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Sorghum Breeding for Improved Drought Tolerance Using Induced Mutation with Gamma Irradiation

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ABSTRACT

Sorghum has a great potential to be grown and cultivated in Indonesia due to its wide adaptability and high productivity. Since sorghum is not a native species of Indonesia, the genetic variability of this crop in Indonesia is low, thus, plant breeding program is required to support national sorghum development. The objectives of this study were to develop superior genotypes to improve sorghum production and quality for food, animal feed and bioethanol industry. Sorghum production is aimed at optimal use of unproductive or marginal land such as that of drought prone areas. Sorghum breeding through induced mutations was conducted at Center for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN). Durra variety was used as parental materials in the breeding program. Induced mutation was made by gamma irradiation on seed treatments. The optimal radiation dose was found to be between 300-500 Gy. Through selection processes and direct screening for drought tolerance in Gunung Kidul district, ten putative mutant lines had been selected. In dry season, the mutant lines B-68, B-72, B-95 and B-100 produced grain yields of 4.55, 4.50, 4.20 and 4.62 ton ha^{-1}, respectively. These yields were significantly higher than the original parent Durra (3.50 ton ha^{-1}) and the control check varieties UPCA (2.68 ton ha^{-1}) and Higari (3.75 ton ha^{-1}). The mutant lines B-68, B-72, B-94, and B-100 were drought tolerant genotypes having a relatively higher drought index. These mutant lines were promising for further sorghum breeding and development in accordance with attempts of increasing land productivity of drought prone areas. Sorghum cultivation in such areas would promote land conservation and support sustainable agriculture development in the region.

Keywords: drought tolerance, induced mutation, mutant lines, sorghum

INTRODUCTION

According to the Statistics Central Bureau, total Indonesian population will reach 316-350 million people in 2025 (BPS, 2007). In this situation, rice demand (as staple food) will increase to 65.9 million tons. If rice production is just the same as in 2005, then Indonesia will face food deficit of 13.1 million tons, leading to food crisis in 2025. This is not exaggeration since rice is the main food crop grown by farmers, and other food crops remain insignificant compared to rice. Rice plant requires much water for its growth and it is mostly grown in the irrigated paddy fields. Arable land in Indonesia is actually dominated by dry land farming areas. Meanwhile, rice production in the country tends to decrease due to several reasons such as intensive expanding of land use changed to non-agricultural purposes (Suwarno *et al.*, 2004; Mattjik, 2007). Also, the fact that water scarcity still becomes problem in some agricultural areas will make dry land farming systems become more reliable to be developed in order to support sustainable agriculture and food security in the country. Developing dry land farming systems should be focused on crops that require less water. Sorghum is a suitable crop for dry land farming system in Indonesia owing to its wide adaptability, drought tolerance, high yielding and big potential use for food, animal feed and bioethanol industry (House, 1985).

Sorghum (*Sorghum bicolor* L.) is a cereal crop that is usually grown under hot and dry conditions. According to House (1985) sorghum might be originated from the headwaters of the Niger River in Africa. Archaeological evidence suggested that the practice of sorghum domestication was introduced from Ethiopia to Egypt about 3000 B.C. Now about 80% of sorghum cultivation is found in the Africa and Asian regions, however, the world sorghum production is still dominated by the USA, India, Nigeria, China, Mexico, Sudan and Argentina (ICRISAT/FAO, 1996).

In many countries sorghum is generally used as food source, animal feed, and raw materials for industries. As global food source sorghum ranks the fifth after wheat, rice, corn, and barley. U.S. GRAIN COUNCIL (2005) mentioned that grain sorghum is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world. Based on the form of its spike and basic spikelet, sorghum is classified into 5 races namely *Bicolor*, *Guenia*, *Caudatum*, *Kafir*, and

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Durra. Race Durra has white color of grains is the one that is commonly cultivated as grain sorghum and used as food source. Among race Durra, there is a variety in high sugar content in its stalk, a type known as sweet sorghum. In many countries, sweet sorghum is used for syrup, sugar (*jaggery*), and/or ethanol industry (Rajvanshi and Nimbkar, 2005; Undersander, 1990).

