

Application of induced mutation technique to improve genetic variability of Indonesian traditional rice varieties

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Abstract. The development of rice genotype is vital to guarantee food security to cope with global climate changes and increasing population growth. Biodiversity is spread throughout the Indonesian archipelago. Many Indonesian local varieties are resistant to biotic and abiotic stresses that may be useful for rice breeding program. They are well-adapted to specific environment and have good aroma and eating quality, but have some weaknesses, such as late maturity, susceptible to lodging, unresponsive to fertilizer and low yield. Induced mutation breeding is useful for increasing genetic variability to develop genotypes with several interesting agronomical characters and yield. Center for Isotope and Radiation Application, National Nuclear Energy Agency (CIRA-NNEA) has been conducting the induced mutation technique for genetic improvement of rice. Pandan Putri, an early maturing mutant variety that was derived from irradiated Pandan Wangi variety from Cianjur area, is one of successful improvements of traditional rice varieties using mutation technique. The other success stories are described in this paper. It is concluded that nuclear technology can be applied for induced mutation breeding to improve several agronomical traits.

Keywords: nuclear technology, induced mutation, traditional rice, genetic improvement.

1. Introduction

Rice (*Oryza sativa* L.) is the primary food crop for most of Asian countries. In Indonesia, rice plays an important role in providing food and nutritional security and eradicating poverty. To achieve stable growth in rice production in keeping with the increasing population, a strong effort is required to boost productivity, break yield barriers and provide safety against fluctuations in climatic conditions. Securing the availability of rice production and ensuring sustainable community food needs in Indonesia can be done through the cultivation of modern and traditional rice varieties. Modern rice varieties are obtained through the stages of rice breeding activities which are then released nationally. Most of the traditional rice varieties existed in Indonesia belongs to the *tropical japonica* sub-species (known as *javanica*) [1]. Until now, most of these traditional rice varieties continue to be cultivated in several regions in Indonesia.

Based on the number of existing traditional rice germplasm, it is estimated that only 10–15% of these varieties are continuously planted by farmers. In the future, this number is likely to decline if there is no systematic effort to preserve local rice varieties [2]. Extensive genetic diversity in traditional rice is a genetic potential that controls several important traits. Therefore, traditional



varieties are very useful in rice breeding activities to obtain superior traits and to expand the genetic background of superior varieties to be produced [3].

Traditional rice varieties have been reported to have resistance to various environmental stresses including resistance to pest and diseases and are adapted to specific locations. Therefore, they have been cultivated for generations by most farmers, although it has some morphological characters such as relatively long growth duration [4]. Traditional rice that still survives to date is a cultivar produced by natural selection for decades or even hundreds of years, so that it generally has good characteristics preferred by the community such as delicious rice taste and resistance to abiotic stresses [5].

Genetic erosion of traditional rice germplasm in Indonesia began after the green revolution program implemented by the government at the end of the 1960s. The introduction of high-yielding varieties which tends to have shorter harvesting age triggered mass extinction of traditional varieties because many farmers turned to these superior varieties [6]. The yield of traditional varieties was far lower than modern varieties, and thus they were no longer planted by farmers and started to extinct. To anticipate their extinction whilst preserving their positive characteristics and increasing its economic value, breeding activities need to be carried out to improve their genetic without changing other positive properties.

Cross-breeding (or recombinant breeding), which is based on hybridization of different genotypes followed by trait selection, has become a common practice in plant breeding. Further advancements in plant breeding for the induction of genetic alterations through physical and chemical mutagen laid the foundation of another type of plant breeding known as mutation breeding. The variation so created is further amplified by recombination of alleles on homologous chromosomes and their independent assortment at meiosis [7]. The most noticeable effects of irradiation on the transmission of the genetic materials are its inhibiting action on meiosis. Spontaneous and induced mutations are the primary source of all genetic variations existing in any organism, including plants [8].

Induced mutations with mutagenic agents have been used to create genetic variations from which desired mutants can be selected. Mutation breeding has become a very productive breeding tool that offers the possibility of inducing desired attributes that are not found in nature or that have been lost during domestication. Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through mutagenesis [7]. The possibility of increasing the genetic variability of rice varieties by means of ionizing radiation had more consideration from plant breeder point of view [9]. This paper summarizes the development and achievement of mutation induction techniques in improving the genetic background of Indonesian traditional rice.

