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EFFECT OF GAMMA IRRADIATED POLLEN ON PURPLE CORN (*Zea mays* L.)

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SUMMARY

Corn is the most productive cereal crop in the world. It is suitable to be planted at the area of high temperature, as ear (cob) maturation is determined by the accumulation of heat gained by the plant. In Indonesia, field corn is the second staple food following rice, though the purple corn has not been found abundantly in this region. This research was carried out during two planting seasons (March to December 2014) at the Brawijaya University, Malang, East Java, Indonesia. Most of the current research discussed about the anthocyanin as favorable antioxidant for health and is reported to have for specific anti-cancerous effect. Efforts were made to determine the trait changes in purple corn plant following pollen mutation with gamma ray irradiation. The observations regarding amylopectin and anthocyanin contents including morphological and agronomic traits were recorded. The pollen of 40 purple corn genotypes have been irradiated with gamma irradiation with the given doses of 0 Gy, 25 Gy, 50 Gy, and 100 Gy per 10 genotypes at National Nuclear Energy Agency (BATAN), Indonesia. After pollination with irradiated pollen, 200 generated seeds in each irradiation level were used for planting material. Gamma irradiation affected on purple corn crops will all given doses except 0 Gyand observed some unique plants of curly glume stalk at 25 Gy, three-pronged ears at 50 Gy, and an extra-ordinary long for ear stalk at 100 Gy. The regression of gamma irradiation dose with amylopectin was $y = -0.001x^2 + 0.171x + 40.17$ (R2 = 0.802), while that with anthocyanin was $y = -0.001x^2 + 0.026x + 0749$ (R2 = 0.617).

Keywords: Purple corn, gamma irradiation, pollen mutation, anthocyanin

Key findings: Gamma irradiation corns at doses of 25 Gy, 50 Gy and 100 Gy on pollen for generating M1 plants has affected to change several quantitative and qualitative characters in purple. Different dose gave different influence either on quantitative or qualitative traits. Dose 25 Gy was found effective to increase almost 1 ppm anthocyanin content from control. Dose 50 Gy typically changed on amylopectin about 3% higher and leaf length with 5 cm longer than control. However, 100 Gy prolonged ear stalk for about 4 cm. Some unique putative mutants were found in this research, i.e. three-pronged ear, ear stalk and curly glume stalk.

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INTRODUCTION

Corn is an essential and the most productive cereal crop in the world as it is suitable at high temperature area especially for its ear maturation which is determined by heat accumulation gained by the plants. The planted areas of corn in the world cover more than 100 million ha that spread over 70 countries of mostly tropical and subtropical climates including 53 developing countries (Dowswell *et al.*, 1996). In Indonesia, corn is the second staple food following rice especially for people in Eastern Java and in most East Nusa Tenggara (NTT) province. It is presumed that more than 55% domestic demand of corn is used for feed and the rest is for other industries, food and seeds (Kasryno *et al.,* 2007).

Currently, purple corn has attracted the attention of most people in the world due to its antioxidant flavonoid, including anthocyanin which is good for human health. The people of Peru have used purple corn as a special drink (Chicha Morada) made with water of boiled corn food stuff for centuries. Food research informed that purple corn contains antioxidant, which is able to inhibit carcinogen that induced tumor on rats, and mentioned that anthocyanin is a very favorable antioxidant with anti-cancerous properties for health (Jones, 2005). Purple corn also plays an important role in industrial sector. Anthocyanin derived from purple corn has been applied as coloring substance for drinks, jellies, and candies in Japan. Purple corn has approached the classification as food of integral functional component from diet that provides very essential nutrient and energy. Some researchers on food and nutrient have endeavored to look for functional foods in almost all over the world from variances of plant, including purple corn that contains high anthocyanin (Aoki *et al.,* 2002).

