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Mutation breeding in underutilized cereal crops: a review

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Abstract

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Mutation breeding is a method of plant breeding using physical and chemical mutagenic induction to generate genetic diversity. This approach is taken when the genetic diversity of the desired trait is narrow. Mutation breeding in major cereal crops such as rice, wheat, and corn have been widely carried out, but underutilized cereal crops are rarely carried out. The purpose of this literature review was to discuss the development and techniques of mutation breeding in underutilized cereal crops i.e. sorghum, millet, and adlay. Article searches were carried out on various research articles and review articles. Mutation breeding has been widely carried out, especially on sorghum and millet, but little has been done on adlay. Mutagens that were widely used were gamma-ray irradiation and EMS. The TILLING approach was used to speed up the molecular selection process. The Rapid Cycling Method was also used to accelerate the purification process of mutant lines in each generation. Mutation breeding produces a number of mutant varieties in underutilized cereal crops. Several techniques can be used to speed up the breeding process.

Keywords: adlay; millet; mutation breeding; sorghum; underutilized cereal

Introduction

Changes in an individual are needed in order to be able to face environmental changes and evolutionary processes. These changes take place slowly and are influenced by genetic factors. Hugo de Vries (1901) first coined the term mutation as a sudden change in heritable genetic material that is not caused by recombination or segregation. Mutations that arise spontaneously occur are very rare and random. According to Acquaah (2012), the occurrence of spontaneous mutations is 10^{-6} per locus. The use of radiation was proposed by de Vries (1901) to induce mutations in plants and animals. This was proven by Gager & Blakeslee (1927) by inducing Radium-rays on *Datura stramonium* and Muller (1927) by inducing X-rays on *Drosophila*.

Freisleben and Lein proposed the term mutation breeding in 1944 namely induction and development of mutant lines for plant improvement. Mutation breeding in a broad sense includes the exploration of nature as a source of natural mutants and the development of varieties with multiple sources of mutations (Shu et al., 2011). The first mutant plant variety was released in Indonesia in 1936, namely “Chlorina” variety of tobacco which was the result of X-ray induction (Tollenaar, 1938). Mutant varieties induced by chemical mutagens were released for the first time in the United States in 1966, namely “Luther” variety of barley. Furthermore, mutation breeding was developed for various commodities with various methods (Shu et al., 2011).

Hybridization is one of the methods used in plant breeding. This method takes time to combine and stabilize the desired characters. Mutation breeding methods can reduce the time of the breeding process and can increase the choice of the desired mutant. Mutation breeding is one of the methods of plant breeding by induction of physical and chemical mutagens to generate genetic variation. The next generation can inherit this variation through hybridization (Shu et al., 2011). Breeding through mutation induction is done by generating maximum genomic variation with a minimum decrease in growth rate. Mutation induction has resulted in great genetic diversity and plays an important role in plant breeding programs (Sikora et al., 2011).

Techniques and methods for developing major cereal crops through mutation induction have been widely published. However, the availability of information about this in underutilized cereal crops is still limited. Identification and evaluation of relevant literature in research related to mutation breeding processes have been carried out to write this article. This review article consists of various literature studies derived from research data and reports related to mutation breeding in underutilized cereal crops, especially sorghum, millet, and adlay. The search engines used to search for material (articles) are google.com and scholar.google.com. The literature studied was of publications from 1900 to 2022. The purpose of this review paper is to provide an overview of mutation breeding techniques and methods in the development of underutilized cereals, especially sorghum, millet, and adlay.

Mutation Breeding in Sorghum

Sorghum (*Sorghum bicolor* L. Moench.) was one of the first plants to be domesticated for human use (de Wet, 1978). Over time, sorghum undergoes a process of evolution and selection both naturally and through human intervention. The evolutionary process resulted in 5 basic races, namely bicolor, guinea, caudatum, kafir, and durra (de Wet, 1978).

Sorghum is a very productive plant, which can be used as a source of food, animal feed, alternative energy, or for industrial purposes. This plant is able to adapt to nutritional deficiencies, aluminum stress, drought, high salinity, standing water, and high temperatures (Tari et al., 2013). Sorghum plants in dry areas can produce seeds with productivity between 1 – 5.9 tons/ha (Ghasemi et al., 2012) and potentially up to 20.1 tons/ha (Dahlberg et al., 2011). According to USDA (2022a), world sorghum production reaches 65,589,000 MT with the five largest producing countries namely the United States (17%), Nigeria (10%), Ethiopia (8%), Sudan (8%), and Mexico (7 %).