Sorghum has a high yield potential, comparable to rice, wheat, and maize. On a field basis, yields have exceeded 11 ton ha^{-1}, with above average yields ranging from 7-9 ton ha^{-1} where water is not limited. In the areas where sorghum is commonly grown, yields of 3-4 ton ha^{-1} are obtained under normal condition (House, 1985). Sorghum is also known to have wide adaptability, ranging from lowland, medium to highland altitude. Highest yields are usually obtained from varieties maturing in 100-120 days. Late-maturing varieties tend to be appropriate for forage crop.

Sorghum is not Indonesian origin, therefore, its genetic variability in Indonesia is still limited. Some sorghum genotypes have been introduced from other countries e.g. from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Through plant breeding programs, the Ministry of Agriculture has released some sorghum varieties such as *UPCA*, *Keris*, *Mandau*, *Higari*, *Badik*, *Gadam*, *Sangkur*, *Numbu* and *Kawali* (DEPTAN, 2003). These national varieties have big potential to be cultivated and developed in arable lands during normal growing seasons. Further sorghum breeding is needed especially to obtain drought tolerant genotypes that can overcome problem of water shortage during prolonged dry seasons.

Sorghum has been reported to have high tolerance to adverse conditions such as drought (House, 1985). Compared to maize, sorghum has a more extensive and fibrous root system. The plant roots penetrate a greater volume of soil to obtain water. Fertilizer, even under low rainfall conditions, encourages root development, hence the root are able to extract water from a greater volume of soil. Sorghum requires less water for growth than other cereal crops. A study shows that sorghum requires 332 kg of water per kg of accumulated dry matter, while maize requires 368 kg of water, barley 434 kg, and wheat 514 kg. Compared to maize, sorghum is also more tolerant to water lodging, salinity, and aluminum toxicity (Rana and Rao, 2000).

For the areas which has drought problem such as that in Gunungkidul District of Yogyakarta Province, sorghum could be the choice to be grown especially during dry season. Sorghum breeding to improve its yield in drought prone areas is of importance for increasing the overall agricultural production in the region. Sorghum biomass that can yield up to 90 tons ha^{-1} could be suitably used for animal feed, while sorghum grain of white type is commonly used as food source since it has good nutritive values (DEPKES RI, 1992). Sorghum grains are also commonly used as raw material for industry such as ethanol, beer, wine, syrup, paint, glue, and modified starch industry (ICRISAT, 1990).

Sorghum can also be used as source of energy. Countries like USA, India and China, have developed sorghum as bioethanol (biofuel). In the USA sorghum can produce up to 10,000 L ethanol ha^{-1}, while in China 7,000 L ethanol ha^{-1} and in India 3,000-4,000 L ethanol ha^{-1}. In India, an improved, pressurized, multifuel (kerosene, ethanol or diesel) mantle lantern producing light output of 1,250-1,300 lumens (equivalent to that from a 100 W light bulb) called "Noorie" was developed. A pressurized alcohol stove with a heating capacity of 3 kW for 85% (v/v) ethanol concentration with a thermal efficiency of 30-50% was also created (Rajvanshi and Nimbkar, 2005). For Indonesia, it is a big challenge to develop sorghum for bioetahnol industry in dry land farming system in order to anticipate future energy crisis. Research toward that objective is being conducted through collaboration of sorghum breeding institutions with some relevant private industries.

MATERIAL AND METHOD

Sorghum breeding by using mutation techniques had been carried out at the Center for Research and Development of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN), Indonesia since 2000. Sorghum Durra variety from ICRISAT was used as starting breeding material. The dry seeds of sorghum Durra variety with water content of 12% were irradiated with Gamma rays emitted from Cobalt-60 source in the Gamma Chamber 4000A. The dose levels of 0-1000 Gy, with increment of 100 Gy, were used in order to estimate appropriate radiation dose for breeding purposes. Response of sorghum growth in the M1 generation was studied by best-fitting curve software, and the LD-20 and LD-50 values were estimated. The dose level between these two LDs was then used in generating plant population in further breeding program as suggested by IAEA (1977).