Starch also plays an important role in purple corn chemistry. According to Rubi (2009), starch is beneficial and flexible polymer, however, for the last few years it has affected morphological and functional properties of flour product. Corn has more diverse types than other starch sources, starting from low to high amylopectin content. It is categorized into four types based on its starch property, such as field corn contains 74-76% amylopectin, waxy type contains 99% amylopectin, Amylomaize contains 40-70% amylose and sweet corn contains 22. 8% amylose (Richana and Suarni, 2007). Hybrid purple corn is intermediate type between waxy and amylomize type. Purple corn with high amylopectin are more smoothies and sticky in nature.

The new and broad genetic variation in purple corn can be achieved through irradiated pollen both form quality and quantity perspectives. Gamma irradiation is one of essential physical mutagene on mutation of crops (Savaskan and Toker, 1991). Application of gamma irradiation in crop breeding has been performed on either somatic or reproductive organ tissue to increase genetic variance (Sawangmee *et al.*, 2011). Genetic variance as a result of gamma irradiation may provide wider opportunities for advancement in crop breeding, so that the required properties can be inherited. Van-Harten (1998) informed that irradiated pollen, due to sensitivity, could be used to solve ovule fertilization problem.

Effectiveness of gamma irradiation is depended upon dose or a unit for absorbed energy, so that it can be measured with gray (Gy) equivalent to 1 $J.kg^{-1}$. A dose unit of irradiation has been established as well, 100 rad = 1 Gy, or 1 krad = 10 Gy (Kovacs and Keresztes, 2002). So far, some researches have proven that gamma irradiation may affect the contents of carbohydrate and flavonoids too (Liu *et al.*, 2011, Kovacs and Keresztes, 2002, Siavash *et al.,* 2011). The morphological and agronomical characters are very common to be observed in order to realize the mutation effect on any crop. Therefore, a research project was planned with the objectives determine the trait changes in mutant first generation (M1) of purple corn plant following pollen mutation with gamma irradiation.

MATERIAL AND METHODS

Research on gamma irradiation was conducted during two planting seasons (March-July and August December 2014) at Laboratory of Research and Technological Development, Center for Isotope and Irradiation (PAIR) BATAN Pasar Jumat, Jakarta, Indonesia. Planting was done at the Experimental Station of Brawijaya University, Malang Regency, East Java, Indonesia with 300 m a.s.l for altitude and about 2150 mm for precipitation per year.

The adequate quantity of pollens were harvested from 40 plants of purple corns then mixed and cleaned with tea sieve to separate them from their pollen tubes and other wastes. The cleaned pollens were divided into four treatments, in which each of them comprised of four parts. Each part was put into plastic bag that had been filled with silica gel. To keep pollen viability during time to irradiation, they were put into Styrofoam box which had been filled with dry ice and then sealed tightly. Due to distance, gamma irradiation was then conducted a day after harvest. Pollens were irradiated with different doses of gamma radiation from Cobalt 60. The doses used were 0 Gy, 25 Gy, 50 Gy, and 100 Gy. After having gamma irradiation, each treated pollens were applied for hand pollination on a unit experiment consisting of 40 plants in the same day or a day after harvest. Forty five days after pollination generated seeds from each treatment were harvested and processed for planting materials.

The research comprised of two parts, i.e. field experiment for growing seeds regenerated from irradiated pollen fertilization, and analysis of anthocyanin and amylopectin contents on the first generation of mutant (M1) seeds. Anthocyanin and amylopectin analysis were conducted according to the modified method of Wuttisela *et al.* (2008). As the research applied an explorative analysis, a genetic observation was also performed on morphological and agronomical characters for each individual plant, which may have favorable genetic potency, well growth, as well as unique traits in M1 plants. Fourteen traits observed in the experiment were days to emerging plumulae, days to tasseling, days to silking, ear height, plant height, leaf length, leaf width, leaf number, days to harvest, ear stalk length, ear length, ear diameter, seed number per cob and number of seed rows.

The experiment was carried out in randomized complete block (RCB) design with three replications. Normality data were firstly examined using Kolmogorov-Smirnov test and the normal data was then tested with Analysis of Variance (ANOVA). Tukey test was also applied after conformity of significance ($P < 0.05$). Data of amylopectin and anthocyanin contents were analyzed using simple correlation analysis using software Origin-8.

