18.2
40	3	0	0	0	0	100

Each growth variable can be evaluated its position on each cluster using Anova analysis followed by DMRT. The control treatment data is separated from the irradiated shoot data which is considered as a separate cluster so that the Gamma irradiation treatment results can be compared with the control. Based on average data of *A. annua* shoots in each cluster, it is shown that cluster 1 (3.9 cm) had the lowest shoot height significantly different from other clusters. The height of control cluster (6.3 cm) was not significantly different from the clusters 2-5 (Figure 2). Cluster 3 had the highest shoot height, but was not significantly different from clusters 2, 5 (7.2 and 7.0 cm, respectively) and control cluster.

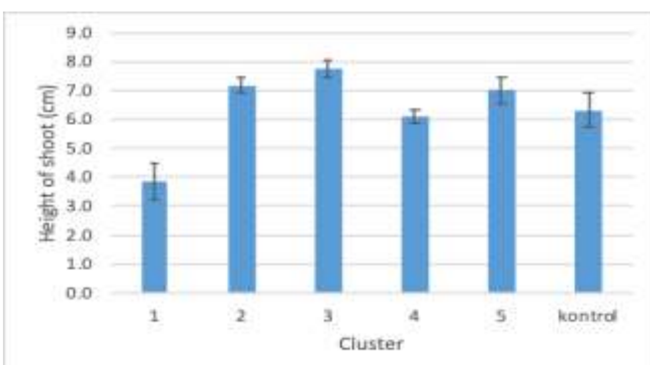

Figure 2. Height of *A. annua* shoots in each cluster

The number of *A. annua* leaves in cluster 2, 3, 5 were 13.5; 13.6; 13.9 leaves, respectively that was not significantly different from control (14.0), but was significantly different from the clusters 1 and 4 which were 11.7 and 11.5 respectively (Figure 3). The cluster 5 (4.4) had the highest lateral shoot number which significantly differed from control and other clusters (Figure 4). Cluster 1 (0.4) had the lowest lateral shoot number which did not significantly differed from clusters 3 and 4 (0.9 and 0.4, respectively) and control (0.5 shoots).

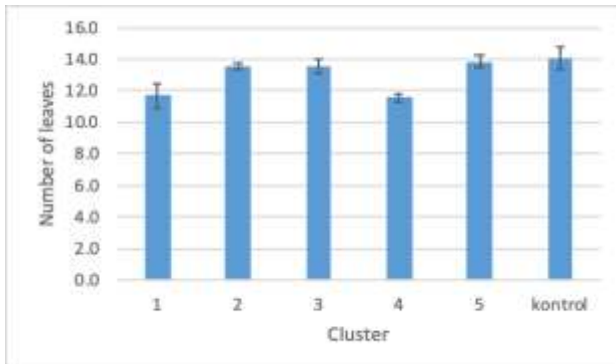

Figure 3. Number of *A. annua* leaves in each cluster

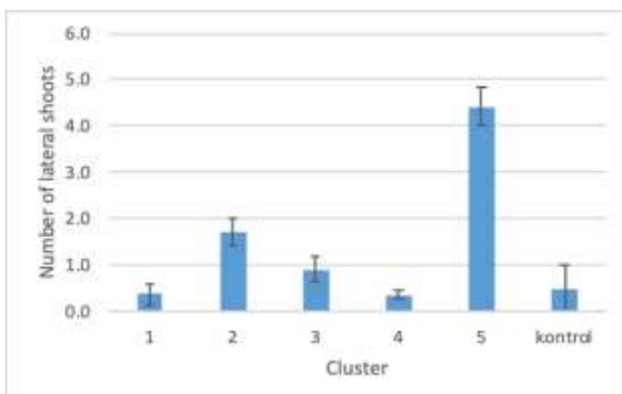

Figure 4. Number of *A. annua* lateral shoots in each cluster

The clustering based on the 4th leaf blade and petiole length. The results showed that all clusters of Gamma ray irradiation eg clusters 1-5 were not significantly different. Clusters 2 and 4 had averaged value of 0.6 cm which significantly lower keaf blade values than that of the cluster control (0.9 cm) (Figure 5). Based on the length of the 4th petiole, the value of clusters in A. annua varied. Cluster 3 (0.8 cm) had the highest petiole which not significantly different from the control (0.8 cm), but it was significantly different from cluster 1 (0.5 cm). This cluster had low petiole length and not significantly differed from clusters 2, 4 and 5 (Figure 6).