Some M1 plants were harvested to generate about 4,000 M2 plants. Individual plant selections based upon phenotypic variations were started in the segregating M2 population, focusing on agronomic and yield characters. The parental variety Durra and two national varieties (UPCA and Higari) were used as controls. The screening for drought tolerance was a combination of indirect selection (PEG method) and direct selection in the field. The PEG method, a concentration of 25% polyethylene glycol was used and operated in seedling stage. According to Singh and Chaudhary (1998), PEG could reduce water potential equivalent to natural drought condition so that water absorption by roots could be affected. In direct selection, the plants were grown directly in drought prone areas during dry season. Production of total biomass, yield and its components were used as criteria for selection.

A total number of 170 selected tolerant plants from the PEG method were then transplanted to the field for seed multiplication. Direct drought tolerance test for these selected plants was conducted in the field in Gunungkidul during dry season in 2002 (M4), 2003 (M5), and 2004 (M6), respectively. Sowing time was adjusted to the end of rainy season i.e. in April where average rainfall was about 253 mm (Table 1). Artificial irrigation was given only in early growth stage to stimulate seed germination. One month after sowing, the irrigation was stopped and the plants were entirely exposed to the natural drought condition. Condition of average rainfall and rain day at the experimental location is presented in Table 1. Agronomic data of plant height, number of leaves and grain yield were measured and used as criteria for drought tolerance. The growth and yield of the selected drought tolerant plants were also evaluated in the field condition during rainy season. Sowing time as adjusted to early rainy season i.e. in December where average rainfall was about 201 mm (Table 1). Randomized block design was used in this experiment. Drought index was calculated by dividing the grain yields under drought to that of rainy condition. The drought index measures relative sensitivity of genotypes to water stress response with regard to grain yield production.

Table 1. Average rainfall and rainy days during the field trials in Gunungkidul District, Yogyakarta Province (BPP Semanu, 2005)

Month	Average	
	Rainfall (mm)	Rainy days
January	310.3	18
February	329.0	19
March	280.3	15
April	253.0	9
May	58.6	3
June	67.0	4
July	38.0	2
August	14.1	1
September	6.1	1
October	85.6	5
November	112.8	8
December	201.4	15
Total	1.756.2	100

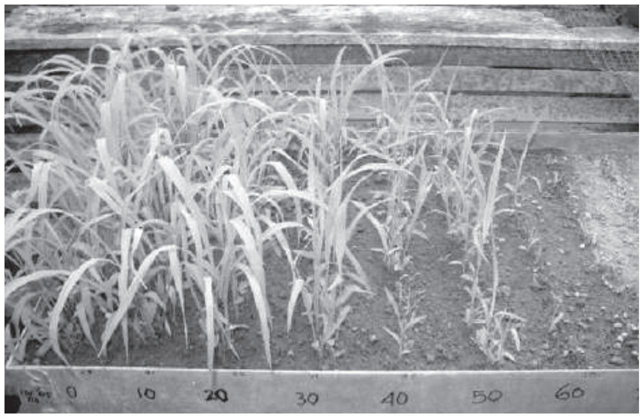

Figure 1. Visual performace of gamma irradiation effects on sorghum growth in the M1 generation

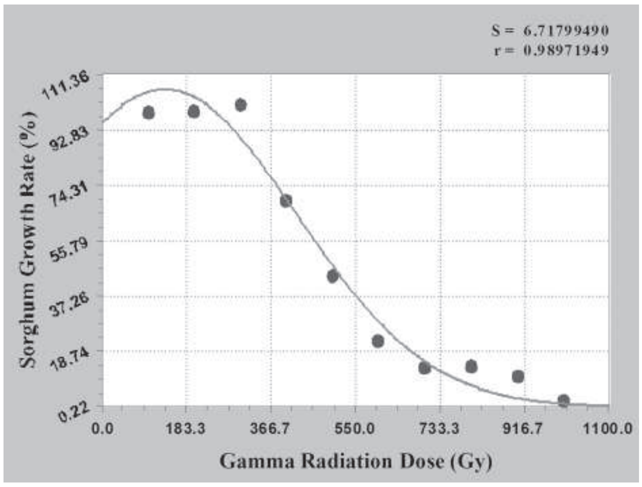

Figure 2. Effect of gamma irradiation on growth rate of sorghum Durra variety in the M1 generation. There was polynomial fit $Y = a + bx + cx^2 + dx^3$ with a = 99.3389, b = 0.1068, c = -0.0006 and d = 4.2185.