RESULTS

Based on Kolmogorov-Smirnov analysis (Table 1), all data were spread normally except days to silking resulted from 50 Gy irradiation, so that they were analyzed using ANOVA. The Tukey test in Table 2, showed that different doses of gamma irradiation have no affected on seed per ear, number of seed rows, leaf width, leaf number, ear height, ear diameter, ear length, plant height, days to tasseling and days to silking, but differed on leaf length and ear stalk length. Leaf length treated with dose of 0 and 25 Gy did not differ from one to another with 85.66 to 86.63 cm length, but it was found that 50 and 100 Gy irradiation was higher than earlier doses which reached about 91.68 to93.49 cm length.

In the contrary, seeds regenerated from pollination by 100 Gy irradiated pollens resulted longer ear stalk than others did that reached 14.35 cm length. For days to harvest, all doses except 50 Gy did not show any significant effect. Application of dose 50 Gy retarded germination so that the days to emerging plumulae was 0.4 days longer than other treatments.

The round shape was dominant with more than 70 % for first leaf with all given doses (Figure 1). The remains were intermediate or spatula-like shape. At 100 Gy, the first leaf with sharpened tipwas also observed (Figure 1). In addition, observation on anthocyanin coloration in stem resulted that the higher dose applied for irradiation, the lower anthocyanin coloration existed. Here, 0 Gy gave about 15% anthocyanin, while doses 25, 50 and 100 were 7%, 5%, and 3%, respectively. In line with observation on anthocyanin coloration of stem, coloration of glume also revealed in the same regulation. Higher dose of gamma irradiation lead to decrease in coloration of glume. The 15% glume coloration was found from 0 Gy, while doses 25, 50 and 100 constributed 10%, 5%, and 4%, respectively.

Table 1. Normality test on data of Kolmogorov-Smirnov.

Parameter	Dose	Significance	Parameter	Dose	Significance
Seed number per cob	0	0.000*	Plant height	0	0.668
	25	0.940		25	0.777
	50	0.396		50	0.671
	100	0.107		100	0.736
Number of seed row	0	0.363	Number of leaf	0	0.044*
	25	0.843		25	0.075
	50	0.274		50	0.307
	100	1.000		100	0.756
Leaf length	0	0.681	Ear height	0	0.317
	25	0.766		25	0.233
	50	0.735		50	0.848
	100	0.344		100	0.603
Leaf width	0	0.949	Days to harvest	0	-
	25	0.537		25	0.892
	50	0.290		50	0.648
	100	0.172		100	0.665
Ear stalk length	0	0.645	Days to tasseling	0	0.942
	25	0.782		25	1.000
	50	0.088		50	0.780
	100	0.392		100	1.000
Ear diameter	0	0.000*	Days to silking	0	0.780
	25	0.424		25	1.000
	50	0.886		50	0.000*
	100	0.972		100	0.298
Ear length	0	0.277	Days to emerging plumulae	0	0.391
	25	0.782		25	0.915
	50	0.556		50	0.629
	100	0.323		100	0.691

*= Data is abnormally spread

Table 2. Result of Tukey test on each parameter.

Dose	Seeds per Ear	Number of Seed Row	Leaf Length	Leaf Width	Ear Stalk Length (cm)	Ear Diameter (cm)	Ear Length (cm)
0	46.00	11.10	85.66a	8.57	9.78a	3.98	13.72
25	53.03	11.27	86.63a	8.70	9.55a	2.80	13.82
50	49.73	10.70	93.49b	8.67	10.92a	3.51	13.71
100	55.53	11.50	91.68ab	8.75	14.35b	2.73	15.47
Tukey	NS	NS	6,423	NS	2,384	NS	NS

Dose	Plant Height	Leaf Number	Ear Height	Days to Harvest	Days to Tasseling	Days to Silking	Days to Emerging Plumulae
0	114.83	10.21	117.83	86.00a	44.27	45.70	3.27b
25	123.68	9.53	123.76	85.81a	44.00	45.30	3.15a
50	116.95	9.28	126.03	86.92b	45.57	47.27	3.33c
100	124.95	9.30	119.04	85.64a	43.60	45.53	3.24b
Tukey	NS	NS	NS	0.398	NS	NS	0.054

Numbers followed by different letter in the same column show significant difference on Tukey test, while numbers followed by the same letter or without letter in the same column show insignificant difference.

