Sorghum Mutation Breeding for Improving Tolerance to Abiotic Stresses brought about by Climate Change

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Fig 1. Sorghum seedling growth on very dry soil

The limiting factors for dryland agriculture development in Indonesia are mostly related to abiotic stresses i.e. drought problems in the eastern and soil acidity in the western part of the country. To face the worsening conditions brought about by climate change and variability, a crop was sought that would require less agricultural inputs, being drought tolerant and having a good adaptability with high economic value. The choice fell on sorghum (Sorghum bicolor (L.) Munch). Sorghum is a cereal crop and recognized as a source of food, feed, fuel and fiber (4Fs).

For Indonesia, sorghum is still regarded as a minor crop and its cultivation was limited and mostly grown by the local farmers in a specific region. Sorghum is not of Indonesian origin so that the available plant genetic variability was low. Attempts to increase sorghum genetic variation was achieved through a mutation breeding program which was conducted at the Center for Isotope and Radiation Application (CIRA), the National Nuclear Energy Agency (BATAN), with the support of the Plant Breeding and Genetics sub-program of the Joint FAO/IAEA Centre through the national IAEA TC projects INS/5/030 and INS/5/039, the regional IAEA/RCA TC projects RAS5040, RAS5045, RAS5056 and RAS5070 and the IAEA CRP D1.50.13 (Contract Code: 16947). Further support was given by FNCA for drought tolerance in soybean and sorghum, and from JSPS for irradiating sorghum seeds with ion-beams in Japan. The breeding objectives were to improve sorghum genotypes for their yield and quality, and tolerance to adverse conditions brought about by climate change, such as prolonged drought and soil acidity.

Mutation Breeding

Fig 2. Gamma irradiator facility at CIRA, BATAN

A gamma irradiator with a Cobalt-60 source, available at CIRA, BATAN, was used to irradiate sorghum seeds in a mutation breeding program. Some breeding materials were introduced from ICRISAT as well as from China through the exchange agreement within IAEA TC project RAS5040. Sorghum seeds were irradiated with gamma rays at dose levels of 0-1000 Gy, with increments of 100 Gy and the resulting M1 plants were grown in the greenhouse.

This radiosensitivity experiment was used to estimate the optimal irradiation doses i.e. the doses that could produce the highest genetic variability in the M2 population. Based on the M1 growth rate data, analysis using best-fitting software found that the optimal irradiation doses for sorghum were around 250-400 Gy.

Fig 3. Sorghum response to gamma irradiation

Fig 4. Mutant selection started in the M2

The experiment revealed that M2 populations derived from the optimal irradiation doses (250-400 Gy) presented the highest genetic variance. They become the base populations to start selection. The total M2 phenotypic variance consists of genetic and non-genetic or environmental variances. The latter usually can be estimated from the parental variance, assuming that the parental variety is homozygous. The presence of genetic variation in the M2 assured that selection would be possible to improve some characters of the selected plants.

Plant selection in the M2 was mostly carried out on the basis of relative phenotypic changes compared to the control plant (un-irradiated parent). The selected plants were then grown in the M3 generation for seed multiplication and further selection through different plant screening protocols, which would depend on the breeding objectives. In case of sorghum breeding, screening was made to search for putative mutants tolerant to abiotic stress i.e. drought and soil acidity, and with high yield and good quality of products.

Screening for Drought Tolerance

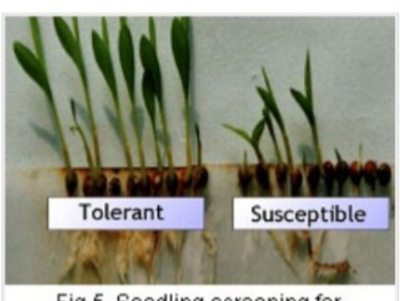

Fig 5. Seedling screening for drought tolerance

Screening for drought tolerance was conducted by using a combination of indirect screening (using the PEG method) in the laboratory and direct screening in the field. Plants were grown in hydroponic media containing 25% polyethylene glycol (PEG) and after 3 weeks, observations were made for seedling growth.

The tolerant plants showed normal growth and it was easy to distinguish from the susceptible ones. According to Singh and Chaudhary [1], 25% PEG can reduce water potential equivalent to natural drought condition so that water absorption by roots is affected. Thousands of plants were screened at the seedling stage, and the tolerant ones were transferred to the field to multiply seed. Field selection was continued for searching for plants having good agronomic performance like plant stature, growth duration, resistance to insect and disease with high grain and biomass yield. These promising mutant lines were then evaluated in replicated yield trials, including the parental and standard check varieties. Some of the promising mutant lines were then included in the national multi location trials which were required before submission to official release by the Ministry of Agriculture.

Fig 6. Drought tolerant mutant in the field

Screening for Acid Soil Tolerance

Fig 7. Seedling screeing for acid soil toletance

According to Kochian, et al [2], unfertile acid soil is associated with low soil pH and high level of soluble aluminium that can inhibit root growth, restricting access to water and nutrients (known as Al-toxicity). Screening for acid soil tolerance is usually conducted by indirect selection using the $AlCl_3$ method. Sorghum seedlings of the mutant and control plants were grown in hydroponic media containing 148 μM $AlCl_3$ and after 3 weeks, variations in the root system were observed. The plants having robust root growth were assumed to be tolerant, selected and transferred to the field for seed multiplication. Further evaluation was done by growing the selected plants in real acid soil fields at Taman Bogo, Lampung Province (Sumatera) where the soil condition is classified as very acid, with soil pH at 4.2 and Al-saturation at 39%. Tolerant plants, showing good agronomic performance with high yield were then selected and classified as promising mutant lines.