RESULTS AND DISCUSSION

The visual effects of gamma irradiation on sorghum seedling growth in the M1 generation are shown in Figure 1. The sigmoid growth curve was studied further by best-fitting model for the relationship between radiation doses and survival rates. It was found that the 3rd degree polynomial fitted the model (r=0.9897, Figure 2). From this model, it was estimated that the LD-20 = 344 Gy and LD-50 = 504 Gy. This interval doses might be used in determining optimal radiation doses for breeding purposes. For further breeding program, attention should be focused on population derived from the dose within the interval.

Further sorghum breeding was focused on population derived from radiation dose of 400 Gy. From this population, a total number of 170 plants were selected for drought tolerance in seedling stage in the greenhouse. These 170 selected plants were grown in drought prone areas in Gunungkidul for direct field test during dry seasons. Through field screening and selection processes in successive generations, ten drought tolerant mutant lines were identified. These mutant lines were B-68, B-69, B-72, B-75, B-83, B-90, B-92, B-94, B-95, and B-100. Their agronomic data and yield in dry and rainy seasons are presented in Table 2. Some of the mutant lines had agronomic and yield performances significantly better (higher) than those of the original variety (Durra) and the control national check variety (UPCA and Higari). Some visual performances of some mutant lines are presented in Figure 3.

In dry season, a significant increase in grain yield of about 20-30% compared to the original variety was found in mutant line B-68, B-72, B-95 and B-100 (Table 2). Moreover, B-100 line also had more leaves than the original variety Durra. These results suggested that B-100 was a promising line to be developed as dual purpose sorghum, as grain and forage sorghum, especially when grown in dry

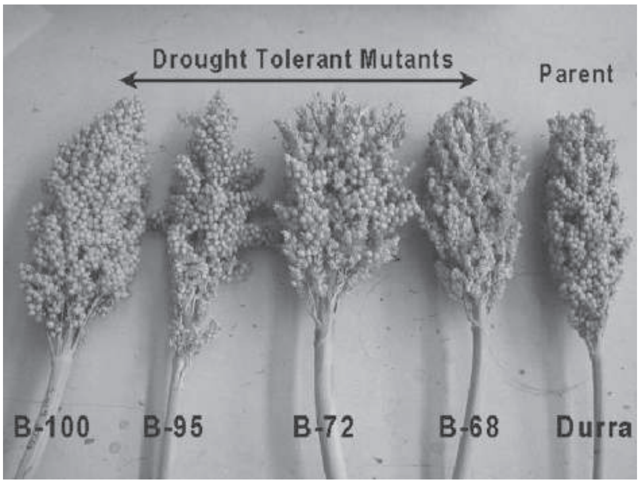

Figure 3. Visual variation on head size and form of some sorghum mutant lines derived from Gamma irradiation with the dose of 400 Gy

season. Since sorghum cultivation are mostly intended only for dry season, thus, the mutant line B-68, B-72, B-95 and B-100 are promising for further development as new grain sorghum varieties particularly for drought prone areas such as those found in Gunungkidul district. All these sorghum mutants have character of white grain color, the type that is desirable for food source. Thus, these promising lines have a good prospect for alternative food source during dry season, while their stovers (stem and leaves) can be used for animal feed or forage.

Table 2 also shows that in general, sorghum could grow and yield better in rainy season rather than in dry season. In rainy season where soil moisture is sufficient, sorghum line B-68, B-72, B-83, B-90, B-92, B-95 and B-100 produced grain yield up to 6.00, 6.21, 6.11, 5.92, 5.87, 6.49 and 6.51 ton ha^{-1}, respectively. These yields were significantly higher compared to their original variety Durra (5.00 ton ha^{-1}) and the national check varieties UPCA (4.34 ton ha^{-1}). From these data it could be concluded that the mutant line B-68, B-72, B-95 and B-100 were promising not only in dry season but also in rainy season. Therefore, these promising lines are now included in the national multi-location trials in collaboration with some Local Agricultural Offices (Dinas Pertanian and BPSB).

