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Combination of Somaclonal Variation and Mutagenesis for Crop Improvement

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ABSTRAK

Perbaikan Tanaman Melalui Kombinasi Variasi Somaklonal dan Mutagenesis. ***Endang G. Lestari.*** Perbaikan tanaman melalui mutgenesis dapat mengubah satu atau lebih sifat-sifat atau karakter tertentu pada tanaman dalam upaya memperbaiki mutu tanaman. Kultur jaringan dapat meningkatkan efisiensi teknik dan mempercepat program pemuliaan tanaman melalui pembentukan keragaman diikuti seleksi dan multiplikasi genotipe yang diperoleh. Pada tanaman yang diperbanyak secara vegetatif, kombinasi perlakuan mutasi dan variasi somaklonal sangat efektif dalam program pemuliaan tanaman. Pada tanaman yang diperbanyak melalui biji, kombinasi dengan kultur antera untuk pembentukan tanaman dihaploid sangat menjanjikan. Teknik tersebut dapat mempersingkat program pemuliaan, karena dari keragaman dalam populasi yang ada dapat dilakukan seleksi dan pembentukan genotipe dihaploid serta multiplikasi pada genotipe terpilih yang diinginkan.

Kata kunci: Mutasi, perbaikan tanaman, variasi somaklonal.

ABSTRACT

Combination of Somaclonal Variation and Mutagenesis for Crop Improvement. ***Endang G. Lestari.*** Mutation-based plant improvement, which changes one or a few specific traits of a cultivar, can contribute to crop improvement. Tissue culture increases the efficiency of mutagenic treatment to induce variations. *In vitro* culture in combination with induced mutation can speed up the breeding program by generating variability, followed by selection and multiplication of the desired genotypes. In many vegetative propagated crops, mutation induction in combination with *in vitro* culture techniques can be the most effective method for plant improvement. In seed propagated species, the application of mutation coupled with doubled haploid systems seems to be highly promising in crop improvement. This approach speeds up the breeding program through generation of variability followed by selection of homozygousity and rapid multiplication of desired genotypes.

Keywords: Mutation, plant improvement, somaclonal variation.

INTRODUCTION

One of the aims of *in vitro* technique is to for enhance plant genetic variability, such as through somaclonal variability (Predieri, 2001). Ahloowalia (1986) stated that genetic changes could take place *in vitro* (Karp, l994) due to the presence of mutative cells. Mutation is a sudden and random genetic material changes and functions as the basic variation sources for living organism to be inherited to the next generation. The mutation can occur in nature spontaneously or by induction using mutagens. This process will result in genetic variations that are applied by breeders in their breeding program.

In vitro culture combined with induced mutation, particularly through the use of gamma ray had been proven speed up the breeding program to produce genetic variations or for multiplication (Ahloowalia *et al*., 2004; Maluszynski *et al*., 1995). These techniques have been applied both in the generative and vegetative plant stages. More effective results will be obtained particularly when applied on self-pollinating plants due to their narrow genetic variations, between 0-1% (Baenziger and Peterson, 1992).

Materials that induce mutations are called mutagens that can be divided into three groups, namely colchicines, physical mutagen, and chemical mutagen. Colchicines functions in hampering the spindle thread formation in the anaphase of the cell division. The physical mutagens include ultra violet light, gamma-rays, and x-rays. The chemical mutagens include Ethyl Methane Sulfonate (EMS) and Diethyl Ethan Sulfonate (DMSO). The physical mutagen is commonly used in the form of ionizing radiation, while the chemical mutagen is commonly used in the form of chemical solution (IAEA, l977; Van Harten, l998).

In addition to mutagens mentioned above, *in vitro* selection is one of the somaclonal variation method. Its effectiveness and efficiency are due to its ability to change the plant into the desired characters, either by applying a selection agent onto the culture media or by giving a certain condition to change the somaclonal with the required character.

