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Review Paper

Mutation Breeding of Mushroom by Radiation

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Abstract - Mushrooms belonging to macrofungi have been consumed by humans for their nutritional and medicinal values for centuries throughout the world. Mushroom farming is practiced in more than 100 countries of the world, with production increasing at a rate of 7% per annum. High yield and good quality are always the principal goals for agriculturally important crops, including mushrooms. Several breeding methods are employed for strain improvement such as mass selection based on the natural chance mutation and induced mutation (mutation breeding), protoplast fusion technology, cross breeding and transgenic breeding. However, mutation breeding has shown prominent success in crop plant improvement. Though several-hundred mutant crop varieties have been developed around the world, the mutation breeding of mushrooms is limited. This review paper explores the potential application of radiation on the development of mutant varieties of mushrooms for breeding with desired traits such as better quality and productivity.

Key words : Mushroom, Mutation breeding, Radiation mutagenesis

INTRODUCTION

Mushrooms are fruit bodies of macrofungi and labeled as natural and healthy food products. According to Chang and Miles (1992), a mushroom is "a macrofungus with a distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand." These fungi mostly belong to the class of basidiomycetes, and some fungi to the class of ascomycetes. Mushrooms are categorized into four groups (Okhuoya 2011): edible mushrooms, which are fleshy and edible (e.g. *Agaricus bisporous*, *Lentinus edodes*, *Volvariella volvacea*, *Auricularia auricular*, *Pluerotus squarrosullus*, *P. tuberregium*, *Termitomyces africanus*, etc.); poisonous mushrooms, which have proven to be, or are suspected of being, poisonous (e.g. *Amanita phalloides*, *A. verna*, *A. virosa*, *A. muscaria*, etc.); medicinal mushrooms, which are considered to possess medicinal applications (e.g. *Ganoderma lucidum*, *Pleurotus tuberregium*, *L. edodes*, *Schizophylum commune*, etc.); and miscellaneous/other mushrooms, the properties of which remain less well-defined (e.g. *Stereum hirsutum*, *Phallus indusiatus*, etc.). For millennia, mushrooms have been used as a food and valued as medical provisions, and recently, their consumption has been increasing, involving a great number of species apart from the popular white button mushroom, *A. bisporus*, which is the most cultivated mushroom in the world (Mattila *et al.* 2001). Mushroom farming is currently practiced in more than 100 countries around the world, with an estimated total production of over 12 million tons (Suman and Sharma 2007). Its production is increasing at a rate of 7% per annum (Bhupinder and Ibitwar 2007). The oyster mushroom, *P. ostreatus*, is the second-most cultivated mushroom worldwide (Kues and Liu 2000; Berne *et al.* 2008) and comprises about 40 species (Jose and Janardhanan 2000). A great number of bioactive substances present in good varieties of mushrooms hike human biological functions and make people

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healthier. Mushrooms are known not only for their culinary and medicinal properties, but also for their potential applications in multifarious fields, including important industrial production and environmental conservation.

BREEDING AND GENETIC IMPROVEMENT OF MUSHROOMS

The principal goals of mushroom breeders and mushroom-related research are to increase the yield and quality of mushrooms, as well as reduce the production costs. Mushroom breeding does have problems, including storage, senescence-induced browning, and disease resistance. Various improvements, such as a shorter growth cycle and the prevention or reduction of spore formation, should be considered for better mushroom breeding. A lot of breeding methods exist, including mass selection based on natural chance mutation, programmed mutation through the use of radiation or chemicals, crossbreeding, and transgenic breeding. Two other methods successfully employed for breeding are protoplast fusion or protoplast mutagenesis and spore germination (Horgen *et al.* 1991; Kerrigan *et al.* 1992).

Protoplast fusion has been used to improve strains for the desired traits among edible mushrooms in higher basidiomycetes (Chang *et al.* 1993). This has been a potentially feasible method used to create mushroom hybrids, especially when conventional methods cannot be achieved. As a conventional hybridization, protoplast fusion can be performed interspecifically (Takehara *et al.* 1993; Matsumoto *et al.* 1997), intraspecifically (Kiguchi and Yanagi 1985; Toyomatsu and Mori 1987), intergenerically (Eguchi *et al.* 1993; Zhao and Chang 1996) and even interheterogenerically (Toyomatsu and Mori 1987; Eguchi and Higaki 1995). Djajanegara and Masduki (2010) carried out protoplast fusion between white and brown oyster mushrooms to obtain high productivity and a long storage life. Protoplast fusion was used between *Laetiporus sulphureus* having antithrombosis activity and the commonly cultivated basidiomycete *Hypsizygus marmoreaus* to obtain cultivable basidiomycetes capable of producing an anti-thrombin substance to cure thrombosis (Okamura *et al.* 2000). The regeneration of protoplasts is a time consuming and difficult process. The success of protoplast technology depends on the optimum conditions for the efficient regeneration of protoplasts (Reyes *et al.* 1998).

Traditional crossbreeding is an inefficient method to improve mushroom strains. Transgenic breeding is a new way for the genetic improvement of mushrooms to increase their yield and quality. While there are presently no commercially available transgenic mushroom strains, a lot of promising research is being conducted in this direction. The recombinant DNA technology for producing transgenic mushrooms has created numerous possibilities and opportunities. Transformation is an important tool in transgenic breeding and functional genomics studies. Many approaches have been introduced through this technique, such as eliminating, destructing, or silencing a gene to reach different targets or needs (Kwan and Chum 2008). Transformation techniques employed with other filamentous fungi can also be adapted for mushrooms (Van de Rhee *et al.* 1996). Various techniques such as $CaCl_2$, PEG - polyethylene glycol (fusogenic agents) mediated integration (Sato *et al.* 1998; Li *et al.* 2006), restriction enzyme-mediated integration (REMI) (Sato *et al.* 1998; Hirano *et al.* 2000; Irie *et al.* 2003; Joh *et al.* 2003), electroporation/electrofusion (Tamai 1991; Kuo *et al.* 2004; Dhitaphichit and Pornsuriya 2005; Kim *et al.* 2010), *Agrobacterium tumefaciens*-mediated transformation (ATMT method) (Mikosch *et al.* 2001) and particle bombardment (Sunagawa and Yumi 2002; Guo *et al.* 2005) have been used to incorporate DNA into protoplasts, mycelium, or basidiospores (Chakravarty 2011). Adachi *et al.* (2002) recently demonstrated that the transposon arrayed gene knockout system was a highly efficient method for gene identification and targeted gene disruption for genetic modification in filamentous fungi.