Breeding through mutation induction in sorghum plants is still little done. This can be seen from the number of mutant varieties recorded in the Mutant Variety Database (MVD). Until 2021 there were 17 mutant varieties of sorghum, of which the first released mutant sorghum variety was Jinza 1 in China in 1970 and the last one was Samurai 1 and Samurai 2 in Indonesia in 2014 (IAEA, 2022).

Mutation breeding of sorghum in Indonesia was reported by Human et al. (2020). Breeding was carried out at the Center for Isotope and Radiation Applications and Technology, National Atomic Energy Agency (BATAN). This breeding is carried out to support the national program for food diversification in the context of food and energy security. The breeding program is carried out to improve yield and quality as well as tolerance to environmental conditions due to climate change, especially drought. The parent used was the Zhengzu variety, which is an introduced variety from China. Mutagenesis was carried out using Co-60 gamma irradiation at a dose of 0 to 1000 Gy with a dose range of 100 Gy. The radiation sensitivity test of the M_1 generation greenhouse by planting seeds on sandy soil. Plant growth was measured for each treatment dose and the optimal dose was determined based on the Lethal Dose (LD). LD_{20} and LD_{50} of 250 and 400 Gy are optimal doses because they show the highest diversity in this range.

Selection against drought stress in the M_2 was carried out indirectly using a Polyethylene Glycol (PEG) solution and directly in the field. The seeds were grown in 25% PEG solution (permanent wilting point) and the selected seedlings were then planted in the field for seed propagation. Selection in the field is carried out in the dry season. The breeding program was continued by planting selected mutants to obtain homozygous lines. The homogeneity and stability tests were carried out on the M_5 and homozygous lines with the desired traits were nominated as promising lines. Yield test and seed quality analysis were carried out before the promising line was proposed for varietal release.

Samurai-1, Samurai-2, and Pahat are varieties resulting from mutation breeding that have been released as adaptations to drought. The three varieties were the expected lines from the evaluation, namely Patir-1, Patir-4, and ZH-30. Samurai-2 and Pahat are recommended as food sorghum while Samurai-1 is recommended as sweet sorghum because they have high tannin content in seeds. Patir-1 has a yield of 6.1 $t.ha^{-1}$, Patir-2 is 6.4 $t.ha^{-1}$ and Pahat 5.8 $t.ha^{-1}$. The three varieties have yielded 38-52% higher than their parents. Pahat has an early age (89 days) with short stems and resistance to fall and is easy to harvest. While Samurai-1 has a high sugar content (12 Brix).

Mutation induction of sorghum plants using chemical mutagens has produced mutants that are useful in sorghum plant breeding programs. Xin et al. (2008) used ethyl methanesulfonate (EMS) to induce the inbred line BTx623. A total of 1600 mutant lines have been generated from this treatment. Phenotypic changes were observed in the M_2 and M_3. Targeting Induced Local Lesion IN Genomes (TILLING) was used to screen for new genes induced by mutations. Two brown midrib (bmr) phenotypes which are traits related to changes in lignin content and increased digestibility for forage were isolated from the five identified mutations. The TILLING approach was also carried out by Blomstedt et al. (2012) to select sorghum mutants. The TILLING approach was used to speed up the selection process. For this approach to be feasible, the source of the plant genome and the target gene for the desired trait are known.

Furthermore, Xin et al. (2009) reported a population of generation M_3 mutant inbred BTx623. In this generation, a phenotype emerged with the characteristics of brown midrib (BMR), erect leaves (erl), many tillers (mtl), and late flowering (LFL). These traits can be used as genetic resources for the functional genome of sorghum and bioenergy research.

Sequencing of the whole genome of sorghum has been carried out by Jiao et al. (2016). Sequencing was performed on 256 phenotypes of the M_4 mutant line from BTx623 seeds and revealed more than 1.8 million mutations affecting more than 95% of the genes in the sorghum genome. Eight genes potentially influencing drought tolerance were identified by reverse genetics, three of which had allelic mutations and two of which showed proper co-segregation with the desired phenotype. The results of the sequencing of the mutant lineage can be used as a source of the sorghum mutant library for functional gene validation. This is useful for accelerating the breeding of sorghum.

