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ADAPTABILITY OF MUTANT GENOTYPES OF ARTEMISIA (*Artemisia annua* L.) AS RESULT OF GAMMA IRRADIATION IN THREE LOCATIONS WITH DIFFERENT ALTITUDE

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ABSTRACT

The objective of this study was to identify the adaptability of twelve artemisia mutant genotypes, which were planted in three locations with different altitude, as a result of gamma irradiation. Randomized Complete Block Design (RCBD) was applied in this research with three replications as blocks. The genotypes 1B, 1C, 1D, 2, 3, 4, 5A, 6B, 7A, 8, 14, 15 and two control genotypes as parent genotype from seed and from *in vitro* were used. The genotypes were planted in three different locations such as Mount Putri, Cianjur (1450 m above sea level), Pacet, Cianjur (950 m above sea level) and Cicurug, Sukabumi (540 m above sea level). Based on the method of postdictive and predictive success, the model used was AMMI2 which was able to explain up to 100% of interaction-influenced variation. The genotypes which were found stabile and adaptive in these three locations were 1B, 1C, 1D, 6B and 15. Genotypes 3 and 7A were adaptive specifically in Pacet area, 5A was adaptive for Gunung Putri while genotype 4 was for Cicurug only.

Keywords: AMMI, *Artemisia annua*, mutant genotype, adaptability

INTRODUCTION

Artemisinin is the active compound that is efficacious and effective antimalarial against Plasmodium parasites resistant to chloroquine (Paniego and Guiletti, 1993). This compound is produced by Artemisia (*Artemisia annua* L.) from temperate regions. This plant can grow well in the highlands of 1000-1500 m above sea level, therefore its geographical distribution is very limited (Gusmaini and Nurhayati, 2007). Meanwhile in Indonesia, an area with this height is usually planted with vegetables, so there is competition between the cultivation of vegetable crops with these plants.

Many efforts are needed to improve the adaptability of this crop, especially for the cultivation in the lowland, and one of them is through mutation breeding. Artemisia was irradiated with gamma rays on its buds, causing the diversity in either qualitative or quantitative characters (Syukur *et al*., 2008). The mutant genotype of artemisia needs to be evaluated and selected in order to obtain artemisia that has broad adaptability, especially for the cultivation below 1000 m asl.

The application of plant breeding could not be separated from the influence of the existing environment, since the growth of plant is a function of genotype and environment (Allard, 1960). The appearance of plant depends on the genotype, living environment and the interaction between genotype and environment. Specific crop response to the diverse environments resulted in the interaction between genotype and environment (GxL). The big effect of interaction will directly reduce the contribution of genetics in the final performance (Gomez and Gomez, 1985). Therefore, plant development is directed to create plant variety that is adapted to the widely varying environmental conditions (Pfeiffer *et al.,* 1995). According to Zhenghao *et al*. (2007), the content of artemisinin in Artemisia is influenced by climatic conditions, planting time, harvesting time, temperature, light and water.

Several methods to explain and interpret the genotype response to environmental variation had been developed. One method that can be used is the method called *additive main effects multiplicative interaction* (AMMI), as applied by Sumertajaya (1998), Kusumaningsih (2004), Sujiprihati *et al*. (2006), and Syukur *et al*. (2010). In this method, the analysis was done by combining the additive effect on analysis of variance and the multiplicative effect on principal components analysis (Mattjik and Sumertajaya, 2006).

The objective of this research was to study the adaptability of twelve mutant genotypes of artemisia resulted from gamma irradiation treatment at three sites with different altitude.

MATERIALS AND METHODS

This research was conducted from February to December 2009. The cultivation was conducted in three different locations: Gunung Putri Cianjur, Cianjur Pacet and Cicurug Sukabumi. Gunung Putri experimental field is located at an altitude of 1450 m above sea level (asl) with the soil type of andosol and a slope of 45^0 and pH 6.5. Pacet experimental field is located at an altitude of 950 m asl with the soil type of andosol having a pH of 6-7. Cicurug experimental field is located at an altitude of 540 m asl, with the soil type of andosol having a pH of 5-6.