MUTATION BREEDING

Mutation breeding is a process by which mutant variants with desirable traits are developed without altering the remaining genotype through physical, chemical and biological mutagenic agents. The mutation breeding method has attracted the interest of breeders for many decades (Fahad and Salim 2009). Mutations may induce one or more changes in the characteristics of the mutated organisms at the gene level, which will be passed to the next generation (Carlile and Watkinson 1994). Mutations are applied to mushrooms to obtain better quality and productivity. A desired mutant characteristic can be economically beneficial, for example, resistance to fungicide, higher yield, etc. Plant mutation

breeding through radiation has been investigated for a long time in many countries. Liu *et al.* (2004) observed that mutation breeding has become one of the most fruitful and widely used methods for crop improvement, and have played an important role in crops, especially in food crop production in China. From 1930 ~ 2004, more than 2,250 mutagenic plant varieties have been released, and have been derived either as direct mutants (70%) or from their progenies (30%) (Maluszynsk *et al.* 2000). According to FAO-IAEA (http://mvgs.iaea.org/PDF/Mutation%20Induction%20for%20Breeding% 20101.pdf), about 3,088 mutant varieties have been registered worldwide as of 2009. Two-hundred and forty-two direct-use mutant varieties generated using irradiation, chemical mutagenesis, and somaclonal variations have been registered in Japan (Nakagawa 2009). New crop plant mutant varieties provide useful gene resources for the security of food resources, environmental conservation, and the promotion of new industries. This phenomenon is not only meant for crop plants alone, but is also suitable for the mushroom industry.

Mutagen is a natural or artificial agent that can alter the structure, function, or sequence of DNA. Mutagenic agents are mainly categorized into two types, physical mutagens (radiation including non-ionizing radiation, i.e., UV, and ionizing radiation, i.e., X-rays, gamma rays, alpha, beta particles, and ions) and chemical mutagens (bromouracil, aminopurine, ethylmethane sulfonate, diethyl sulfate, methyl methane sulfonate, ethyleneoide, ethylene imine, N-nitrosoethyl urea, N-nitrosomethyl urea, diepoxy butane, nitrosoguanidine, etc.). Mutations have also been generated through biological mutagens, such as T-DNA insertions and transposon tagging. Each mutagen has particular features for mutation induction. Chemical mutagenesis usually results in a single nucleotide substitution (point mutation), predominately G : C to A : T transition mutations, in the target genome. Radiation mutagenesis from non-ionizing radiation (UV) induces pyrimidine dimers in DNA, which is a result of a covalent attachment between adjacent pyrimidines in one strand, and finally causes errors in DNA replication. Ionizing radiation produces a range of damage to cells or other genetic material primarily due to the production of free radicals (*OH or hydroxyl radical) by removing electrons from their molecules (water). Free radicals possess unpaired electrons and are chemically very reactive, and will interact with DNA, proteins, and lipids in cell membranes. Ionizing radiation produces a range of effects on DNA, both through free radical effects and direct action, which include breaks in one or both strands, deletions, duplications, inversions, translocations, damage to/loss of bases and the cross linking of DNA (Kaur *et al.* 2011). Recently, new physical mutagens, such as ion beam radiation and cosmic rays, have been proven to be effective for inducing mutations. Ion beams are different from other physical mutagens such as gamma or X-rays in that they not only involve energy transfer (as gamma or X-rays), but also mass deposition and charge exchange; hence, this may result in complex DNA damage and changes (high percentage of double-strand breaks and subsequent chromosome aberrations) that are not found when gamma or X-rays are used. Ion beams are produced by particle accelerators, i.e., cyclotrons. Typical ions used for irradiation on biological samples are neon-20, nitrogen-14, carbon-12, lithium-7, argon-40 and iron-56. A wider mutation spectrum and less collateral physiological damage are commonly reported for ion beam radiation when compared to other mutagens, which is considered an important advantage (Kazama *et al.* 2008; IAEA 2009).

RADIATION VS. CHEMICAL MUTAGENESIS

Chemical mutagenesis is recognized as a valuable supplement to conventional breeding methods for crop improvement (Dhanavel *et al.* 2008), and plays a vital role in enhancing genetic variability in higher plants (Ricardo and Ando 1998; Kumar *et al.* 2009). However, chemical mutagens are less suitable due to several disadvantages such as uneven penetration, low reproducibility, and health risks upon handling (Kang *et al.* 2011). Several studies have shown that chemical mutagens are more effective than physical mutagens (Rao and Rao 1983; Kumar 1988; Dhanayanth and Reddy 2000), while other studies have shown that the opposite is true (Tara and Dnyansager 1980; Zeeral 1991). The IAEA (2009) reported that among the officially released mutant varieties in more than 60 countries throughout the world, close to 90% were developed through irradiation (e.g. fast neutrons, X-rays, more than 64% through gamma irradiation). Gamma radiation can change the genetic diversity of filamentous fungi and induce positive (Boominathan *et al.* 1990) or negative mutants (Liwicki *et al.* 1985; Zolan *et al.* 1988) of specific genes. Gamma radiation is often applied to eukaryotic organ-

isms. Among 242 mutant varieties, about 61% of mutants are induced through gamma irradiation in Japan. This high percentage of gamma irradiated mutants indicates that mutation breeding via gamma irradiation is an effective and highly successful approach for the generation of commercial cultivars (Nakagawa 2009). Gamma radiation is an effective ionizing radiation due to its energy level, which has the ability to penetrate better into the cell walls of mushroom mycelia and target cells (Esser 1971). It has also been proven that low-energy ion beam irradiation causes a higher rate of mutation and a wider mutational spectrum with lower damage (Yu 2006). In several microbiology studies, it is evident that the production of higher metabolites is mostly achieved through radioisotope mutagenesis (Slater 2000). The IAEA (1992) reported that irradiation with an average dose of 10 kGy shows no toxicological hazards and introduces no specific microbial or nutritional problems.