2. Morpho-agronomic characteristics of Indonesian traditional rice varieties

Indonesia, known as an agrarian country, is located around the equator. Its tropical climate with only two seasons has made this country rich in biodiversity including traditional rice germplasm. Abundant rice genetic resources are found in Indonesia. Almost every region has more than one traditional rice varieties that have been cultivated for generations. For example, Kewal variety from Banten, Barak Cenana from Tabanan (Bali), Jembar from West Java, Pandan Wangi from Cianjur (West Java), Rojolele from Klaten (Central Java), Kuriak Kusuik from West Sumatra, Siam Datu from South Kalimantan, etc.

The existing traditional rice varieties are cultivated by the farmer's community and controlled by the government. Therefore, traditional varieties are more adaptable to climate change than introduced varieties. Approximately, 3,800 local rice germplasms are registered by Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development [10]. "Fur" or "Gundil" is an example of Indonesian rice variety known by the peasant community and widely cultivated in Java, Lombok, Bali, Sumbawa, and other several remote areas.

Generally, local farmers cultivate the traditional rice varieties in unfavorable growing environments, such as dry land in hilly areas, acidic dry land, swampy land, "lebak" or lowlands that are often flooded, tidal swamps that tend to be saline and other marginal lands. Therefore, it is possible to make the traditional rice varieties more adapted and tolerant to biotic and abiotic stresses with good

taste according to people's preferences. Several traditional rice varieties have been identified as having resistance to biotic stresses, such as rice gall midge, brown planthopper, bacterial leaf blight, orange leaf disease, leaf blast, neck blast, *Rice stripe virus* and rice tungro disease, and abiotic stresses, such as drought, Al toxicity, Fe toxicity, salinity, low temperature and shading [3].

Majority of Indonesian traditional rice have long panicles, low tiller number, round seeds which make them hard to fall, wide leaves, intermediates amylose content and photoperiod insensitive [1]. Rice belonging to the *javanica* group has usually long hair and dense grain (*sericeous*), and a tail/hair at the end of the grain [6].

Each traditional variety adapts well to the area where the plant originates, with the taste of rice according to the preference of the local community and has a specific aroma. Other characteristics are strong and deep rooting, but not responsive to fertilizer application. Resistance to plant pests and diseases is present in many traditional rice and wild rice [11]. A list of characterized and identified 456 traditional rice accessions from Indonesia with resistance to pests and plant diseases have been published [3].

The traditional rice are commonly late maturity and tall, which makes them susceptible to lodging and produce low tiller number. In general, traditional varieties have also low yield potential and less ideal plant architecture. Traditional rice is commonly cultivated in suboptimal land such as dry land of ex-forests in an effortless way with no chemical inputs for fertilizers and pesticides.

Farmers usually prepare seeds for the next planting season in a traditional way by relying on their own crops. Thus, the quality of the seeds, especially the level of purity, is very low, which affects production. Due to low purity of seed, the appearance of traditional rice varieties in the field in terms of plant height, harvesting days, grain shape and color, seem to be diverse [12]. Efforts have been made by the regional government to improve the seed purity of traditional rice and release them as superior varieties. Until 2012, a total of ten local rice varieties have been purified and released in Indonesia (Table 1).

Table 1. Local varieties that have been purified and released by regional government in 2004–2018 [12].

Traditional varieties	Province	Year of release
Rojolele	Central Java	2003
Pandan Wangi	West Java	2004
Anak Daro	West Sumatra	2007
Kuriek Kusuik	West Sumatra	2009
Junjung	West Sumatra	2009
Caredek Merah	West Sumatra	2010
Lampai Kuning	West Sumatra	2014
Siam Mutiara	South Kalimantan	2008
Siam Saba	South Kalimantan	2008
Cekow	Riau	2012
Karya	Riau	2012

The regional government's aims for the release of traditional rice varieties are (1) to obtain the legality that traditional varieties deserve to be regional superior varieties with specific site, (2) to obtain legality for efforts to produce certified seeds, (3) to obtain equal rights in the use of qualified seeds, and (4) to increase the benefits and economic values of local variety seeds for the community and local government.

3. Induced crop mutagenesis

Crop mutation breeding based on mutation techniques approach has been used for more than 50 years, and more than 1,000 cultivars of 44 crop species have been released [13]. Mutagenesis is a powerful tool that has been used to create genetic materials for plant breeding. The primary aim in mutation breeding is to develop and improve well adapted plant varieties by modifying one or two major traits to increase their productivity or quality through inducing physical or chemical mutagens in seeds and other planting materials [14].