SOMACLONAL VARIATION AND *IN VITRO* SELECTION

Somaclonal Variation

Somaclonal variation is a genetic variation in a plant occured during *in vitro* or cell culture (Karp, l994; Ahloowalia, l986). The variation can take place due to several factors, such as (1) chromosome multiplication, (2) change in chromosome structure, (3) somatic cross-breed or change of chromatic sister, (4) gene amplification and deletion, (5) jumping particles, and (6) chariotype change (Larkin and Scowcroft, l981; George and Sherrington, 1984; George, l993; Maluszynski *et al*., l995; Duncan *et al*., l995), as well as a change in promoter sequence and deletion of the introns (Ahloowalia, 1997). Mutation is expected to occur to one gene toward the expected character (Bozorgipour and Snape, l997). Meanwhile, somaclonal variation could take place at the dominant or to the recessive gene including the sole gene (Broertjes and Van Harten, l988).

Factors that could effect somaclonal variations in the cultured cells are ages of culture, sources of explants, pathways of regeneration, genotypes of the donor plant, environmental conditions during the culture, types and concentrations of plant growth regulators in the culture media, and presence or absence of *in vitro* selective agents (Larkin and Scowcroft, 1981; Maliga, l984; Smith *et al*., 1993). Genetic variations are sources for plant breeding and biotechnology that provide an easy access to innovative genetic variations to support the breeding program (Anwar *et al*., 2010).

In Vitro Selection

Through *in vitro* selection, cell population resulted from the somaclonal variation could be selected for their particular characteristics such as tolerances to drought, low temperature, A1 stress, acid soil, plant diseases. Recently, the global warming has caused decrease in the food production. To overcome this problem, new improved varieties must developed for tolerance to the new environmental condition, particularly the high temperature.

In vitro selections of particular cells to improve tolerance to water stress have been reported such as in *Vigna radiata* L. (Gulati and Jaiwal, l993), *Capsicum annum* L (Santoz-Diaz and Ochoa-Alejo, l994), and *Brassica juncea* (Gangopadhyay *et al.,* l997). Shoot regenerants with drought tolerance has been obtained from *Nicotiana plumbaginifolia* (Sumaryati *et al*., l992), *Sorghum bicolor* L. (Duncan *et al*., l995; Gupta, l997), rice (*Oryza sativa* L.) (Adkins *et al*., l995), and *Lycopersicon esculentum* (Bressan *et al*., 1981).

Lack of water is one of the most important environmental disturbances that influence the distribution of many species from year to year. The tissue culture method is a novel approach to solve this problem. The main idea is to use cultured cells as the selection units instead of the whole plants. This method is based on spontaneous or induced mutation *in vitro* followed with selection of resistant cells on a medium containing a selective agent and subsequent regeneration of the surviving resistant cells.

Polyethylene glycol, a non-penetrable and non-toxic osmotic compound, lowers water potential of the medium and has been used to stimulate drought stress in plants. Genetic improvement through *in vitro* selection of Satsuma *mandarin* orange (*Citrus unshiu* Marc.) for tolerance to water stress resulted in downsizing of the fruit but improving the fruit water content (Morinaga and Rykes, 2001).

COMBINATION OF SOMACLONAL VARIABILITY AND MUTAGENESIS

Tissue culture has a potential for improving effectiveness of mutation induction in several aspects. Primarily, it offers several choices of plant material to be treated with the mutation induction technique. For examples, *in vitro* axillary buds, organs, tissues, and cells are more suited than *in vivo* buds. Tissue culture also allows for handling of a large population for mutagenic treatment, selection, and cloning of selected variants (Jain, 2001). It also offers a rapid execution of propagation cycles of subculture aimed to distinguish the mutated from the non-mutated sectors (Ahloowalia, 1998). Frequency of somaclonal variation is determined by a number of factors, including genotypes, sources of explants, duration of culture, and composition of medium (Skirvin *et al*., 1994; Duncan, 1995).

PHYSICAL AND CHEMICAL MUTAGENESIS

Both physical and chemical mutagens contain nuclear energy to change the plant genetic material, and the process is called physical and chemical mutagenesis.

Physical Mutagenesis

The types of irradiation potentially available for mutagenesis are ultraviolet radiation (UV light) and ionizing radiation (using x-rays, gamma-rays, alpha and beta particles, proton, and neutrons). The effect of

UV light on DNA is to create pyrimidine dimers that act in blocking the DNA transcription and replication with only a small portion of it is mutated (Britt, 1996). Ionizing radiation penetrates deeper into the plant tissue and can induce various types of chemical changes. Britt (l996) provided a complete overview on changes that can occur at gene, chromosome, and genome levels, including chromosomal break down, inversion, duplication, translocation, and point mutation. The x-rays and gamma-rays have been most widely used in ionizing radiation and most effective in fruit breeding.