MUTATION BREEDING BY RADIATION

1. Protoplast mutagenesis

Protoplast mutagenesis through UV irradiation was demonstrated in Jisongrong mushrooms (*Agaricus blazei*) by Zhang *et al.* (2004). The mutant strain has good yield, i.e., both the biomass and exopolysaccharide content increased 6% over the parent strain. The mutant strain has good stability even after transferring for ten generations. Kaur *et al.* (2011) genetically improved the third-most popular mushroom in India, *Calocybe indica* (milky mushroom), through protoplast mutagenesis. In this study, mutation was induced by UV and chemical mutagens. Among 30 putative mutants, four showed maximum biological efficiency in terms of yield (maximum 98%) and enzyme studies compared with the parent. The fungicide resistant *A. bisporus* mutant strain was produced by UV irradiation (Elliot and Langton 1981).

A high yielding *G. lucidum* strain was obtained by UV mutagenesis. Its mycelia yield was raised by 21%, and the content of extracellular polysaccharide was 31% higher than the control (Lixin *et al.* 1998). UV induced mutagenesis of Ganoderma's protoplast was carried out (Gang *et al.* 2001). The dry weight and polysaccharides contents of the UV mutants are higher than in the original strain. Auxotropic and morphological mutants have been obtained through the UV mutagenesis of protoplasts from *V. volvacea* monokaryon (Mukherjee and Sengupta 1986). Toyomasu and Mori (1987) and Homolka *et al.* (1988) obtained auxotrophic mutants of *Pleurotus* sp. and *Oudemansiella musida* by proplast mutagenesis.

2. High temperature tolerant mushroom mutants

Since *P. ferulae* is a low-temperature type edible mushroom, it cannot be planted in all seasons. It was found that higher temperatures prevent bud formation and fruiting body growth, and finally result in decreased mushroom production or even no harvest at all. To achieving a year-round production of *P. ferulae*, high temperature tolerant *P. ferulae* strains were developed by Chen *et al.* (2008a). Conidiophores of wild type *P. ferulae* were implanted with nitrogen ions at a dose of $1.5 \times 10^{15} \sim 1.5 \times 10^{16}\ cm^{-2}$. It was found that during riper periods, the surface layer mycelium of the mutant in a mushroom bag was neither aging nor growing tegument even at above 30°C. The mycelium-endurable temperature of the mutant was increased by 5°C compared to that of the wild-type strain. The fruiting body growth temperature of the mutant was 18～22°C during the daytime and 8～14°C at night. The highest growth temperature of the fruiting body of the mutant was increased by about 7°C with respect to that of the original strain. This characteristic (high temperature tolerance) has been found to be stable for three generations of sub-culturing.

3. Low-sporing mushroom mutants

Traditionally, mushrooms produce billions of spores floating in the air, which cause health problems such as lung allergies and fever attacks (Chakravarty 2011). During the cultivation of edible mushrooms including *P. pulmonarius*, the enormous number of spores produced by fruiting bodies adversely affects the health of mushroom farmers, mushroom cultivation facilities, and the genetic diversity of natural populations (Okuda *et al.* 2009a). *Pleurotus* continuously release spores into the atmosphere, and the antigens present on the walls of the spores cause respiratory allergies. Moreover, these spores settle on the fruit bodies, germinate, and form a velvety film, which gives an unpleasant appearance to the mushrooms. Spore allergy is one of the most important limiting factors for the large scale cultivation of this species. Sporeless/low-sporing strains of *Pleurotus* (*P. florida* and *P. sajor-caju*) were developed at the Indian Institute of

Horticultural Research, Bangalore using UV mutation to alleviate the spore allergy problem. Due to the inability of UV rays in breaking the multicellular mycelia into single cell units, the mutation of the mycelium did not yield the desired results. In contrast, mutation of the *P. sajor-caju* spores yielded an extremely low-sporing mutant after 75 min of exposure. Through investigations focusing on 10 generations, it was found that the mutant was stable and has a low-sporing characteristic (Ravishankar *et al.* 2006).

Okuda *et al.* (2009a) constructed a primary genetic linkage map and identified the locus associated with the sporulation-deficient (sporeless) mutation of *P. pulmonarius* using 150 progeny isolates derived from a cross between sporeless and wild-type isolates. The construction of this *P. pulmonarius* genetic linkage map and identification of markers that are closely linked to the sporeless locus will facilitate marker-assisted selective breeding of a sporeless strain with economically important traits. Two sequence-tagged site (STS) markers for the sporeless trait from *P. pulmonarius* sporeless mutant, TMIC-30058 (has a high potential for breeding sporeless cultivars), were described (Okuda *et al.* 2009b). Moreover, Okuda *et al.* (2011) studied the rapid detection of a sporeless trait from *P. pulmonarius* culture extracts through real-time PCR, and concluded that this method enables reducing the time and labor for screening the sporeless trait, suggesting its availability for the efficient breeding of sporeless cultivars.

4. High biological efficient mushroom mutants

Lignocellulolytic mutants of *P. ostreatus* were developed by irradiating the mycelia with gamma-rays (1 ~ 2 kGy). The selected irradiated strains were able to form fruiting bodies and grew similarly to the control. The extracellular enzyme activities in a liquid media of the isolated strains were up to 10-times higher than the control. Genetic similarities of the irradiated strains ranged from 64% to 93% of the control. From these results, it seems that the gamma radiation induces genetic diversity and the mutants can be used to recycle the lignocellulosic waste (Lee *et al.* 2000).

Mutation using ^{60}Co gamma-ray on *P. floridae* yielded 4 putative mutants, which showed higher productivity compared to the control. Higher productivity is reflected by a significantly higher amount of fruiting bodies, higher fresh weight and dry weight yields of three successive flush periods. The average diameter of the fruit bodies of the mutant was bigger compared to the control (Djajanegara and Harsoyo 2009). Phenolic compounds increased after a 2 kGy dose of gamma irradiation when the samples were compared with the control over a nine-day storage period in *A. bisporus* (Beaulieu *et al.* 1999).