Among different type of physical mutagen, gamma rays are widely employed for mutation studies because they have shorter wave length and possess more energy per photon than x-rays, and penetrate deeply into the tissue. Gamma rays have been a popular method to improve the qualitative and quantitative characters for many crops [9]. Most of the gamma ray irradiation doses were acute. High doses of radiation caused higher sterility of M_1 plants, but in some cases, low doses of irradiation stimulated the growth and yield of M_1 plants [15]. Irradiating seeds with suitable doses of gamma rays produces physiological or genetical changes in plant tissue which may affect the yield of plant [16].

Increasing the genetic variability of rice varieties through ionizing radiation is more preferred by plant breeder to start plant mutation breeding. Until now, mutation breeding technique has become a very productive breeding tool that offers the possibility of inducing desired attributes that are not found in nature, or that have been lost during domestication. There are two major outcomes resulted from mutation breeding: improved varieties that are directly used for commercial cultivation and new genetic stocks with improved characters or with better combining ability of traits.

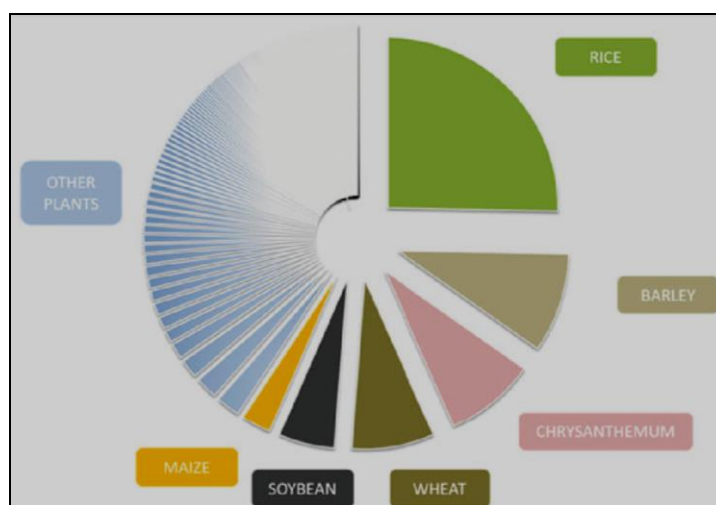


Figure 1. Pie chart representing officially registered mutant crop varieties. The Mutant Variety Database contains 3,222 entries out of which 2,456 are seed propagated and 367 are vegetatively propagated plants [17].

Over 3,220 crop varieties that have been released through induced mutation technique are being grown in different countries of the world. The majority (80%) of this crop varieties are seed propagated, with almost half of it (48%) are cereals (Figure 1, [17]). Among cereals, rice is the top rank compared to the other crops, whereby 443 rice cultivars have been developed by mutation breeding using EMS, fast neutron and gamma irradiation [18].

Many rice mutants have been developed with new genetic variation and improvement in plant characteristics, both in quantitative and qualitative traits. Numerous studies have reported that many mutant genes controlling important traits like plant height, tiller number and panicle length have been cloned and characterized at the molecular level [19–22]. Babaei et al. [23] also reported that rice mutants have been useful for genetic and physiological assessments of yield-limiting factors.

Dwarfism is one of the important agronomic traits that play a part in increasing rice yield. As many as 80 dwarf mutants of rice have been reported including six high-tillering dwarfs [24]. The following characteristics in rice mutants were subsequently developed: early maturity, endosperm quality, elongated uppermost internode, genetic male sterile, improved nutritional quality because of low phytic acid, giant-embryo mutants of potential interest to the rice oil industry, and adapted Basmati and Jasmine germplasm [25]. In China, the most widely grown rice cultivar, Zhefu 802, induced from Simei 2 by gamma rays, has a relatively short growing period (105–108 d), high yield potential, wide adaptability, high resistance to rice blast and cold tolerance [26].

Basically, plant mutation breeding is an accelerated breeding method which generally takes 7–9 years as opposed to 10–15 years of conventional breeding to produce new cultivar in annual crop. This is because mutation breeding improved an already preferred cultivar for a certain trait. Once mutants have been identified in a population, they can be deployed directly and indirectly in breeding programs [27].