Materials used in this study were 12 genotypes of Artemisia selected from the irradiated Artemisia which was grown in Gunung Putri Cianjur (year 2008), namely genotypes 1B, 1C, 1D, 2, 3, 4, 5A, 6B, 7A, 8, 14 and 15. Control genotypes used were those parent genotypes originally planted from seed and those from *in vitro* culture.

Randomized Complete Block Design (RCBD) was used with three replications as a block; replications were nested within location. The treatments consisting of 14 genotypes were randomly assigned to each block, so that there were 42 experimental units at each location. Each experimental unit consisted of five plants.

Seeds used for seedbed were produced from self-treatment from selected artemisia grown in Gunung Putri. The seeds were planted in mixture of compost-soil medium. One month seedlings on the seedbeds were transplanted to small polybags, and again were transplanted to the field after 1 month in the polybags.

Artemisia was planted in beds with a spacing of 1.2 x 1:25 m in Gunung Putri, 1 x 2.1 m in Pacet, and 1 x 1:15 m in Cicurug. The difference of planting space was due to the availability of land in each experimental field. Maintenance activities including fertilizing, installing stakes, and pest control were done. Fertilization was performed twice, at planting day and two months after planting with a dose of 3.5 grams of urea, 1.5 g KCl, and SP18 3 grams per plant. Installing the stakes was done two months after planting in order to prevent the plants from falling down, weed control was done manually, and pest control was done if the symptoms of plant pest and disease were noticed.

The harvesting was conducted when the plants reached 10-50% of flowering; this was done since the most numerous content of artemisinin occurred in the early phases of flowering. Harvested plants were weighed on their wet weight, and then separated between the stems, leaves and flowers. Drying was done by using direct sunlight. Observations and data analysis were conducted based on a dry weight of leaves since it represents the objective to be achieved in the production of Artemisia plants.

Quantitative data were analyzed using analysis of variance (ANOVA). When the result showed significant effect, then further tests were conducted using Duncan's Multiple Range Test (DMRT) at 5% level. Adaptability analysis was performed using AMMI (*Additive Main effect and Multiplicative Interaction*). AMMI adaptability analysis results were displayed by using the bi-plot to see any genotypes that were stabile at all sites or at specific locations. The analysis of these data was done using SAS 9 software facilities.

RESULTS AND DISCUSSION

Combined analysis of variance showed that the location, genotype and interaction of genotype by location were significantly different. It made possible to do analysis of AMMI. It is clear that the effect of location gave the biggest share to the diversity, followed by the effect of genotype, and then by the genotype and environment interaction effect (Table 1.).

The result indicated that the weight of artemisia dry leaf was strongly influenced by the factors of location, genotype, and interaction between genotype and environment (location). According to Vargas *et al*. (1998), significant interaction between genotype and environment will affect the plant expression, which means that the same genotype will produce different response in different environment.

The content of artemisinin in the leaves and flower of artemisia was 0.01-1.1% of the dry weight of leaves and flowers. Therefore, the cultivation of artemisia was directed to the production of leaf dry weight and artemisisin content (Namdeo *et al.,* 2006). Generally, genotype 5A had a higher leaf dry weight than control genotype from *in vitro* (KI), but did not differ in the control genotype from seed (KB).

The leaf dry weight of genotype 15 in Cicurug, genotype 5A in Gunung Putri and genotype 5A in Pacet were higher than the other two control genotypes (KI and KB). The average dry weight of leaves in Gunung Putri and Pacet was higher than that in Cicurug. The average dry weight of leaves of artemisia was 4910.8 kg/ha or 9.4 tonnes/ha (Table 2.).

In Cicurug (altitude 540 m asl), artemisia could be cultivated using these selected genotype in order to obtain the high dry weight of leaf. Artemisia which was grown in Pacet had no different in dry weight of leaves with those planted in Gunung Putri, but it had faster time to be harvested than those in Gunung Putri (data not shown). This means that Pacet Research Field (altitude of 950 m above sea level) is an appropriate location for the cultivation of artemisia as it could produce leaves dry weight of artemisia up to 7.38 tons/ha in 3-4 months, so that people can do cultivation of artemisia more than one in a year.