Pleurotus mushroom strains (*P. sajor-caju* strains, *P. columbinus* and *P. hybrid*) were subjected to gamma and UV irradiation to induce mutants with improved characteristics in terms of good yield and adaptability to a wider range of temperatures. Mycelial plugs of the parent strains were subjected to gamma radiation ranging from 5 to 400 Gy using a ^{134}Cs radioisotope. Even at a dose of 400 Gy, no mortality was induced in the *Pleurotus* strains, and all irradiated mycelial plugs successfully showed growth onset and mycelium development. However, beyond 200 Gy, the rate of mycelial growth was reduced during Petri dish incubation. Gamma irradiated (400 Gy) strains took double the amount of days to reach a radial mycelial diameter of 50 mm than that of non-irradiated or doses of less than 300 Gy. Certain stimulatory effects of gamma irradiation were noted on the mycelial growth rate and yield of fruiting bags at different doses. However, these effects were not consistent. Similarly, for UV irradiation, the multispore suspensions of the parent strains were subjected to UV-ray exposure times varying from 3 to 20 hrs. After plating, vigorously growing mycelia were obtained. Decreased viability was noted in several strains after repeated subcultures and storage. Viable UV-irradiated strains exhibited similar stimulatory effects as in gamma irradiation at certain exposure times (Beejan and Nowbuth 2009).

Sommer *et al.* (2010) investigated the impact of gamma irradiation on nucleotides and on the free amino acids tyrosine and phenylalanine in fresh mushrooms, *A. bisporus*. Irradiation significantly reduced the guanosine diphosphate concentration (22%). Adenosine monophosphate showed a marked reduction (46%) only at 5 kGy. Guanosine monophosphate, tyrosine, and phenylalanine were not affected by gamma irradiation.

Huang and Mau (2006) obtained higher antioxidant activities in gamma irradiated *A. blazei* than those of the non-irradiated version, and also observed that gamma-irradiation not only evidently maintains the antioxidant properties of *A. blazei* but also enhances its antioxidant properties to some extent. Up to 20 kGy of irradiation did not remarkably affect

the amounts of total antioxidant components in *A. blazei*.

5. Mushroom mutants developed by ion implantation

Lu *et al.* (2008) implanted *Lentinula edodes* with various nitrogen ion dosages and investigated the growth rate of mycelium, mycelial biomass, and lentinan (LNT) contents. The curves of implantation all take a "saddle shape" with increasing N^+ ion implanted doses. While the growth rate of mycelium is high under a low dosage of N^+ implantation, with an increment of dosage, the growth rate shows a downtrend, and then begins rising when the dosage reaches 1.5×10^{16} cm^{-2}. The mycelia biomass and contents of LNT rise first, and then fall with the increment of dosage, both reaching maximum at a dosage of 4×10^{15} cm^{-2}. This seems to be an effective way of mutation breeding through N^+ ion implantation. It has also been found that the polysaccharide yield of the *Pleurotus* mycelium mutants induced by ion beam implantation increased by 75% compared to that of the initial strain (Wang *et al.* 2009a). The UV and He-Ne laser mutated mycelia of *Polyporus umbellatus* showed a high mycelial growth rate and extracellular polysaccharide yield. A mutant strain (LU-7) of *P. umbellatus* was screened out. Its mycelial dry weight and extracellular polysaccharide yield were increased by 25% and 83%, respectively, compared with the parent strain. The subculture of ten generations proved that the mutated strain has relative good genetic stability (Yin *et al.* 2008). A single ion beam mutation breeding method for *P. ferulae* was explored. Conidiophores and mycelium mono-cells of *P. ferulae* were separately treated by nitrogen ion (1.5×10^{15} $cm^{-2} \sim 1.5 \times 10^{16}$ cm^{-2}) implantation. It was distinct to erode the conidiophores and mycelium mono-cells of *P. ferulae* by low-energy N^+ beam implantation, and the energy-effect and dose-effect of the cell livability were prominent. It is clear that the ion beam implantation mutation method is single and feasible to operate for large-type fungus mutation breeding (Chen *et al.* 2008b). A high laccase producing *P. ostreatus* mutant was produced by exposing the basidiospores of *P. ostreatus* to an N^+ beam. The maximum laccase activity of the mutants increased up to 7.78 U g^{-1}, which is 2.8-times of the original stain (Wang *et al.* 2009b). The effect of low-energy ion implantation on the *Pleurotus* polysaccharide was studied by Fu *et al.* (2005). It was observed that the intracellular and extracellular polysaccharide content were increased by 20% and 19% when compared to their parent strains.

6. Genetic similarity of mycelia and basidiospores on irradiation

The genetic similarity of mycelia and basidiospores was altered according to the gamma radiation dose, with 22 ~ 25% changes for basidiospores and 23 ~ 36% for mycelia (Lee and Chang 1999), which shows that the basidiospore may be more radioresistant than the mycellium of *P. ostreatus* and that the genetic similarity of the mycelium of *P. ostreatus* can be changed more easily than that of the basidiospore through gamma radiation. The genetic diversity and phylogenetic relationships among *P. ostreatus* strains mutated by a proton beam was investigated (Kwon *et al.* 2007). Based on an AFLP DNA polymorphism analysis, the dissimilarity among the induced strains increased when increasing the dose. When using more than 400 Gy, the genetic dissimilarity of the irradiated strains was 46 ~ 58%. Kwon and Kong (2006) reported that the optimum proton-beam dose as a mutation source was between 500 Gy and 750 Gy, and 400 Gy and 1,000 Gy, for the basidiospores and mycelia of oyster mushrooms, respectively. Chang *et al.* (2003) concluded that the mutational hotspots for the gamma radiation may be in gene (s) within cells when studying the mutational spectra in the manganese (II) peroxidase gene (*mnp*) of the *P. ostreatus* mutants induced by gamma irradiation (^{60}Co). These mutation-spectra were predominantly A : T to G : C transitions (50%).

Mutant strains of *Pleurotus citrinopileatus* with a high growth rate (21% more than the parental strain) were obtained through ^{60}Co-gamma irradiation. The results of an antagonistic test, esterase isozyme, and peroxidase isozyme electrophoresis revealed significant genetic differences between the mutant strain and its parent stain. After six generations of continuous cultivation, the mutant strain kept good growth vigor, thereby displaying high genetic stability (Cai *et al.* 2009).