4. Mutation breeding in Indonesian traditional rice varieties

In Indonesia, plant mutation breeding technique was applied in rice since early 1960's by Center for Isotope and Radiation Application, National Nuclear Energy Agency (CIRA-NNEA), the only institute that is engaged in rice mutation breeding in Indonesia. Significant achievements were made from the early stages. In 1982, Atomita 1, the first mutant rice variety in Indonesia, was officially released. This mutant variety was produced from seed irradiation treatment of Pelita 1/1 with gamma rays at a dose of 0.2 kGy. Screening for biotic stress was started in M_2 generation and followed by selections of mutants with desirable morphological and agronomical traits in advanced generations. Atomita 1 has contributed more than 10% of the total rice varieties after 1982. Its contribution has been increasing until these years (Table 2).

Mutation breeding based on nuclear technique has the following advantages in rice improvement in Indonesia: (1) almost all characters in rice could be improved as long as their variations exist in nature, (2) both allelic and non-allelic mutations to those already known in the germplasm collection could be induced, and (3) mutation techniques are useful, particularly for the improvement of traditional varieties for specific traits that could not be improved using cross-breeding [28].

The mutation breeding can effectively change a few traits without changing other characteristics that have been preferred. This method is useful for the improvement of local rice varieties that are already popular in certain areas because of preferred taste of rice by the local community and good adaptability in the area, but are very late in maturity and have low and unstable yielding ability. In addition, high plant architecture makes it unable to stand down and hard to fall especially before harvest. Susceptibility to lodging can reduce the yield both in quantity and quality. Attempts to improve these defects through cross-breeding have failed due to the inability to keep the desired quality preference [28].

Pandan Putri variety is an example of a successful improvement of traditional rice varieties using mutation breeding. This variety is the improvement of Pandan Wangi, a local rice variety, through seed radiation with gamma rays at a dose of 0.2 kGy. Pandan Putri is 45 days earlier but with appearance and taste of rice that are not different from the wild type, Pandan Wangi. The improvement activities of local rice varieties with mutation breeding are growing in line with the rampant efforts of the regional government to purify and release local rice varieties to support regional food self-sufficiency programs by utilizing local wisdom.

Table 2. Genetic improvement in rice mutant varieties developed using mutation breeding in Indonesia (1982–2016).

Name	Year	Parent and treatment	Improved character(s)	Status
Atomita 1	1982	Pelita I/1; 0.2 kGy	Resistant to brown planthopper (BPH) biotype 1 and green leafhopper (GLH); early maturity	Released
Atomita 2	1983	Pelita I/1; 0.2 kGy	Resistant to BPH biotype 1; tolerant to saline soil; early maturity	Released
Atomita 3	1990	Mutant line No. 627/103/PsJ; 0.2 kGy	Resistant to BPH biotype 1 and biotype 2; early maturity	Released
Atomita 4	1991	Cisadane; 0.2 kGy	Early maturity; tolerant to high Fe soil (poor soil drainage)	Released
Situgintung	1992	Seratus Malam*; 0.2 kGy	Resistant to BPH biotype 1; intermediate resistant to BPH biotype 2	Released
Cilosari	1996	Mutant line of Seratus Malam (SM 268/PsJ) × IR36	Tolerant to bacterial leaf blight (BLB)	Released
Meraoke	2001	F ₁ seeds (Atomita 4 × IR64); 0.2 kGy	Slender seed; tolerant to BLB strain IV; early maturity	Released
Woyla	2001	F ₁ seeds (Atomita 2 × IR64); 0.2 kGy	Slender seed; tolerant to BLB strain IV; early maturity	Released
Kahayan	2003	F ₁ seeds (Atomita 4 × IR64); 0.2 kGy	Tolerant to BLB strain IV	Released
Winongo	2003	F ₁ seeds (Atomita 3 × IR64); 0.2 kGy	Big slender seed; tolerant to BLB strain IV	Released
Diah Suci	2003	F ₁ seeds (Cilosari × IR74); 0.2 kGy	Slender seed; tolerant to BLB strain IV	Released
Mayang	2004	F ₁ seeds (Cilosari × IR74); 0.2 kGy	Big and slender seed; tolerant to BLB strain IV	Released
Yuwono	2004	IR64; 0.1 kGy	Tolerant to BLB strain IV	Released
Mira-1	2006	Cisantana; 0.2 kGy	Resistant to BPH biotype 1 and 2	Released
Bestari	2008	Cisantana; 0.2 kGy	High yield; resistant to BPH biotype 1 and 2; intermediate resistant to BPH biotype 3	Released
Pandan Putri	2010	PW 1-PsJ; 0.2 kGy	Early maturity; aromatic; semi-dwarf	Released
Inpari Sidenuk	2011	Diah Suci; 0.2 kGy	High yield; slender seed; early maturity	Released
Mugibat	2012	Cimelati; 0.2 kGy	Tolerant to blast fungus race 133; intermediate tolerant to blast fungus race 033 and race 173; intermediate resistant to BPH biotype 1, 2 and 3; long grain and slender seed	Released
Sulutan Unsrat 1	2012	Super Win; 0.2 kGy	High yield; high protein content; intermediate resistant to BPH biotype 1 and 2; tolerant to BLB strain III	Released
Sulutan Unsrat 2	2012	Super Win; 0.2 kGy	High yield; intermediate resistant to BPH biotype 1 and 2; tolerant to BLB strain III	Released
Mustaban	2016	Kewal; 0.2 kGy	High yield; slender seed; early maturity; semi-dwarf	Released
Mustajab	2018	Jembar; 0.2 kGy	Plant architecture; semi-dwarf; high yield; resistant to BLB strain III; medium resistant to BPH biotype 1	Released