One of the first steps in mutagenic treatment is estimation of the appropriate dose of mutagen to apply. The unit of the dose of radiation energy absorbed is Gy (Gray), which is equivalent to 1 J kg^{-1} or equivalent to 100 rad. The choice of the dose of mutagen to apply for the highest probability of success for useful mutant rescue is left to the breeder's experience on the specified plant material, its genetics and physiology. Radio-sensitivities of plant materials varied with species and cultivars, physiological conditions of plant and plant organs, as well as with manipulation of the irradiated plant material before and after treatment with the mutagen. Correlations between the physiological status of the plant and its radio-sensitivity is often determined by water content of the plant tissue, since water molecules are most frequently become primary targets of the ionizing radiation. To avoid negative effects of mutation, most researchers preferred the use of relatively low doses of irradiation (Predieri, 2001).

For breeding purposes, the mutagen used need to have low somatic but strong genetic effects to the plant. After treatment, however, growth of the culture needs to be sustained as to overcome primary irradiation injuries and to allow for evaluation of treatment efficiency. The culture can either be irradiated during the growth proliferation or then transferred rapidly to a fresh medium to avoid the formation to toxic compound (Ahloowalia, l998).

The plant tissue irradiation is affected by hyperhydricity that can increase the frequency of induced mutation (Cassells *et al*., 1998). Variants of internode length or fruit colour of trees derived from shoots irradiations with 30 and 40 Gy were identified in the field. Radio-sensitivities of micro-cuttings have also been investigated through tissue culture of pear (*Pyrus communis* L.) cv. 'Doyenne du Comice'. Proliferating cultures occurred through x-rays irradiations at doses ranging from 10 to 60 Gy.

Chemical Mutagenesis

The chemical mutagens most commonly used for mutation induction in plants belong to the class of alkylating agents, such as ethyl methane sulfonate (EMS); diethyl sulphate (DES), ethylene mine (EI); ethyl nitroso urethene (ENU), ethyl nitroso urea (ENH), methyl nitroso urea (MNH), and azides. As compared to the physical mutagens, chemicals mutagens may give rise relatively more to gene mutations than to chromosomal changes. Assessments of LD_{50} for the chemical mutagens are done by varying concentrations and duration of the mutagen treatments, solvent used (e.g. DMSO), and pH of the solution. EMS have been used for induced mutation on banana shoot tips followed by regenerating adventitious buds. Chemical mutagens has also been used in banana (*Musa* spp. AAA group) to produce tolerant variants to fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*) (Predieri, 2001). Purwati (2006) obtained 9 highly susceptible and one susceptible banana variants to *F. oxysporum* f. sp. *cubense* infection from banana somaclones by mutation induction with EMS.

Colchicines as Mutagen

Colchicines have been widely used in increasing the level of ploidy (Van Harten, 1998). This mutagen is important in banana cross-breeding. Colchicines was used to obtain tetraploid banana by immersing shoot tips of the diploid clone 'SH-3362' in a liquid Murashige-Skoog (MS) medium containing 0-1% (2.5 mM) colchicines.

Mechanisms that govern induction of somaclonal variation during cell culture include gene amplification, single nucleotide base change, transposon migration, altered methylation status, chromosome instability, chromosome inversion, single gene mutation, translocation, cytoplasmic genetic changes, ploidy changes, as well as rearrangement and partial deletion of chromosome (Evans and Sharp, 1986; Maliga, 1984; Shoemaker *et al*., l981; Cassels *et al*., 1991). Some of the consequences of the high mutation frequency are chimeras, pleiotropy, instability, and epigenetic variations (Cassels *et al.,* 1991; Jones, 1990; Smith *et al.,* 1993). Useful variations must be stable, durable, and inherited in the Mendelian fashion, while not altering other agronomic or economic importance traits of the donor parents (Cassels *et al*., 1991; Smith *et al*., 1993).