In the dry season, the mutant line B-95 and B-100 had plant height of 160.4 cm and 160.3 cm respectively, and significantly shorter than the Durra variety. This semi-dwarf stature is an advantage in term of lodging resistance. Focusing on sorghum use for ruminant feed, however, the mutant line B-69, B-90 and B-100 might be appropriately developed further as forage crop because they had higher number of leaves. Forage sorghum could be of important for the local farmers to suffice their animal feeds, thus, it might help them reduce the cost for buying corn leaves.

Relatively high drought index value was found for the mutant lines B-68 (0.758), B-72 (0.725), B-94 (0.730), and B-100 (0.710). This high drought index indicates that these mutant lines were more tolerant to the condition of water stress with regard to the ability of producing grain yield. In other words, their grain yield production was reduced only less than 30% under drought condition. Durra variety described by ICRISAT as highly tolerant to drought and this was confirmed by relative high drought index in this experiment (0.700). This trait remained consistently inherited from Durra to some of its mutant lines.

It is important to keep the promising mutant lines in the sorghum germplasm collection for further breeding program or other research. Some of these promising lines are to be

Table 2. Average agronomic and yield data of the mutant lines and control varieties in rainy and dry seasons

Lines	Rainy season			Dry season			Drought index
	PH	NL	Yi	PH	NL	Yi	
B-68	179.7	8.2	6.00*	164.3	7.6	4.55*	0.758
B-69	187.6*	8.9	5.75	163.5	8.1*	3.87	0.673
B-72	175.2	8.8	6.21*	166.3	7.5	4.50*	0.725
B-75	183.5*	9.5*	5.48	167.2	7.0	3.38	0.617
B-83	177.7	9.6*	6.11*	169.3*	7.9	3.83	0.627
B-90	178.0	9.1*	5.92*	162.0	8.5*	3.22	0.544
B-92	179.0	8.5	5.87*	162.2	7.8	3.45	0.588
B-94	177.3	9.7	5.23	162.3	7.8	3.82	0.730
B-95	178.8	8.5	6.49*	160.4*	7.7	4.20*	0.647
B-100	170.6*	9.4*	6.51*	160.3*	8.5*	4.62*	0.710
Durra	175.1	8.2	5.00	165.2	7.5	3.50	0.700
UPCA	171.9	7.5	4.34	166.0	7.1	2.68	0.618
Higari	168.1	7.7	5.60	156.7	6.5	3.75	0.670
LSD 5%	5.2	0.71	0.75	4.00	0.43	0.68	

*Significantly different compared to the original variety Durra; PH = Plant height; NL = Number of leaves; Yi = Grain yield (ton ha^{-1})

submitted for official release as new sorghum varieties in Indonesia. Multi location trials of these promising mutant lines were conducted in several provinces starting in 2005 before submission to official release to the Ministry of Agriculture. Cultivation of drought tolerant sorghum would be expected to help farmers increase productivity of their marginal land during dry season, so that it would increase the overall agricultural production and ensure food and feed security. Furthermore, sorghum cultivation would also promote land conservation and sustainable agricultural development in the region. Sorghum development program in Indonesia is also aimed at supporting the government policy in renewable energy source i.e. to develop sorghum for bioethanol.

CONCLUSIONS

Research on sorghum breeding using mutation techniques found that the optimal radiation dose of gamma irradiation was around 300-500 Gy. Through selection processes and direct screening for drought tolerance, a number of putative mutant lines were obtained. In dry season, the mutant line B-68, B-72, B-95 and B-100 had significant increase in grain yield of about 20-30% compared to the original variety (Durra). Meanwhile, the mutant line B-69, B-90 and B-100 had more leaves so that they are promising and recommended for fodder. The mutant lines B-68, B-72, B-94, and B-100 were drought tolerant genotypes whit relatively high drought index. These mutant lines were promising to be investigated and developed further in accordance with attempts of increasing productivity of marginal land in drought prone areas. Sorghum cultivation in such areas would also promote land conservation and sustainable agriculture development in the region.

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