The new dwarf sorghum [*Sorghum bicolor* (L.) Moench) mutant $BTx623_{dw5}$ has been released by USDA-ARS under No. Reg. GS-787, PI 688506. Pygmy sorghum mutant isolated from the mutated BTx623 mutant library. The dwarf phenotype in the dw5 mutant is caused by a single nuclear gene mutation and is inherited recessively. The dw5 mutant was 72–74 cm tall compared to its parent BTx623 which was 155 cm (Chen et al., 2019). USDA–ARS has also released three new genotypes of sorghum (*Sorghum bicolor* L.) brown midrib (bmr) BTx623bmr12-30 (No. GS-790, PI 699404), BTx623bmr12-34 (No. GS-789, PI 699405), and BTx623bmr12 -35 (Reg. No. GS-788, PI 699406). The bmr12 can be extensively used in forage breeding programs for animal feed. The bmr12 mutant flowered 4–5 days earlier than the inbred BTx623 strain. This bmr12 mutant also had 14–24% greater total seed protein than the inbred BTx623 strain (Emendack et al., 2022).

The development of mutation breeding techniques is carried out to ease the work in the breeding process. Indriatama & Anisiyah (2020) developed *rapid cycling techniques* that effectively shorten the purification of mutant sorghum lines by four weeks per generation compared to conventional methods. This method applies a combination of field application and in vitro culture. Seeds were harvested at the age of 10 days after flowering and immediately cultured on tissue culture media. With this technique, plantlets can be acclimatized at the age of 7 days and can then be grown in the field. Sorghum grown with conventional methods can only be harvested 28 days after flowering and can only be planted as seeds. Thus we can speed up the process of breeding sorghum mutants. This method has the potential to be applied to other commodities so that it can accelerate the mutation breeding process (Figure 1).

Mutation Breeding in Millet

Millet is one of the oldest foods known to man. Millet belongs to the Graminae family. The term millet refers to

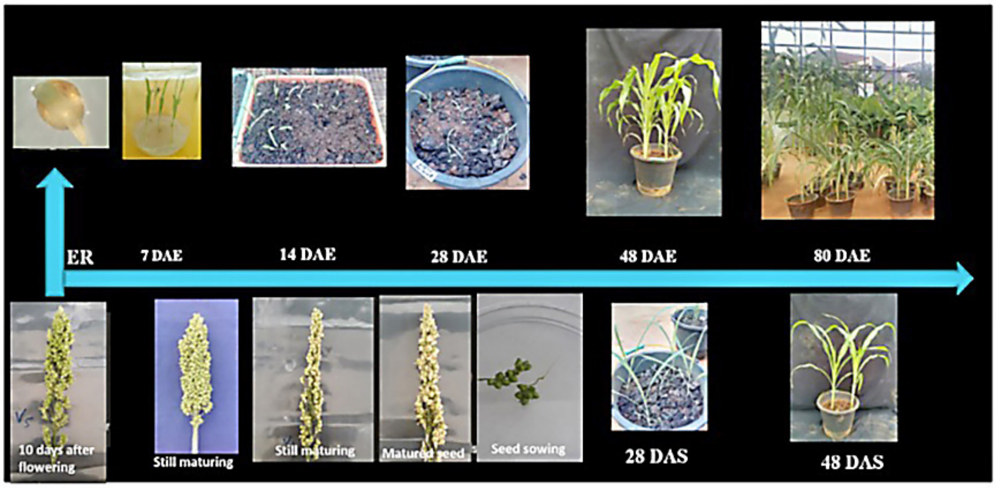

Fig. 1. Rapid cycling method (Indriatama & Anisiyah, 2020)

several types of small-seeded annual grasses belonging to species under five genera namely, Panicum, Setaria, Echinocloa, Pennisetum, and Paspalum in the Paniceae tribe and one genus Eleusine, in the Chlorideae tribe (Karuppasamy, 2015).

Millet has a unique feature of resistance to adverse environmental conditions, especially infertile soil conditions. Barley is able to adapt to soil conditions with low nitrogen and phosphorus content (Nadeem et al., 2020), drought (Lapuimakuni et al., 2018), and high salinity (Ardie et al., 2015). This plant also has the potential as a model crop to develop cereal crops, animal feed, and bioenergy (Doust et al., 2019).