7. Irradiation for maintaining the postharvest quality of mushrooms

Several reports have suggested that irradiation treatment is highly effective in inhibiting physical changes associated with postharvest deterioration and maintaining a fresh product appearance (Kader 1986). Doses of gamma irradiation

inhibited cap opening, stalk elongation, and browning; reduced the level of microbial contamination; and generally extended the shelf life of *A. bisporus*, with no detectable impact on organoleptic attributes (Gill *et al.* 1969; Lescano 1994). Furthermore, gamma irradiation extended the storage life of *P. sajor-caju* (Roy *et al.* 2000) and *P. pulmonarius* (Xia *et al.* 2005) without adversely affecting key nutritional components. Radiation treatments also delayed the cap opening and browning, and lowered the rates of decomposition and weight loss, in a straw mushroom, *V. volvacea* (Ye *et al.* 2000; Liu *et al.* 2003).

Ionizing radiation has been a common practice for extending the storage life in mushrooms. The storage life of button mushroom fruit bodies increased from 8 weeks to 12 weeks through radiation at 1.5～3 kGy (Maha and Pangerteni 1989). Van-Logten *et al.* (1971) stated that there were no apparent harmful effects of irradiated mushrooms on animals in respect of growth and food intake, survival, blood chemistry and morphology, organ weights, histopathogenecity, and reproduction. In 1969, the Health Authorities of the Netherlands cleared mushrooms irradiated at a maximum dosage of 250 K rad for human consumption (Chang and Quimio 1982).

In *P. nebrodensis*, an irradiation dose of 1.2 kGy significantly delayed (by 6～9 days) the onset of fruit body softening, splitting, and browning compared with non-irradiated controls. Irradiation with 1.2 and 1.6 kGy also had a positive effect on other indicators of mushroom tissue senescence, resulting in smaller decreases in soluble protein levels and more protracted increases in proteinase activity. Peak levels of polyphenoloxidase activity, widely recognized as causing postharvest browning of mushroom tissue, were also significantly lower in fruit bodies exposed to 1.2 kGy compared with non-irradiated controls (Xiong *et al.* 2009).

Xing *et al.* (2007) showed that the exposure of *Hypsizygus marmoreus* fruit bodies to 0.8 kGy ^{60}Co gamma irradiation was clearly beneficial in maintaining the postharvest appearance of mushroom sporophores. The smallest increases in proteinase activity were recorded in samples dosed with 0.8 and 2.0 kGy, and levels of superoxide dismutase were significantly higher in samples exposed to 0.8 kGy compared with non-irradiated controls. Gamma irradiation was reported to delay the appearance of unsightly aerial hyphae on the surface of *H. marmoreus* fruit bodies during postharvest storage at 4°C.

CONCLUSION

As the consumption of mushrooms for their nutritional and medicinal properties is increasing all around the world irrespective of religion, community, culture, and food habits, research has to be focused on the breeding of high yield and good quality mushroom strains to meet the world's requirements. Mutation breeding is a modern breeding method. Among the various methods of mutagenesis, radiation mutation is a very promising method, and further exploration has to be made in terms of development of superior mushroom strains with desired traits, from theory to practice.

REFERENCES

Adachi K, Nelson GH, Peoples KA, Frank SA, Montenegro-Chamorro MV, Dezwaan TM, Ramamurthy L, Shuster JR, Hamer L and Tanzer MM. 2002. Efficient gene identification and targeted gene disruption in the wheat blotch fungus *Mycosphaerella graminicola* using TAGKO. *Curr. Genet*. **42**:123-127.

Beaulieu M, Daprano MBG and Lacroix M. 1999. Dose rate effect of γ irradiation on phenolic compounds, polyphenol oxidase, and browning of mushrooms (*Agaricus bisporus*). *J. Agric. Food Chem*. **47**:2537-2543.

Beejan PHF and Nowbuth R. 2009. Use of irradiation for the induction of mutations in oyster mushrooms for improvement of strains. pp. 289-292. *In*: Induced plant mutations in the genomics era (Shu QY *ed.*), Food and Agriculture Organization of the United Nations, Rome.

Berne S, Pohleven F, Turk T and Sepcic K. 2008. Induction of fruiting in oyster mushroom (*Pleurotus ostreatus*) by polymeric 3-alkylpyridinium salts. *Mycol. Res*. **112**:1085-1087.

Bhupinder K and Ibitwar BB. 2007. Mushroom cultivation and processing. *Science Tech. Entrepreneur* March issue:1-12.

Boominathan K, Balachandra DS, Randall TA and Reddy CA. 1990. Nitrogen-deregulated mutants of *Phanerochaete chrysosporium*-a lignin-degrading basidiomycete. *Arch. Microbiol*. **153**:260-265.

Cai DH, Zhang QS, Dong HX, Wang XL, Liu SF and Wang YJ. 2009. Breeding of a novel *Pleurotus citrinopileatus* strain. *Food Sci*. **21**:301-304.

Carlile MJ and Watkinson SC.1994. The Fungi. p. 482 Academic Press, London.

Chakravarty B. 2011. Trends in mushroom cultivation and breeding. *Aust. J. Agric. Eng*. **2**:102-109.

Chang HH, Lee YK, Kim JS, Lee KS and Cho KS. 2003. Muta-

tion spectrum of manganese (II) peroxidase gene in the *Pleurotus ostreatus* mutants induced by gamma radiation. *J. Microbiol*. **41**:52-57.

Chang ST, Buswell JA and Miles PG. 1993. Genetics and breeding of edible mushrooms. pp. 324. Gordon and Breach Science Publishers, Philadelphia.

Chang ST and Miles PG. 1992. Mushroom biology - a new discipline. *The Mycologist*. **6**:64-65.

Chang ST and Quimio TH. 1982. Tropical mushrooms biological nature and cultivation methods. pp. 493. The Chinese University Press, Hong Kong.

Chen H, Wan H, Zhang J and Zeng X. 2008a. Mutation-screening of *Pleurotus ferulae* with high temperature tolerance by nitrogen ion implantation. *Plasma Sci. Technol*. **10**:503-505.

Chen H, Wu BS, Lv J, Zeng XX. 2008b. Bioeffect and mutation breeding method of *Pleurotus ferulae* by ion beam implantation. *Biotechnol*. **4**:38-40.

Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M and Thilagavathi C. 2008. Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). *Afr. J. Biotechnol*. **7**:4116-4117.