Figure 1. First leaf corn: Unique type (1), 100 Gy (2), 50 Gy (3), 25 Gy (4) and 0 Gy (5).

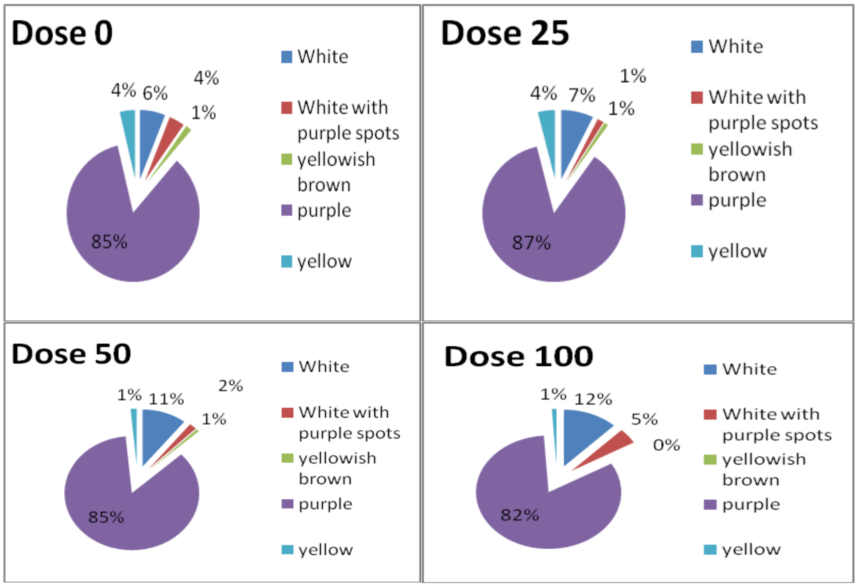

Figure 2. Distribution of seed colors as the response of gamma irradiation doses.

The seed color variation with most dominant purple color was observed in each given irradiation doses (Figure 2). In among doses treated, except a minute percentage of seed color variance, most of the seeds (87%) were found in purple color. Interestingly, the greatest percentage of white and purple-spotted color was gained by 100 Gy. Of the ear tip shape observation, the dominant rounded ear tip shape with cylindrical, conical–cylindric and cylindric were observed. The most dominant rounded ear tip shape with 46% was found from 25 Gy compared to other irradiation doses. Dominant kernel types in this purple corn was96 % flint which normally distributed in each irradiation dose. However, it was found in 100 Gy that almost 32 % plant have dent kernel type (data un-published). Their seed row formation was dominantly regular form, and dose 100 had the most regular formation.

Unique putative mutant

A three-pronged ear as a unique putative mutant was found only at dose 50 Gy (Figure 3). At dose 100 Gy, it showed the highest mean (29 cm) for the ear stalk length, while the average length for contol plants was only 14 cm. Another putative mutant was curly glume which found only in dose 25 Gy (Figure 3). The shape was quite different from those likes in any other dose.

Correlation between gamma irradiation and amylopectin and anthocyanin contents

Coefficient determination between gamma irradiation dose (X) and amylopectin content (Y) has been resulted with $R^2 = 0.802$ (Table 3 and 4) and equation gamma irradiation dose-amylopectin content was quadratic $Y = -0.001x^2 + 0.171x + 40.17$ (Figure 4). For gamma irradiation dose- anthocyanin content, the R^2 value was lower than that with amylopectin. It was $R^2 = 0.617$ with the equation $Y = -0.001x^2 + 0.026x + 0.749$ (Table 3 and 4). Both equation showed a hiperbolic effect of irradiation dose, so that the optimum dose of gamma irradiation was 25 Gy that reached 1.60 ppm anthocyanin.