The Promising Mutant Lines

Fig 8. Tolerant vs susceptible mutants in field

From this mutation breeding program, a number of sorghum mutant lines were identified to have high tolerance to drought [3] and soil acidity [4] with improved grain yield and quality. Some promising mutant lines were investigated under national multi-location trials as one of the requirements for releasing crop varieties by the Ministry of Agriculture. The multi-location trials were conducted in 12 agro-climatically different regions and in 2 (rainy and dry) seasons using the randomized block experimental design. The mutant genotype stability analysis was done using the Eibenhart and Russel method [5] which is primarily based on genotype and environment interactions for the main parameters (grain and biomass yields). Statistic data of all observed agronomic and quality characters were analysed and reported in a proposal for varietal release. The proposal was presented and defended in front of the Variety Releasing Team which is under the management of the Ministry of Agriculture.

The Released Mutant Varieties

Fig 9. Released sorghum mutant variety PAHAT

Three mutant varieties of sorghum, PAHAT, SAMURAI 1 and SAMURAI 2, were officially released by the Ministry of Agriculture. These mutant varieties were grain sorghum, drought tolerant, of semi-dwarf stature, early maturing, high yielding, and recommended for dry season cultivation especially in drought prone areas of the eastern part of Indonesia such as East Java, Nusa Tenggara Barat, Nusa Tenggara Timur, Maluku and Irian Provinces. Meanwhile, some sorghum mutant lines having characteristics of acid soil tolerance are currently under multi-location trials.

Fig 10. Panicle variation among the mutant lines

The acid soil tolerant mutant lines are mostly late maturing, of tall stature with high biomass production (known as forage sorghum), but some with high sugar content in their stems (known as sweet sorghum). Forage sorghum will be suitable for animal feed while sweet sorghum can be developed for bioenergy (bioethanol) production, particularly in the areas with acid soil condition in the western part of the country.

Mutant Variety Extension

The released sorghum mutant varieties were extended by stakeholders, including farmers, local agricultural offices and some private companies. As an example, the PAHAT variety is currently grown by the private company PTPN XII, in Banyuwangi, East Java. This company processed flour out of sorghum grain and from thereon many kinds of sorghum food products, like cookies, which are now available on the local market. Meanwhile, plant stover (stem and leaves) is used for feeding their livestock (cattle and cow).

Fig 11. Mutant variety grown by the company

Fig 12. Sorghum flour produced by the Company

According to the U.S. Grain Council [6], sorghum flour has good nutritive values (349 kcal/100 gr) as it contains carbohydrates (70.6%), fats (3.4%), proteins (9.1%),, dietary fibres (4%) and tannins (0.06%). Through market promotion, sorghum is being introduced as a healthy food because it has a low glycaemic index so that its products become popular to people suffering from diabetes.

The glycaemic index indicates the food's effect on a person's blood glucose (also called blood sugar) level. Besides, its tannins function as anti-oxidants that can lower unsaturated fat and cholesterol.

Fig 13. Cookies made from sorghum flour

Fig 14. Sorghum cookies low in glycaemic index

Other Use of Sorghum

Sorghum grain and stover are also commonly used for animal feed (poultry and ruminants). The so-called sweet sorghum with high sugar content in its stem juice is usually used for making liquid sugar or syrup, or being fermented to produce ethanol and used as bioenergy provider (bioethanol). Meanwhile, sorghum fibre is a potential material for making various industrial products such as pulp, paper or particle boards.

Fig 15. Chopped sorghum stover for feed

As animal feed, sorghum grain which is rich in carbohydrate, protein, fat and minerals is usually used as alternative poultry feed while its stover is for ruminant feed such as cattle. This is of importance, especially in drought prone areas, where other forage crops are difficult to grow for animal husbandry. Mutation breeding to improve sorghum characteristics related to animal feed has been conducted at CIRA, BATAN, and some promising mutant lines are now being proposed for official release to the Ministry of Agriculture.

Sweet sorghum is one type that has high sugar content in its stem juice, ranging from 10% to 24% brix. It is a potential material to make liquid sugar or syrup, or to be fermented to produce ethanol. Bioethanol made from sweet sorghum has been investigated and promoted in many countries such as the USA, China, and India [7, 8] as a renewable energy source. It is environmentally friendly and can be utilized as fuel for cooking stoves.

Fig 16. Sorghum stover ready for long storage

Fig 17. Feeding cattle with sorghum stover

Fig 18. Sweet sorghum for bioethanol

Mutation breeding to improve sorghum characteristics related to bioethanol production was conducted at CIRA, BATAN and one of the promising mutant lines (PATIR-1) has been officially released by the Ministry of Agriculture as a new sweet sorghum mutant variety by the name of Samurai 1. This mutant variety was gown by a private company in collaboration with the local government of East Kalimantan Province and supported by BATAN to produce bioethanol used for cooking stoves made by the company which is now popular in the local farmer community.

Still, sorghum is not yet widely grown in Indonesia and is limited only to a specific region. Sorghum is still a minor crop in Indonesia. In order to disseminate sorghum cultivation technology to wider farmer communities, some local governments collaborating with BATAN arrange sorghum field days to promote sorghum cultivation and technology transfer to the farmers. In the future, it is hoped that sorghum cultivation in the unproductive lands, e.g. drought prone areas, will impact positively on increasing land productivity, improving soil fertility, promoting sustainable agriculture development and ensuring food and energy security in the country.

Fig 19. Bioethanol made from sweet sorghum

Fig 20. Cooking stoves use sorghum bioethanol

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Fig 21. Sorghum field day in Gunungkidul, Yogyakarta Province

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