Dhanayanth KPM and Reddy V. 2000. Cytogenetic effect of gamma rays and ethyl methane sulphonate in chilli piper (*Capsicum annum*). *Cytologia* **65**:129-133.

Dhitaphichit P and Pornsuriya C. 2005. Protoplast fusion between *Pleurotus ostreatus* and *P. djamor*. *Songklanakarin J. Sci. Technol*. **27**:975-982.

Djajanegara I and Harsoyo. 2009. Mutation study on white oyster mushroom (*Pleurotus floridae*) using gamma (^{60}Co) irradiation. *J. Chem. Nat. Resour. Eng*. **4**:12-21.

Djajanegara I and Masduki A. 2010. Protoplast fusion between white and brown oyster mushrooms. *Indo. J. Agric. Sci*. **11**:16-23.

Eguchi F, Fukuzumi T and Higaki M. 1993. Production of new species of edible mushrooms by protoplasts fusion method II. Analysis of the mycelia and basidiospores of a fusant between *Pleurotus ostreatus* and *Agrocybe cylindracea*. *Mokuzai Gakkaishi*. **39**:825-830.

Eguchi F and Higaki M. 1995. Production of new species of edible mushrooms by protoplast fusion method III. Protoplast fusion and analysis of the fusant between *Pleurotus sajor-caju* and *Mycoleptodonoides aitchisonii*. *Mokuzai Gakkaishi*. **41**:342-348.

Elliot TJ and Langton FA. 1981. Strain improvement in the cultivated mushroom *Agaricus bisporus*. *Euphytica* **30**: 175-182.

Esser K. 1971. Application and importance of fungal genetics for industrial research. pp. 83-91. *In*: Proceedings of the symposium on use of radiation and radioisotopes for industrial microorganisms, 29 March-1 April, Vienna, Austria.

Fahad AQ and Salim K. 2009. Mutagenic effects of sodium azide and its application in crop improvement. *World Appl. Sci. J*. **6**:1589-1601.

Fu YQ, Zhang LV and Zeng X. 2005. Low energy ion implantation on the biological effects of *Pleurotus* polysaccharide. *Chem. Biol. Eng*. **5**: DOI:cnki:ISSN:1672-5425.0.2005-05-004.

Gang L, Fan Y, Ruixue L, Zhixiang X and Baojian L. 2001. A study on the breeding of new Ganoderma varities by UV induced mutagenesis. *Acta Microbiol. Sin*. **2**:229-233.

Gill WJ, Nicholas RC and Markakis P. 1969. Irradiation of cultured mushrooms. *Food Technol*. **23**:385-388.

Guo LQ, Lin JF, Xiong S and Chen SC. 2005. Transformation of *Volvariella volvacea* with a thermal hysteresis protein gene by particle bombardment. *Acta Microbiol. Sin*. **1**:39-43.

Hirano T, Sato T, Yaegashi K and Enei H. 2000. Efficient transformation of the edible basidiomycete *Lentinus edodes* with a vector using a glyceraldehyde-3-phosphate dehydrogenase promoter to hygromycin B resistance. *Mol. Gen. Genet*. **263**:1047-1052.

Homolka L, Vyskocil P and Pilat P. 1988. Mutagenization of protoplasts of *Oudemansiella mucida. Appl. Microbiol. Biotechnol*. **28**:166-169.

Horgen PA, Jin T and Anderson JB. 1991. The use of protoplast production, protoplast regeneration and fragment length polymorphisms in developing a systematic and highly reproducible breeding strategy for *Agaricus bisporus*. pp. 62-71. *In*: Genetics and breeding of *Agaricus* (Van-Griensven LJLD *ed*.). Pudoc, Wageningen.

Huang SJ and Mau JL. 2006. Antioxidant properties of methanolic extracts from *Agaricus blazei* with various doses of γ-irradiation. *LWT- Food Sci. Technol*. **39**:707-716.

IAEA-International Atomic Energy Agency. 1992. Irradiation of spices herbs and other vegetable seasonings. A compilation of technical data for its authorization and control. IAEA-TECDOC- **639**:16-17.

IAEA-International Atomic Energy Agency. 2009. Developments in mutation assisted plant breeding. http://www.iaea.org/About/Policy/GC/GC53/ GC53InfDocuments/ English/ gc53inf-3-att1_en.pdf.

Irie T, Sato T, Saito K, Honda Y, Watanabe T, Kuwahara M and Enei H. 2003. Construction of a homologous selectable marker gene for *Lentinula edodes* transformation. *Biosci. Biotechnol. Biochem*. **67**:2006-2009.

Joh JH, Kim BG, Chu KS, Kong WS, Yoo YB and Lee CS. 2003. The efficient transformation of *Pleurotus ostreatus* using REMI Method. *Mycobiol*. **31**:32-35.

Jose N and Janardhanan KK. 2000. Antioxidant and antitumour activity of *Pleurotus florida*. *Curr. Sci.* **79**:941-943.

Kader AA. 1986. Potential applications of ionizing radiation. *J. Am. Food Technol*. **40**:117-121.

Kang SY, Ha BK and Kim DS. 2011. Mutation breeding. pp. 150-161. *In*: Radiation Technology and Its Applications (Yoo BD *ed*.), Nuclear Training and Education Center, Korea.

Kaur J, Sodhi HS, Kapoor S, Khanna PK and Jaswal RK. 2011. Strain improvement of specialty mushroom, *Calocybe indica*, through mutagenesis. *Appl. Biol. Res*. **13**:62-69.

Kazama Y, Saito H, Yamamoto YY, Hayashi Y, Ichida H, Ryuto H, Fukunishi N and Abe T. 2008. LET-dependent effects of heavy-ion beam irradiation in *Arabidopsis thaliana*. *Plant Biotechnol*. **25**:113-117.

Kerrigan RW, Baller LM, Horgen PA and Anderson JB. 1992. Strategies for the efficient recovery of *Agaricus bisporus* homokaryon. *Mycologia* **84**:575-579.

Kiguchi T and Yanagi SO. 1985. Intraspecific heterokaryon and fruit body formation in *Coprinus macrorhizus* by protoplast fusion of auxotrophic mutants. *Appl. Microbiol. Biotecnol*. **22**:121-127.