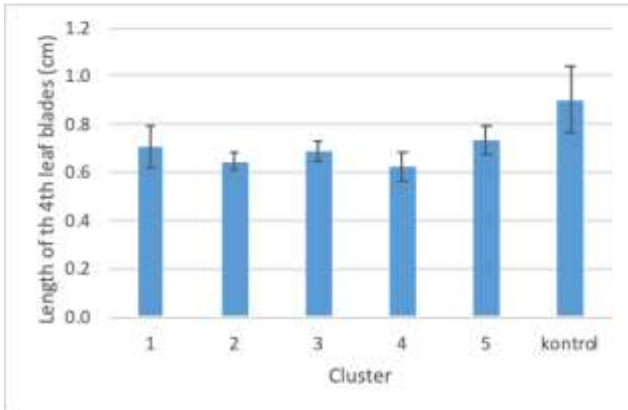

Figure 5. Length of the 4th *A. annua* leaf blades in each cluster

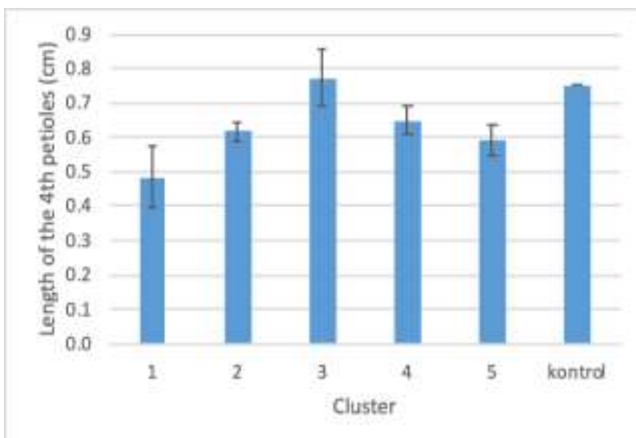

Figure 6. Length of the 4th *A. annua* petioles in each cluster

The number of roots of the five clusters *A. annua* as a result of irradiation with Gamma rays also varied. Cluster 3 (13.2) had the highest number of roots compared to other clusters. Cluster 1 (0.8) had the lowest number of roots. The cluster control (9.5) was not significantly different from cluster 2 (9.8) (Figure 7).

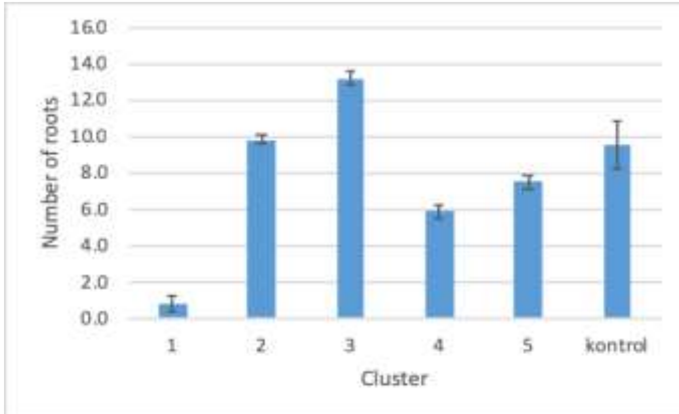

Figure 7. Length of *A. annua* roots in each cluster

Discussion

In general, plant breeding aims to improve existing crop varieties to be superior in some traits, such as to produce plants that are more tolerant to biotic or abiotic stresses, produce higher, and have superior quality. To achieve this goal, several reports have been published in related with increase in genetic diversity so that the selection of plants can be done in accordance with the desired plant breeding purposes. Increased plant genetic diversity can be carried out through a number of plant breeding methods such as hybridization methods, introduction, polyplodization and mutations (Crowder, 2006). Gamma ray irradiation was performed on *A. aanua* to obtain mutants with high artemisinin levels.

Gamma irradiation affects plant growth and development by affecting cytology, morphogenetic changes in cells (Mahamune *et al.*, 2011) and plant tissues (Aruna & Srinath, 2014). The diversity of growth *of A. annua* clones resulting from irradiation showed that mutation process by Gamma ray irradiation changed in plant growth both in positive and negative ways. This is beneficial for the selection of high artemisinin levels with high plant growth.

Gamma ray irradiation in *A. annua* had a positive influence on number of lateral shoots and the number of roots. Both parameters in clusters 3 and 5 were higher compared to other clusters. Meanwhile, the shoot height, leaf number, leaf length and length of petiole in cluster of control plants were not significantly different with that in clusters 3 and 5. These results indicated that the radiation dose had an effect of increasing growth (Sakr *et al*., 2013) or inhibiting plant growth (Hasbullah *et al.*, 2012). The results showed that the clones of *A. annua* mutants in cluster 3 were selected clones with better growth characteristics compared to control plant clusters. This finding is beneficial for selection of superior clones for the genetic improvement purposes.

Based on Ward analysis, Cluster 3 of *Artemisia annua* was the highest growth in terms of shoot height, length of the petiole and the number of roots compared to other clusters, whereas cluster 5 was the best for lateral shoots. The *A. annua* mutant having high growth consisted of 14 plants i e 5 Gy 3.a, 5 Gy 4.a, 5 Gy 19.a1, 10 Gy 10.a *, 10 Gy 13.a, 10 Gy 37.a, 20 Gy 1.a, 20 Gy 6.a, 20 Gy 16.a, 30Gy 12.a, 30 Gy 15.a, 30 Gy 18.a, 30 Gy 28.a and 30 Gy 38.a, respectively.

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