P3TIR/P.53/2000

DEVELOPMENT OF MUTATION TECHNIQUE AND TISSUE CULTURE FOR VARIETY IMPROVEMENT OF CINGER AND PATCHOULY.

Ismiyati Sutarto, Masrizal and Yuliasti

DEVELOPMENT OF MUTATION TECHNIQUE AND TISSUE CULTURE FOR VARIETY IMPROVEMENT OF GINGER AND PATCHOULY

Ismiyati Sutarto, Masrizal and Yuliasti

Center for Research and Development of Isotope and Radiation Technique
National Nuclear Energy Agency, Jakarta, Indonesia.

Abstract

Ginger (*Zingiber officinale* Rosc.) and patchouly (*Pogostemon cablin* Benth) are important commodities for export. Conventional breeding by means hybridization of these varieties is very difficult due to their narrow genetic variability and inability to flower. Tissue culture combined with irradiation of gamma (γ) rays could increase genetic variability in vegetatively propagated plants. A series of experiment consisted of two commodities, ginger and patchouly was conducted by exposing ginger shoot tips and patchouly leaf cuttings to γ rays at the doses 3, 6, 9, 10, 12, 15 and 20 Gy (ginger), and 15, 30, 45, 60 and 75 Gy (patchouly). Irradiated ginger shoot tips and patchouly leaf cuttings were plated in the MS basal medium with supplement of vitamins and plant growth regulators. Ginger exlpants irradiated up to the dose 20 Gy were able to grow. However, the best growth performance was obtained from those irradiated at the dose 9 Gy, and the abnormalities were found in irradiated ginger explants at the doses 10, 12, 15 and 20 Gy. The best growth performance of patchouly explants was indicated by the highest number of shoots and shoot height. All of the shoots emerged from the patchouly explant were able to root. Abnormalities of irradiated pathouly explants were obtained at the doses 60 and 75 Gy and indicated by dwarf shoots and pale leaf color.

Key words: Ginger, patchouly, tissue culture, mutation technique.

Introduction

Ginger (*Zingiber officinale* Rosc.), a spice crop, and patchouly (*Pogostemon cablin* Benth), an oil-producing plant belong to industrial crops beside coconut, tobacco, medicinal and fiber crops. These plants are economically needed to develop since the demand of ginger and patchouly is increasing year by year.

The obstacle faced in improving varieties of ginger and patchouly is the low genetic variability caused by vegatative propagation. Genetic variability in ginger and patchouly could be increased through somaclonal variation, callus culture and mutation technique. *In-vitro* culture is not only used for micro propagation but also for producing better plant character through somaclonal variation. Larkin and Scowcroft (1981) stated that somaclonal variation is genetic variation produced from cell culture. According to Reisch (1983), this variation could be obtained at differentiation stage of cell and increased by applying either physical (x and γ rays) or chemical (EMS) mutagents.

Breeding program in vegetatively propagated crops like ginger and patchouly is very limited. Selection may be done in genetic variability in the nature. Therefore, germ plasm collection with the wide genetic variability is needed. Hybridization is a method in plant breeding to transfer a character from one variety to another variety, i.e. resistance to pests and diseases or increasing yield quality. Hybridization on ginger and patchouly had not been reported. However, an effort towards ginger and patchouly hybridization may be carried out since flower induction and flower biology had been observed properly.

Continuous breeding program based on the wide genetic variability through selection will meet an ideal result, such as: tolerant to environmental changes and diseases (Simmonds, 1986). In contrast, if the genetic variability is narrow, selection will be very slow due to the risk of susceptibility to pests and diseases (Duvick, 1989).

Ginger is an important spice crop and is also used in various medicinal and culinary preparations in Indonesian community. It is vegetatively propagated through underground rhizomes with a very low multiplication rate. Hosoki and Sagawa (1977) and de Lange et al (1987) reported heavy losses in the yield due to bacterial wilt (*Pseudomonas solanacearum*), soft rot (*Pythium aphanidermatum*) and nematodes (*Meloidogyne* spp). In India, rhizome rot caused by *Fusarium oxysporum f. zingiberi* is a serious threat to this crop during storage and under field condition. According to Dohroo (1989), more than 87 % transmission rate of *Fusarium oxysporum f. zingiberi* through rhizome was found in Himachal Pradesh. Because of the disease is mainly transmitted by rhizomes propagated every year, the production of disease-free clones with rapid multiplication rate is necessary for the successful cultivation of this crop. Vegetative propagation through *in-vitro* culture in ginger is needed in order to accelerate plant material production (Mariska and Syahid ,1992; Sharma and Singh, 1997).