Millet plants can produce seeds with a productivity of 4.1 – 4.3 t/ha while hybrid plants can produce 5.9 – 6.1 t/ha (Ning et al., 2015). According to USDA (2022b), world millet production reaches 29 563 000 MT with the five largest producing countries namely India (35%), Niger (13%), Sudan (9%) China (8%), and Nigeria (6%).

Breeding through mutation induction in millet plants has been widely carried out. This can be seen from the number of mutant varieties recorded in the Mutant Variety Database (MVD). There were 5 varieties of pearl millet (*Pennisentum glaucum* L.) released from 1974 to 1982, 3 varieties of proso millet (*Panicum milliaceum* L.) that were released from 1985 to 1993, 21 varieties of foxtail millet (*Setaria italica* (L.) Beauv) released from 1966 to 2009, 2 varieties of finger millet (*Eleusine coracana* (L.) Gaertn) released in 2009, and 4 varieties of Japanese barnyard millet (*Echinochloa esculenta* (A. Braun) H. Scholz) released from 2012 to 2013. Those varieties are the result of direct or indirect mutation breeding with mutagens used to induce mutations, including gamma-ray irradiation, fast neutrons, carbon ion beam, aerospace cosmic radiation, NMU, and DMS chemical mutagens (IAEA, 2022).

In recent years, mutation breeding in millet has continued to be carried out, including proso millet (Bhave et al., 2016; Kate et al., 2018; Francis et al., 2022), foxtail millet (Gopinath et al., 2017; Anittha & Mullainathan, 2018), finger millet (Ambavane et al., 2015; Aviya & Mullainathan, 2018; Waghmode et al., 2020; Ganapathy et al., 2021; Sellapillaibanumathi et al., 2021), pearl millet (Ambli et al., 2016; Addai & Salifu, 2016; Addai, 2018), and barnyard millet (Ramesh et al., 2019). In proso millet, the irradiation dose of 20 kRad is an effective dose to cause genetic diversity (Kate et al., 2018), but the desired properties can be isolated from doses of 40 and 50 kRad, namely early maturity and yield at doses of 20 and 60 kRad (Bhave et al., 2016). In finger millet, the gamma-ray irradiation dose of 600 Gy showed the highest value on physiological and morphological characters (Sellapillaibanumathi et al., 2021; Vasisth et al., 2022). Chlorophyll mutations appeared at doses of gamma-ray irradiation of 400 and 500 Gy and in the Dapoli-1 variety, the mutant for early maturity and high yielding properties could be isolated at doses of 500 and 600 Gy (Ambavane et al., 2015). The heritability of iron, calcium, and protein content was high in the M_3 generation mutant (Waghmode et al., 2020). The heritability and correlation of M_4 generation mutants are high in yield characteristics (Vasisth et al., 2022).

In pearl millet, an increase in the concentration of EMS causes a decrease in the efficiency and effectiveness of the mutagen (Ambli et al., 2016). Gamma-ray irradiation doses of 100 and 200 Gy resulted in an increase in early maturity and yield components (Addai, 2018). In barnyard millet, EMS mutagens and gamma-ray irradiation showed high effectiveness and efficiency at low doses and showed a decrease with increasing doses (Ramesh et al., 2019). In foxtail millet, Anittha & Mullainathan (2018) found that EMS caused more chlorophyll mutations than DES. EMS concentration of 0.45% showed the best results on yield characteristics (Gopinath et al., 2017). The mutant library has been built on a foxtail millet (Sun et al., 2019). The mutant library of WP1 genes was constructed using high-resolution SSR/INDEL molecular markers. Mutant libraries can be used to speed up the research process.

Mutation Breeding in Adlay

Adlay or job's tears is one of the commodities used by humans as a food source. This plant is believed to have originated in tropical and subtropical Asia. This plant has been used since the late Paleolithic period in northern China about 28 000 years ago, then adlay disappeared in the *younger dryas* and reappeared in the early Holocene in the Huai and Yangzhi rivers areas. Adlay began to spread in the Neolithic era about 8000 years ago along with the domestication of millet (Liu et al., 2018). Currently, adlay is widely cultivated in tropical and subtropical areas.