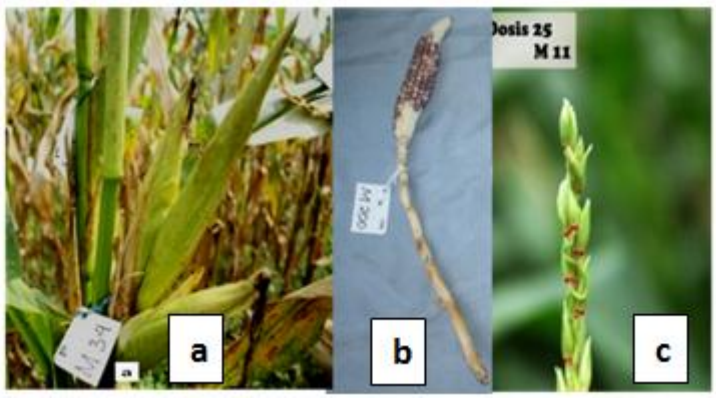

Figure 3. Unique M1 putative mutant of three-pronged ear at dose 50 Gy (a), extra ear stalk at dose 100 Gy(b) and curly glume at dose 25 Gy(c).

Table 3. Anthocyanin and amylopectin contents in M1 seed generated from irradiated pollen.

Dose (Gy)	Row	Amylopectin (%)	Anthocyanin (ppm)
	1	40.97	0.47
0	2	42.66	0.99
	3	38.70	0.39
	1	39.97	1.49
25	2	40.54	1.32
	3	45.70	1.99
	1	41.73	1.76
50	2	46.74	0.97
	3	52.21	0.71
	1	46.98	0.26
100	2	44.53	1.05
	3	43.13	1.08

Table 4. Coefficient of Pearson's correlation (r) between gamma irradiation and anthocyanin and amylopectin contents.

No.	Starch Analyzed	Coefficient of correlation (*r*) by Gamma Irradiation
1	Amylopectin	0.896
2	Anthocyanin	0.785

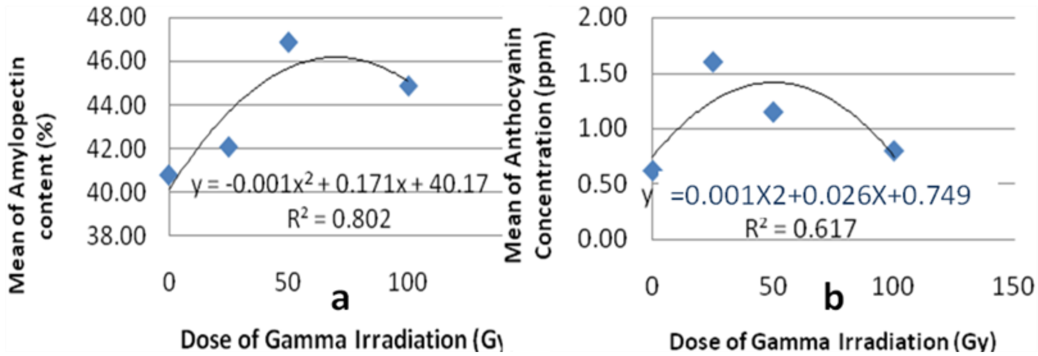

Figure 4. Regression of gamma irradition with amylopectin (a) and with anthocyanin (b).

DISCUSSION

Effect of gamma irradiation on morphological characters

Qualitative characters are easily categorized in noticeable phenotypic categories due to their straight distribution (Falconer, 1989). The observable qualitative characters include colors of anthocyanin in glume and internode, shape of the first leaf tip (shoot), color of anthocyanin of the first leaf cup, angle between leaf and stem, and curving angle of the leaf blade and are controlled by simple one or two genes, recognizable effect, simple inheriting ways and less environmental affect to them (Welsh, 1991).