Collaboration among CIRA-NNEA, Sam Ratulangi University and the North Sulawesi Regional Government, has resulted in the release of Sulutan Unsrat 1 and Sulutan Unsrat 2 varieties. The two varieties were around 25 days earlier than Superwin's original varieties [12]. At present, collaborations are also being carried out by CIRA-NNEA with several regional governments and higher education in Indonesia (Table 3). Through these collaborations, local rice varieties will be genetically improved by means of induced mutation to obtain varieties with comparable maturity and height with modern varieties, and yet have the original taste and aroma. Mutant varieties with such desirable characteristics will benefit farmers because of shorter growth period, better yield quality and quantity, and relatively higher selling price. At the same time, these mutants can preserve the preferred characteristics of the traditional rice varieties which are almost extinct due to difficulties in competing with modern varieties. CIRA-NNEA is also collaborating with local government of Kota Baru, Paser, East Kalimantan and Sijunjung, West Sumatra to improve local rice varieties through mutation breeding.

Table 3. Radiation treatment of 10 traditional rice varieties done by CIRA-NNEA.

Traditional rice variety	Origin	Growth duration (months)	Plant architecture	Radiation treatment
Simera, Karang Dukuh	Jambi	>4	High	0.2 kGy
Siam Datu	South Kalimantan	>6	High	0.2 kGy
Pandan Wangi	Cianjur, West Java	>6	High	0.2 kGy
Superwin	North Sulawesi	>6	High	0.2 kGy
Dayang Rindu	Musi Rawas	>4	High	0.2 kGy
Payo	Kerinci	>6	High	0.2 kGy
Rojolele	Klaten, Central Java	>4	High	0.2 kGy
Beak Sembalun	NTB	>6	High	0.2 kGy
Barak Cenana	Tabanan, Bali	>6	High	0.2 kGy
Palalawank	Landak, West Kalimantan	>6	High	0.3 kGy

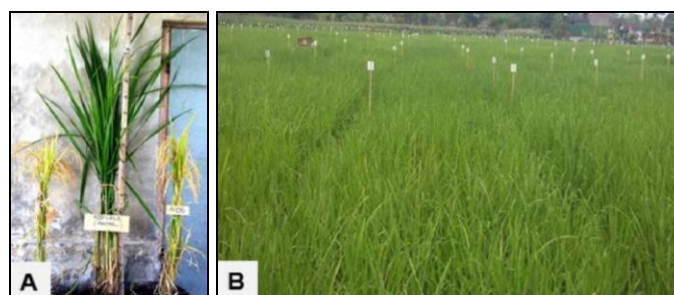


Figure 2. Early and semi-dwarf selected mutant plants (A), early homogeneous and semi-dwarf M₄ lines (B) originating from Rojolele ionized-seed irradiation at a dose of 200 Gy.

5. Conclusions

Mutation breeding has been proved as an effective means to genetically improve Indonesian local rice varieties. This method is useful to change the desired traits without changing preferred traits already present in the rice genetic materials, such as good adaptability in specific regions, flavor and aroma.

Some of Indonesian local rice varieties, such as Pandan Wangi from Cianjur and Superwin from North Sulawesi, have been successfully improved through mutations breeding.

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