GENETICS CHANGES FOR THE DEVELOPMENT OF NEW CROP VARIETIES

This variations produced through crossing are recombined to produce new and desired gene recombinants. When the existing germplasm fails to provide the desired recombinants, it is then necessary to resort to others sources of variation. Since spontaneous mutations occur with an extremely low frequency, the mutation induction techniques provide tools for rapid creation and increase of variability in crop species. Most product of the induced mutation is recessive and deleterious from a breeding point of view. Despite limitations of the induced mutation, it contributed significantly to plant improvement worldwide, and in some cases it has made an outstanding impact on productivities of some crops.

SOME RESEARCH RESULTS

The FAO/IAEA Mutant Varieties Database indicators that contained more than half of the induced mutant varieties were released in the last decade. Of the crop species group, 769 mutant varieties were developed directly from mutant progenies and 506 varieties were dominated with cereals. Rice is in the first place with 318 varieties, followed by barley, wheat, maize, durum wheat, and others (oat, millet, sorghum, rye and dura).

Most of the rice mutant varieties (215) were released as direct mutants, which means direct seed multiplication from selected mutants (Table 1). Semi-dwarfness (126) and earliness (110 varieties) were characters most often selected in the treated population. On the list of improved characters were also desired characters for increasing sustainability in rice production, such as cold tolerance (6), salt tolerance (9), and photoperiod sensitivity (5). In rice, as in other crops, radiation was more often used to generate desirable traits (190 varieties), while 23 rice varieties were induced by chemical mutagenesis (Maluszynski *et al*., l995). Some mutant rice varieties have given considerable economic impacts. Rutger (1992) presented data on 11 mutant varieties that were cultivated annually in areas of over 100.000 ha each. Among them was the Chinese varieties Zhefu, which was grown in 1.400.000 ha area and variety Yuanfengzao with an area of about one million ha.

The first mutant variety released in Indonesia is probably the x-ray induced tobacco variety 'Clorina F1' that was released in 1934. The next mutant variety is cotton variety 'M.A.9' that was developed in India anf released in 1948, almost 15 year later (Maluszynski *et al*., 1995).

According to the Mutant Varieties Database (MVD), the mutation techniques have been applied on crop improvement in more than 60 countries all over the continents. The list of the crops and the plant species in which induced mutations have used led to the release of at least one improved variety that has recently reached 175 entities. This also included the ornamental and decorative plant species category. Table 2 shows some of the mutant rice varieties that have been released.

The mutant species have recently been as valuable as the traditional crops and frequently even more important in the developing countries for foreign currency income. Induced mutation have been proven the most rapid, direct, and cheap approach to develop new and attractive flower or ornamental plant varieties. A total of 552 mutant varieties of 40 ornamental and decorative plant species have been developed using the various induced mutation techniques and propagation systems and released.

Recent developments in tissue culture and molecular genetics have brought new tools for

Table 1. Plant characters that have been improved by induced mutations and number of the mutant varieties available.

Character	Number of mutant varieties
Semi-dwarfness	126
Earliness	110
Tillering	24
Tallness	23
Grain quality	16
Blast tolerance	14
Adaptability	12
Glutinous endosperm	12
Salt tolerance	9
Cold tolerance	6
Photoperiod insensitivity	5
Lateness	2

Sources: FAO/IAEA Mutant Variety Database (1993). (http:/www.mvgs.iaea.org).

Table 2. Mutant rice varieties with improved salt tolerance through induced mutation, country and year of release, and the mutagen used.

Mutant variety	Country of release	Year of release, and mutagen used
6B	Vietnam	1986, cross with gamma ray Induced var atomita 2
A-20	Vietnam	1990, cross with MNH Induced mutant
Atomita 2	Indonesia	1983, gamma rays
Cahangwei 19	China	1978, gamma rays
Emai No. 9	China	1980, gamma rays
Fuxuan No. 1	China	1968, gamma rays
Jiaxuan No. 1	China	1974, gamma rays
Liayoan 2	China	1992, gamma rays
Mohan-CSR4	China	1983, gamma rays

Sources: FAO/IAEA Mutant Varieties Database (1993). (http://www.mvgs.iaea.org).

generating mutations. Somaclonal variation that was generated through an *in vitro* system has also been proven to be useful for development of crop mutants. Different characters can be selected under *in vitro* conditions. Insertion mutagenesis and retrotransposon activation will also contribute soon to the MVD. Nevertheless, mutation techniques are important not only for germplasm enhancement, but also necessary for identification of gene function and structure.