Table 1. Analysis of variance of the combined weight of 14 genotypes of artemisia dry leaves on three sites

Source of variance	Degree of freedom	Sum of squares	Mean Squares	F-Test
Location	2	11561717	5780859	34.24**
Replication/location	6	1012879	168813.2	3.26**
Genotype	13	4284839	329603	6.37**
Location*genotype	26	2765235	106355.2	2.06**

Remarks = **: highly significant at 1% level

Table 2. Dry weight of leaves of artemisia plants in three locations

Genotype	Gunung Putri (kg/ha)	Pacet (kg/ha)	Cicurug (kg/ha)	Average kg/ha)
1B	6083^{bc}	3701^{bcd}	893.2^{cd}	3559^{ab}
1C	5939^{bc}	3012^{bcd}	806.7^{d}	3253^{b}
1D	7495^{ab}	5451^{abc}	2005.8^{ab}	4984^{ab}
2	5633^{bcd}	2682^{cd}	760.1^{d}	3025^{b}
3	5845^{bc}	6846^{a}	1255^{bcd}	4649^{ab}
4	3445^{cd}	2969^{bcd}	1294.7^{bcd}	2569^{b}
5A	10859^{a}	7379^{a}	1731.4^{abc}	6656^{a}
6B	4784^{bcd}	3527^{bcd}	891.7^{cd}	3068^{b}
7A	4868^{bcd}	5629^{ab}	1227.7^{bcd}	3908^{ab}
8	1889^{d}	3150^{bcd}	848.8^{cd}	1963^{b}
14	7402^{ab}	5713^{ab}	936.5^{cd}	4684^{ab}
15	6818^{bc}	5202^{abc}	2241.9^{a}	4754^{ab}
KI	5713^{bc}	2182^{d}	479.1^{d}	2792^{b}
KB	4098^{bcd}	5247^{abc}	1027.4^{cd}	3457^{ab}
Average	5776.5^{A}	4477.9^{A}	4477.9^{B}	4910.8

Remarks = KI: control plant from *in vitro*; KB : control plant from seed. Figures followed by the same letters in the same column are not significantly different according to further Duncan's test at 5% level.Figures followed by the same capital letters in the same row are not significantly different according to further Duncan's test at 5% level.

The analysis of adaptability was performed using AMMI (*Additif Main Effect and Multiplication Interaction*). The result of AMMI stability analysis was displayed using the bi-plot to view genotypes which were stabile in all location or specifically stabile in particular location only. Genotype is stated as stabile if it is closed to the main axis, while it is stated as specific location genotype when it is away from the main axis but located adjacent to the location line.

The results of bilinear analysis toward the matrix of interaction effects of leaf dry weight showed the singular value (eigen vector) as follows: 791.42 and 542.40. Based on these values, the number of components that can be considered for AMMI is the first and the second components. Contributions of variance that could be explained by each KUI (Main Component of Interactions) are 67.95% and 32.05%, respectively. Based on this value of the contribution of diversity, it is evident that these two components can explain the diversity of interaction effects, which is equal to 100%.

Based on the postdictive success method, one significant IAKU1 with the value of 2.59 was obtained by F-Test (Table 3). Based on the method of *predictive success,* the model of AMMI2 has the smallest value of RMSPD that is equal to 896.154. Therefore, the number of component that is the best used for AMMI model is the model of AMMI2. AMMI2 model is capable to explain the diversity of interaction effect up to 100%.

Biplot of AMMI2 as a visualization tool of AMMI analysis can be used to view the stabile genotypes at all sites or specifically stabile at a particular location. Genotype stated to be stabile if it is close to the axis, while it is stated as genotype specific locations when it is far away from the main axis but located adjacent to the location line (Mattjik, 2005). The genotypes are stabile in this three sites based on AMMI are 1B, 1C, 1D, 6B and 15. Genotype 3 and 7A are suitable to Pacet location. Genotype 5A is suitable to the location of Gunung Putri, while genotype 4 is suitable for Cicurug location (Figure 1).