Kim JK, Park YJ, Kong WS and Kang HW. 2010. Highly efficient electroporation-mediated transformation into edible mushroom *Flammulina velutipes. Mycobiol*. **38**:331-335.

Kues U and Liu Y. 2000. Fruiting body production in basidiomycetes. *Appl. Microbiol. Biotechnol*. **54**:141-152.

Kumar S. 1988. Recessive monogenic mutation in grain pea (*Pisum sativum*) that causes pyridoxine requirement for growth and seed production. *J. Biosci*. **13**:415-418.

Kumar VA, Kumari RU, Amutha R, Kumar TS, Hepziba SJ and Kumar CRA. 2009. Effect of chemical mutagen on expression of characters in arid legume pulse-cowpea (*Vigna unguiculata* (L.) Walp.). *Res. J. Agric. Biol. Sci*. **5**:1115-1120.

Kuo CY, Chou SY and Huang CT. 2004. Cloning of glyceraldehyde-3-phosphate dehydrogenase gene and use of the gpd promoter for transformation in *Flammulina velutipes. Appl. Microbiol. Biotechnol*. **65**:593-599.

Kwan HS and Chum WWY. 2008. Molecular analysis and genomic studies of shiitake mushroom *Lentinula edodes*. pp. 35-70. *In*: Mushrooms as fuctional foods (Cheung PCK *ed*.), John Wiley & Sons, Inc., Hoboken, New Jersey.

Kwon HJ and Kong WS. 2006. Proton beam sensitivity of basidiospore and mycelium in *Pleurotus ostreatus*. *Kor. J. Mycol*. **34**:34-38.

Kwon HJ, Park YJ, Yoo YB, Park SY and Kong WS. 2007. Genetic variability and phylogenetic relationship among proton-beam-irradiated strains of *Pleurotus ostreatus*. *J. Microbiol. Biotechnol*. **17**:1041-1044.

Lee YK and Chang HH. 1999. Radiation sensitivity of basidiospore and mycelium in *Pleurotus ostreatus. J. Kor. Nuclear Soc*. **31**:287-293.

Lee YK, Chang HH, Kim JS, Kim JK and Lee KS. 2000. Lignocellulolytic mutants of *Pleurotus ostreatus* induced by gamma-ray radiation and their genetic similarities. *Radiat. Phys. Chem*. **57**:145-150.

Lescano G. 1994. Extension of mushroom (*Agaricus bisporus*) shelf life by gamma radiation. *Post-harvest Biol. Technol*. **4**:255-260.

Li G, Li R, Liu Q, Wang Q, Chen M, Li B. 2006. A highly efficient polyethylene glycol-mediated transformation method for mushrooms. *FEMS Microbiol. Lett*. **256**:203-208.

Lixin L, Shaoqi Z and Ruhua Y. 1998. Mutation breeding and submerged culture of *Ganoderma lucidum. Ind. Microbiol*. 3: DOI:CNKI1.ISSN:001-6678.0.1998-03-002.

Liu C, Xu HQ, Wang H and Cai J. 2003. Fresh keeping of mushroom by irradiation. *Acta Agric. Nuc. Sin.* **17**:363-366.

Liu L, Van-Zanten L, Shu QY and Maluszynski M. 2004. Officially released mutant varieties in China. *Mutation Breeding Review* (*IAEA Newsletter*). **14**:1-64.

Liwicki R, Paterson A, Macdonald MJ and Broda P. 1985. Phenotypic classes of phenoloxidase-negative mutants of the lignin-degrading fungus *Phanerochaete chrysosporium. J. Bacteriol*. **162**:641-644.

Lu G, Zhou H, Chen H, Zhang J and Jie L. 2008. Study of biological effects on *Lentinula edodes* by N^+ ion implantation of low energy. *J. Rad. Res. Rad. Proc.* **2**:85-88.

Maha M and Pangerteni DS. 1989. Preservation of mushroom (*Volvariella volvaceae*) with the combination of heating and irradiation. pp. 1207-1215. *In*: Proceedings of the symposium on applications of isotopes and radiation. 13-15 December. Jakarta, Indonesia.

Maluszynsk, MK, Nichterlein K, Van-Zanten L and Ahloowalia BS. 2000. Oficially released mutant varieties. *Mutation Breeding Review* (*IAEA News Letter*) **12**:1-84.

Matsumoto M, Eguchi F and Higaki M. 1997. Polyethylene glycol centrifugal fusion between *Pleurotus ostreatus* and *Pleurotus sajor-caju*. *Mokuzai Gakkaishi*. **43**:215-219.

Mattila P, Konko K, Eurola M, Pihlava JM, Astola J and Vahteristo L. 2001. Contents of vitamins, mineral elements, and phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem*. **49**:2343-2348.

Mikosch TS, Lavrijssen B, Sonnenberg AS and Vangriensven LJ. 2001. Transformation of the cultivated mushroom *Agaricus bisporus* (Lange) using T-DNA from *Agrobacterium tumefaciens. Curr. Genet*. **39**:35-39.

Mukherjee M and Sengupta S. 1986. Mutagenesis of protoplasts and regeneration of mycelium in the mushroom *Volvariella volvacea. Appl. Environ. Microbiol*. **52**:1412-1414.

Nakagawa H. 2009. Induced mutations in plant breeding and biological researches in Japan. pp. 48-54. *In*: Induced Plant

Mutations in the Genomics Era (Shu QY *ed*.), Food and Agriculture Organization of the United Nations, Rome.

Okamura T, Takeno T, Dohi M, Yasumasa I, Hayashi T, Toyoda M, Noda H, Fukuda S, Horie N and Ohsugi M. 2000. Development of mushrooms for thrombosis prevention by protoplast fusion. *J. Biosci. Bioeng*. **89**:474-478.

Okhuoya JA. 2011. Mushrooms: what they are and what they do. Inagural lecture series 114. 3rd March. University of Benin, Nigeria.

Okuda Y, Murakami S and Matsumoto T. 2009a. A genetic linkage map of *Pleurotus pulmonarius* based on AFLP markers, and localization of the gene region for the sporeless mutation. *Genome* **52**:438-446

Okuda Y, Murakami S and Matsumoto T. 2009b. Development of STS markers suitable for marker-assisted selection of sporeless trait in oyster mushroom, *Pleurotus pulmonarius. Breeding Sci*. **59**:315-319.