During the observation on color of anthocyanin in glume, the dominant percentage was found at dose 0 for about 15%. While at doses 25, 50, and 100 were 10%, 5%, and 4%. On the leaf observation, the percentage of the most dominant shape is round, for about 55%, 56%, 70% and 52% at doses 0 Gy, 25 Gy, 50 Gy and 100 Gy, respectively. The round leaves increased at doses 0-50, but decreased sharply at dose 100 remained 52%. Leaf area is important aspect because it determines the progress of photosynthetic process, as well as respiration and transpiration, which determine the crop's growth and development. The increasing leaf area could increase effectiveness of the crop in intercepting light, so that it will increase the photosynthates. Regarding with previous observation (unpublished) on angle between leaf and stem, the most dominant aspect of small angle was found at dose 25 which reached about 64%. Observation on curving angle of the leaf blade, the acute angle increased at dose 25 for about 76% and dose 100 for about 72%, while at dose 50, the acute angle decreased for about 54%. Leaf that has upright shape showed smaller curving angle. Plants having more upright shape, tended to produce higher weight of seeds. This finding is in closer agreement with the Sutoro *et al.* (1997) who found that the position of the corn leaves on crop, based on angle and its curve, will affect the intercepted light, and finally it will also determine productivity of the crops.

The flowering phase of tassel ranged from 45 to 52 days, as marked by the tassel branch before silking. Tasseling stage started 2-3 days before the silking, in which the plants almost reached maximum height and started to disperse the pollen. During this phase, maximum biomass was produced from the vegetative parts, for about 50% out of total dry weight of the crops, however, N, P, and K were absorbed by the crop for about 60-70%, 50% and 80-90%, respectively (Subekti *et al*, 2011).

Based on results of the research, average of days to tasseling at doses 0, 25, 50 and 100 Gy were 44, 44, 45 and 43 days. Dose 100 is potential to have faster silking than all doses. Meanwhile, average of days to silking at doses 0, 25, 50 and 100 were 45, 45, 47 and 45 days, respectively. The findings from Subekti *et al.* (2011) also revealed that the silking was initiated by the emergence of silk from inside of the ear, which is covered up by the corn husk and started 2-3 days after tasseling.

The tallest crops were found at dose 100 Gy. Height of the crop affects numbers of leaf, but not all higher crops have more leaves. According to Singh (1988) who reported that varied heights of crops are considered as very influential characters. Results of the tukey test on morphological characters showed that each dose has significant difference results as on characters of the plumulae emergence, length of leaf, harvesting time, and length of the ear stalk. On the quantitative character, dose 100 Gy has potential quality in this pollen mutation research. Several changes or putative mutations on ear stalk length, leaf width and crop height were found at dose 100 Gy.

Effect of gamma irradiation on agronomical characters

The types of the corn seed showed that the most dominant type in this research is flint or (pearl) type. Subekti *et al.* (2011) reported that in general, local variety of corn in Indonesia belongs to flint type. Most farmers like this type because it is resistant to pests, which are usually found in the storehouse. Based on results of the observation, the higher dose of gamma irradiation, more variances of the seed types will be resulted. It has been shown by percentage of the seed type with the doses 0, 25, 50 and 100 for about 96%, 96%, 94% and 68%, respectively. However, intermediate and dent like types are the most dominant at dose 100 for about 20% and 12%, respectively. Intermediate type is the integrated type between flint and dent.

F-test results on agronomical characters showed that each dose has influenced on ear height, ear length, ear stalk and the days to harvest. Allison and Watson (1966) reported that dry matters for the corn seed are generally derived from the photosynthetic products after flowering. The quantitative characters, which have no significant differences, include ear diameter, seed rows, and seed weight per ear. Therefore, it showed that the observable variances are relatively uniform.