The fruit industry relies on limited numbers of clonally propagated cultivars established on the recognized fruit quality parameters and consumer familiarity of the product, and is very reluctant to changes. This has limited the use of cross-breeding in fruits, as fruit cultivars are generally highly heterozygous and progenies from cross breeding express a large number of traits that are different from their parents. Other specific problems such as polyploidy, incompatibility, apomixes, and long juvenile period may be used to obtain useful recombination as a laboratories task (Broertjes, l976). In contrast, induced mutation change only one or a few specific traits of an elite cultivar that can contribute to fruit improvement without upsetting requirements of the fruit industry or the consumers.

In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time and fruit ripening, fruit colour, self compatibility, self-thinning, and resistance to pathogens (Donini, 1982). It has also been used in selection of variants for increased resistance to pest, diseases, and herbicides. Induced mutations using gamma-rays have been applied effectively on several *Citrus* spp. Gamma-rays irradiation on bud woods (scions) produced higher frequencies of mutation leading to the production of new variants as compared to the non-treated control. Selection and testing of *Citrus* trees derived from mutations took several years before the plants can be used commercially. In recent studies, induced mutagenesis on *Citrus* could improve characters derived from the original variety three years after mutation of the bud wood, showed in the form of seedless character, as well new flesh and skin colour. Although this has not been the final results, the procedure was much faster than the conventional hybridization technique. In *Citrus,* several attempts have been made at inducing characters variability such as seedless, spineless, and colour changes in fruit and juice (Maluszynski *et al*., 2001). Radiosensitivities (LD_{50}) of acute exposure on *Citrus* that ranges from 40-100 Gy (Sanada and Amano, 1996) are depending on the species and varieties, seeds, floral stage embryos, and *in vitro* material of *Citrus* exposed to the Gamma-rays.

By the mutation technique and *in vitro* selection using fusaric acid 45 mg/l, yellow ambon banana grown in bacterial wilt endemic area could produced fruits (Lestari *et al*., 2006; 2009). In addition, crop irradiation programs that have been done in the National Atomic Energy Agency (BATAN) had successfully produced several superior varieties of mungbean, soybean, rice, and cotton. Some of the rice varieties are Atomita I (1982), Atomita 2 (1983), Atomita 3 (1990), Atomita 4 (1991), and upland rice varieties Situgintung (1992), Cilosari (1996), Woyla and Meraoke (2001), Kahayan, Winongo and Diah Suci (http//www.batan.go.id).

The adaptation and stability tests of selected rice numbers in three locations with different altitudes showed sufficiently significant results. The rice varieties were suitable for planting at Gunung Putri, Cipanas (1200 m above sea level, asl.), Pacet, Cianjur, (950 m asl.) and Cicurug, Sukabumi (540 m asl.). The genotypes that were stable and adaptive to the three locations were 1B, 1C, 1D, 6B, and 15. Genotypes 3 and 7 were adaptive specifically at Pacet area, 5A was adaptive to Gunung Putri, and genotype 4 was adaptive to Cicurug (Syukur *et al*., 2011).

The somaclonal technique shows its advantage particularly in increasing the genetic variation. Using

this technique, therefore, new superior varieties with considerable tolerances to biotic and abiotic stresses have been established. In general, it could be stated that varietal improvement could be quickly conducted by *tissue culture.*

The characters of the new somaclone varieties, however, could not be precisely predicted. A number of mutation treatments need to be given to obtain the desired varieties. Through *in vitro* selection, however, new genetic changes could be directed to the achievement of the desired characters.

CONCLUSION

In an attempt to increase plant qualities such as yield, plant architecture, fruit colour, pest resistance, salt tolerance, drought tolerance, and heat tolerance, application of somaclonal and *in vitro* culture technique proved to be effective for engineering of superior crop varieties.

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