Okuda Y, Matsumoto T and Ninomiya K. 2011. Rapid detection for sporeless trait from *Pleurotus pulmonarius* culture extracts by using real-time PCR. *Mycosci*. **52**:143-146.

Rao GM and Rao VM. 1983. Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. *Cytologia* **48**:427-436.

Ravishankar S, Pandey M, Tewari RP and Krishna V. 2006. Development of sporeless/low sporing strains of *Pleurotus* through mutation. *World J. Microbiol. Biotechnol*. **22**:1021-1025.

Reyes RG, Eguchi F, Ijima T and Higaki M. 1998. Regeneration of protoplasts from hyphal strands of *Volvariella volvacea. J. Wood Sci*. **44**:401-407.

Ricardo M and Ando A. 1998. Effects of gamma-radiation and sodium azide on quantitative characters in rice (*Oryza sativa* L.). *Genet. Mol. Biol*. **21**:244-251.

Roy MK, Chatterjee SR and Bakukhandi D. 2000. Gamma radiation in increasing productivity of *Agaricus bisporus* and *Pleurotus sajor-caju* and enhancing storage life of *P. sajor-caju. J. Food Sci. Technol*. **37**:83-86.

Sato T, Yaegashi K, Ishii S, Hirano T, Kajiwara S, Shishido K and Enei H. 1998. Transformation of the edible basidiomycete *Lentinus edodes* by restriction enzyme-mediated integration of plasmid DNA. *Biosci. Biotechnol. Biochem*. **62**: 2346-2350.

Slater RJ. 2000. Radioisotope technique. pp. 687-728. *In*: Principle and Technique of Practical Biochemistry (Wilson K and Walker J *eds*.), 5th ed. Cambridge University Press, Cambridge.

Sommer I, Schwartz H, Solar S and Sontag G. 2010. Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine and phenylalanine in mushrooms (*Agaricus bisporus*). *Food Chem*. **123**:171-174.

Suman BC and Sharma VP. 2007. Mushrooms cultivation in India. pp. 18-22. Daya Publishing House, Delhi.

Takehara T, Kumata A and Aono S. 1993. Interspecific protoplast fusion between some *Pleurotus* species using auxotrophic mutants. *Mokuzai Gakkaishi*. **39**:855-859.

Tamai Y. 1991. Electrical fusion of edible basidiomycetes interspecific fusant between *Pleurotus* species. *Res. Bull. Coll. Exp. For*. **48**:445-426.

Tara JL and Dnyansager VR. 1980. Comparison of ethyl methane sulphonate and radiation induced meiotic abnormalities in *Turnera ulmifolia* L. var. *angustifolia* wild. *Cytologia* **45**: 221-231.

Toyomasu T and Mori KI. 1987. Intra- and inter-specific protoplast fusion between some *Pleurotus* species. *Agric. Biol. Chemi*. **51**:935-937.

Van de Rhee MD, Graca PMA, Huizing HJ and Mooibroek H. 1996. Transformation of common mushroom *Agaricus bisporus. Mol. Gene. Genet*. **250**:252-258.

Van-Logten MS, Tonkelaar EMD, Van-Esch GJ, Van-Steenis G and Kroes R. 1971. The wholesomeness of irradiated mushrooms. *Food Cosm. Toxicol*. **9**:379-388.

Wang L, Chen H, Zhang J and Zeng X. 2009a. The comparative study on screening of *Pleurotus* polysaccharide high-yield strains by use of ion beam implantation and composite mutagenesis. *J. Radiat. Res. Radiat. Proc*. **4**:248-252.

Wang LS, Zhu HL, Li CF, Cheng MJ and Cai HA. 2009b. Screening of fungal mutant strain with high laccase yield by N^+ implantation and enzyme production condition optimization. *Acta Agric. Nuc. Sin*. **2**:228-234.

Xia ZL, Xiong XY and Jiang XJ. 2005. The studies on *Pleurotus pulmonarius* by ^{60}Co gamma irradiation. *Acta Las. Biol. Sin*. **4**:60-64.

Xing ZT, Wang YS, Feng ZY, Zhao ZH and Liu XH. 2007. Effect of ^{60}Co-irradiation on postharvest quality and selected enzyme activities of *Hypsizygus marmoreus* fruit bodies. *J. Agric. Food Chem*. **55**:8126-8132.

Xiong QL, Xing ZT, Feng Z, Tan Q and Bian YB. 2009. Effect of ^{60}Co γ-irradiation on postharvest quality and selected enzyme activities of *Pleurotus nebrodensis. LWT - Food Sci. Technol*. **42**:157-161.

Ye H, Chen JX, Yu RC, Chen QL and Liu W. 2000. The effect of irradiation on stored straw mushroom and the physiological mechanism. *Acta Agric. Nuc. Sin*. **14**:24-28.

Yin H, Liu YY and Li HB. 2008. Mutation breeding of a medicinal fungus *Polyporus umbellatus* by ultra violet and He-Ne laser irradiation. *J. Northwest Uni. Nat. Sci*. **6**:963-966.

Yokono T, Tamai Y, Azuma T, Sakmna Y, Miura K, Kojima Y, Sunagawa M and Ohmasa M. 2000. Karyotype analysis of interspecific fusants of basidiomycetes by pulsed-field gel electrophoresis. *J. Wood Sci*. **46**:480-484.

Yu ZL. 2006. Introduction to ion beam biotechnology. p.183. Springer Science Business Media Inc. New York.

Zeeral NA. 1991. Cytogenetical effect ofgammarays and ethyl methane sulphonate in brinjal (*Solanum melongena* L.). *Cytologia* **56**:639-643.

Zhang H, Li C, Chen M and Liu C. 2004. Protoplast UV mutagenesis breeding of new strains of *Agaricus* sp. *J. Microbiol*. **6**:56-57.

Zhao J and Chang ST. 1996. Intergeneric hybridization between *Pleurotus ostreatus* and *Schizophyllum commune* by PEG-induced protoplast fusion and electrofusion. *World J. Microbiol Biotechnol*. **12**:573-578.

Zolan ME, Tremel CJ and Pukkila PJ. 1988. Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. *Genetics*. **120**:379-387.

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