As an oil-producing plant, patchouly is important for export commodity. Patchouly plants produce patchoulin oil, as a raw material for parfumes, cosmetics, soaps and medicines.The price of patchoulin oil is determined the oil quality produced. The oil quality is influenced by varieties grown, cultural practices, harvesting and post harvest handling. In Indonesia, genetic variation of patchouly is very narrow due to inability to flower (Hutami *et al*, 1998). Therefore, it is very difficult to carry out selection based on conventional breeding through hybridization in order to obtain new variety with better quality of oil. Patchouly is mainly grown traditionally in Aceh, North and West Sumatera. After harvesting, the growers move to other field by cutting new forest area. This system is very harmful for forest environmental conservation. An effort for improving quality of patchouly oil should be done by planting better variety along with cultural practice and post harvest handling improvement.

The objective of this work is to increase genetic variation in ginger and patchouly in order improve their quality through mutation technique and *in-vitro* culture.

Material and Method

The experiment started from August to December 1999 was conducted at the tissue culture laboratory of Center for Research and Development of Isotopes and Radiation Technique. The explants used for ginger were shoot tips sized 0.4 – 0.5 cm long taken from rhizomes, then exposed to γ rays at the doses 3, 6, 9, 10, 12, 15 and 20 Gy. Whereas the explants for patchouly were leaf cuttings sized 0.4 – 0.5, then exposed to γ rays at the doses 15, 30, 45, 60 and 75 Gy. Each treatment for ginger and patchouly consisted of 8 plates and every plate contained 5 explants.

The explants, both ginger and patchouly were sterilized with alcohol 70 % followed with $HgCl_2$ 0.05 %, and rinsed 5 times with aquadest, plated in the modified MS (Murashige and Skoog) basal medium with the supplement of vitamins (glycine, glutamine and aspraragin) and 0.5 ppm BAP, and subcultured in the flasks every 4 weeks in the same medium.

The parameters observed after 8 weeks subcultured were survival growth rate of the explants, number of shoots emerged from the explants, the shoot height and abnormality.

Because of differences in the number of samples, homogenity of variance was measured by using Chi Square Test for unequal degree of freedom. If computed Chi Square value exceeds the corresponding tabular Chi Square value at the 0.01 level significance, the hypothesis of homogeneous variance is rejected.

Result and Discussion

The best growth performance of ginger explants was shown from those irradiated with γ rays at the dose 9 Gy. It seems that the dose inhibited bacterial and fungal contamination of both the media and the explants and stimulated explants growth indicated by the highest survival rate and number of shoots. Whereas irradiated explants at the dose 3 and 6 Gy were not able to show better growth performance due to bacterial and fungal contamination (Table 1).

Sharma and Singh (1997) reported that more than 40 % ginger explant buds were discarded after 12 days of culture because of bacterial and fungal contamination. Hosoki and Sagawa (1977) also stated that a high degree of contamination in in-vitro culture of ginger after sterilizing with 0.5 % (v/v) sodium hypochlorite for 10 minutes. In the present study, once the contamination-free cultures of ginger buds were established, these were easily maintained by subculturing on the fresh medium.

Abnormalities of the growth performance were obtained on irradiated ginger exlpants at the doses 10, 12, 15 and 20 Gy. Most of the shoots emerged from the explants are dwarf and the leaf color is pale and yellow. Hopefully after acclimatization these abnormalities will be recovery and show more variability. Larkin and Scowcroft (1981) stated that the occurrence of different types of phenotypic diversity or somaclonal

Table 2. The growth performance of irradiated *in-vitro* patchouly leaf cuttings.

Doses of γ rays (Gy)	Survival rate (%)	Number of shoot	Shoot height (cm)	Abnormality
Untreated	95	23.50 ± 5.42	6.04 ± 2.18	None
15	85.0	18.33 ± 2.86	6.83 ± 3.31	None
30	85.0	35.36 ± 6.59	10.00 ± 3.22	Callus formation
45	80.0	28.79 ± 7.49	14.36 ± 5.35	None
60	50.0	22.5 ± 3.70	3.68 ± 0.59	Dwarf, pale
75	20.0	5.33 ± 0.58	2.50 ± 0.50	Dwarf, pale
P 0.01 = 15.09		Comp.Chi Sq. = 24.92	Comp.Chi Sq. = 31.80	

Conclusion

Ginger exlpants irradiated up to the dose 20 Gy were still able to grow. However, the best growth performance was obtained from those irradiated at the dose 9 Gy, and the abnormalities were found in irradiated ginger explants at the doses 10, 12, 15 and 20 Gy. Callus formation and the best growth performance of patchouly explants was obtained from those irradiated at the dose 30 Gy and indicated by the highest number of shoots. All of the shoots emerged from the patchouly explant were able to root, except those irradiated at the dose 75 Gy. Abnormalities of irradiated pathouly explants were obtained at the doses 60 and 75 Gy and indicated by dwarf shoots and pale leaf color. More genetic variability both of ginger and patchouly will hopefully be obtained after acclimatization of the plantlet and planting them in the field condition, in order to select them for desired characters.