The exposure of gamma irradiation by a given dose has changed the seed colors and varied on generation M1 of purple corn. Generation M1 showed white seeds, purple-spotted white, yellowish brown, purple and yellow. Chang and Badernas (1965) stated that mutation may create variations on anthocyanin pigmentation with 3 controller genes including *C-A-P*. Chromagene is controlled by C allele, A allele as activator, P allele controlling location or organs (on the blade as shown by Pl gene), on I allele as inhibitor. Variations of color that may emerge are white, grey, pink, red, and purple.

Correlation of gamma irradiation dose toward amylopectin content

Based on the analysis, positive correlation was found between gamma irradiation dose and amylopectin content. Such positive result showed that gamma irradiation in purple corns increased the amylopectin content at doses 0-50 Gy. Maximum amylopectin content was found at irradiation dose of 50 Gy. Higher gamma irradiation dose, 100 Gy, reduced the amylopectin content. It is supported by Kovacs and Keresztes (2002) in which the apple cells show significant increase of soluble carbohydrate at irradiation doses 1000-2500 Gy. It indicated that irradiation stimulates polysaccharide autolysis in the apple cells. Starch granules or irregular shapes of starch become smaller and more rounded shapes during the degraded process. Research by the findings from Irawati and Atika (2004) showed that the irradiation doses 0, 3000 and 5000 Gy on the pumpkin, showed that the starch level decreased along with the increasing dose of irradiation. It may be caused by depolimerazion process at glicosidic chain, which produces more simple components due to radiation. The stability of the amylopectin content toward gamma irradiation has related to composition of the purple corns.

Correlation of gamma irradiation dose toward anthocyanin content

Gamma irradiation had no significant correlation with anthocyanin content. Correlation between anthocyanin and gamma irradiation was shown by the coefficient value of Pearson's correlation -0.89. The coefficient values of correlation between gamma irradiation dose and anthocyanin content that approach -1, have very close correlation. The higher gamma irradiation dose was applied, the lower anthocyanin content was resulted. Chachin and Ogata (1969) studied the effect of gamma irradiation 2-80 kGy on grapes, apples, and orange juice. Based on results of their research, at dose ≥ 10 ≤ 80 kGy, anthocyanin content in grapes was lost. However, Ayed *et al.* (1999) observed on wastes of mangosteen, the increasing anthocyanin occurred at doses 0-9 kGy, particularly at dose 6 kGy. Meanwhile, in research by Alighourchi *et al.* (2008) on gamma irradiation toward pomegranate juice, average loss of anthocyanin content on all pomegranate juices occurred following the exposure of diverse irradiation doses (0, 0.5, 2, 3.5, 5 and 10 kGy) between 22% and 90%. Pomegranate juice, which was irradiated at doses 0.5 and 10 kGy could maintain 80% and 10%. According to Chang and Badernas (1995), mutation may create variation in anthocyanin pigmentation.

At dose 25 Gy, amylopectin content reach the highest level, 158%, in comparison with dose 0 as control. Moreover, at doses 50 and 100 Gy were 85% and 29%, respectively. These results indicated that stability of amylopectin content toward gamma irradiation has related to composition of the purple corn. Results of the research on purple corns showed that the increasing anthocyanin content would be effective if it was irradiated at dose 25 for about 1.60 ppm on average. The highest anthocyanin content was found at average dose 25 Gy row 3 for about 1.99 ppm, while at doses 0, 50, and 100 Gy, the average content of anthocyanin were 0.62, 1.15 and 0.80 ppm.

CONCLUSIONS

Gamma irradiation affected on purple corns at doses 25 Gy, 50 Gy and 100 Gy. Different dose gave different influence either on quantitative or qualitative traits. Some unique putative mutants were found, such as three-pronged ear at dose 50 Gy, ear stalk, length at dose 100 Gy, and curly glume stalk at dose 25 Gy. Even though gamma irradiation and anthocyanin have negative correlation, at dose 25 Gy, it can be considered as the most effective dose in increasing anthocyanin content. All change from gamma irradiation exposured, need to be observed their beneficary in advance for next breeding program.

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