Adlay is included in the Coix genus, Maydae tribe, Poaceae family (Jain & Banerjee (1974). Adlay is a tetraploid plant with chromosome number 2n = 4x = 20 (Rao, 1976; Arago et al., 1997). Adlay is divided into 4 varieties based on shape and fruit characteristics (*involucre*). *Coix lacryma-jobi* L. var. *ma-yuen* (Romanet) Stapf is a variety that is used as a food source because it has a thin seed coat, while *Coix lacryma-jobi* L. var. *lacryma-jobi*, *Coix lacryma- jobi* L. var. *monilifer* Watt, *Coix lacryma-jobi* L. var. *stenocarpa* Stapf are used as feed and handicrafts (Jain & Banerjee, 1974).

Adlay is a traditional Chinese food and medicine plant with nutritional components and medicinal values (Feng et al., 2020). The nutritional content of adlay is 71.81% carbohydrates, 10.89% protein, 1.83% ash, and 5.18% fat (Mayasti et al., 2021). Adlay can produce seeds with a productivity of 2.9 – 3.6 $t.ha^{-1}$ (Liao et al., 2019) and can reach 6.24 $t.ha^{-1}$ under favorable conditions (Aradilla, 2016). Adlay is cultivated in China covering an area of 73,000 ha with a production of 0.22 million tons. There has been an increase in the area of adlay plant production in the last 10 years (Diao, 2017).

Breeding through mutation induction in adlay is rarely done. This can be seen from the number of mutant varieties recorded in the Mutant Variety Database (MVD). Until 2022, there were 3 mutant varieties of adlay, namely Hatomusume which was registered in 1993, Hatohikari, which was registered in 1996, and Hatoyutaka, which was registered in 2007 (IAEA, 2022).

Hatomusume and Hatohikari are the progeny of the Okayama Zairae variety which were induced by gamma rays of 20 kRad (1 Rad = 0.01 Gy) in 1980 (Nakagawa & Kato, 2017). Okayama Zairae is a local variety developed in Okayama prefecture. This variety has high stature, long life, thick seed coat, uneven ripening age, and unstable yield. In 1992, Hatomusume was introduced in the field but this variety was not satisfactory in terms of size and quality of seeds. In 1995 Hatohikari was released under the registration name Hatomugi Norin No. 2 to compensate for the quality of Hatomusume. Hatohikari were released after the M_{15} generation of irradiated Okayama Zairae variety. The Hatohikari variety has medium age, large seeds, and high yields with good seed quality and processing. This variety is suitable in the medium plains and mountains of Chugoku and is suitable for harvesting using a combined harvester (Ishida et al., 1995).

Research to obtain mutant varieties of Hatoyutaka began in 1988. Hatoyutaka was built from the indirect use of mutant genotypes. Hatoyutaka is a derivative of the Tohoku No.1 line (gamma-ray induction in the TS-kei variety) which has early maturity and short plants with the Oou No.4 line (gamma-ray induction in the Okayama Zairae variety) which has high yields. Selection of early maturity, short stems, and high yields were carried out until 1995. Yield tests were carried out from 1996 to 1998, then from 1999 to 2003, multisite tests were conducted in Iwate, Miyagi, and Akita prefectures. Good performance is shown by the results in Iwate and Miyagi Prefectures. After passing through 15 generations of crosses, in September 2004 the line was registered as a variety under the name Hatomugi Norin No. 4 “Hato Yutaka”. Hatoyutaka has early maturity, short stems, and high yields and is suitable for mechanical harvesting (Kato et al., 2007).

Efforts to breed adlay through mutation induction are still being carried out, although they have not yet achieved the expected results as was done by Shen (2017) through the orientation of gamma-ray irradiation dose and EMS concentration on 2 varieties, each from China and Thailand. In addition, ISSR markers are also used to detect genetic diversity resulting from mutations. Research on mutation breeding in adlay is still rarely done. This opens up opportunities for plant improvement through mutation induction. In addition, molecular markers and other techniques can be used to speed up the breeding process.

Conclusion

Mutation breeding produces a number of mutant varieties in underutilized cereal crops. Gamma-ray irradiation and EMS are the most widely used to induce mutations. Among the underutilized cereal crops discussed, adlay plants produced the least mutant varieties. Molecular markers both genome sequencing and TILLING are very useful to speed up the selection process. In addition, the Rapid Cycling method can also speed up the purification process in each generation. Molecular markers and other techniques can be used to speed up the mutation breeding process.

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