Table 3. Analysis of variance of AMMI towards 14 genotypes of artemisia in three different locations

Source of variance	Degree of freedom	Sum of squares	Mean Squares	F-Test
Location	2	11561717	5780859.0	34.24**
Location/replication	6	1012879	168813.2	3.26**
Genotype	13	4284839	329603.0	6.37**
Genotype*Location	26	2765235	106355.2	2.06**
IAKU1	14	1879042	134217.3	2.59**
IAKU2	12	886194	73849.5	1.43tn
Error	78	4035588	51738.3	
Total	125	23660259		

Remarks = AKU1 : analysis of main component 1 AKU2 : analysis of main component 2 ** : significantly influenced at level 1% tn : not significantly influenced

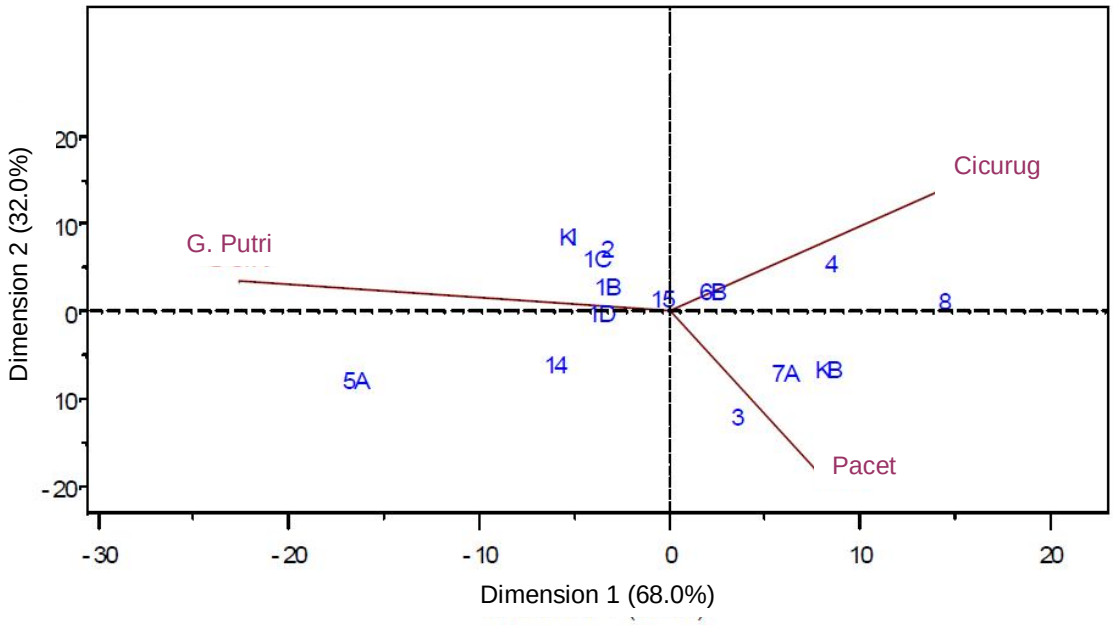

Figure 1. Biplot of interaction effect of AMMI2 model for the data of leaf dry weight of artemisia at three locations (compatibility of model: 100%)

CONCLUSIONS

Genotype 5A in Gunung Putri and genotype 1D and 15 in Cicurug had a higher leaf dry weight than those for the two control genotypes. Genotypes selected in Gunung Putri and Pacet were genotype 1D, 3, 5A, 14 and 15, while the genotypes selected in Cicurug were 1D, 3, 4, 5A and 15.

The genotypes adapted to the three types of altitude based on AMMI method were genotype 1B, 1C, 1D, 6B and 15. Genotype 3 and 7A were suitable to the Pacet location. Genotype 5A was suitable to Gunung Putri location, while genotype 4 was suitable to the Cicurug location.

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