



An Effort of Mutation Breeding by Oryzalin and Gamma Rays on Wild Raspberry (*Rubus* sp.) in Cibodas Botanical Garden

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Abstract

Currently, Cibodas Botanical Garden (CBG) has a domestication programme of wild raspberries (*Rubus* sp.) from Indonesia mountain forest. One of the activities in the domestication programme is fruit breeding. In order to improve the quality of wild raspberry, two mutagens i.e. chemical (oryzalin) and physical (gamma rays) mutagens were used in the breeding programme. Moreover, seed of wild raspberries from the collection of CBG were used in the programme of mutation breeding. The results showed that each species of wild raspberries has different response on the mutagen. Percentage of seed germination were affected by mutagen of oryzalin and gamma rays. *Rubus fraxinifolius* and *Rubus rosifolius* seeds were still able to germinate after treated by 100 μ M of oryzalin. In the other hand, with increase in radiation above 300 Gy a reduction in percentage of germination was observed in M1 generation of *Rubus lineatus* and *Rubus chrysophyllus*.

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INTRODUCTION

Cibodas Botanical Garden (CBG) or *Bergtuin te Tjibodas*, part of Indonesian Institute of Sciences, was established on April 11th, 1852 (Suryana & Widyatmoko, 2013). Cibodas Botanical Garden, an institution for ex situ plant conservation, provides breeders with the basic resources, i.e. genetic variation from wild species for breeding purposes. Based on International Agenda for Botanic Gardens in Conservation (2000), some of major activities of botanic gardens include; conservation biology, environmental education programmes, ethnobiology research, herbarium studies and plant taxonomy, horticultural research and training, laboratory research including in vitro plant cultivation, seed store and tissue banking, tourism, and many more.

Botanical garden has an important role in crop improvement. Moreover, in the domestication process, the useful species were chosen and cultivated, and improved by continuous selection. Through that process, the genetic improvement of those species have been achieved by selection, first from natural seedling population and then from the field that continued unique genotype fixed by vegetative propagation (Lestari & Surya, 2016). Since 2014, wild *Rubus* domestication programme was ongoing projects in CBG. This project was focusing on utilizing and developing wild *Rubus* from Indonesian mountain forests.

Plant breeding is a deliberate effort by humans to nudge nature, with respect to the heredity of plants, to an advantage. The changes made in plants are permanent and heritable (Acquaah, 2012). Plant breeding is the purposeful manipulation of plant species in order to create desired genotypes and phenotypes for specific purposes. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques. Several techniques such as hybridization, mutation, selection, molecular marker were applied in the plant breeding programme.

Wild raspberries (*Rubus* sp.) are collection of Cibodas Botanical Garden (CBG). Currently, CBG has been collected 12 species i.e. *R. accuminatisimus*, *R. alceifolius*, *R. alpestris*, *R. chrysophyllus*, *R. ellipticus*, *R. elongatus*, *R. fraxinifolius*, *R. lineatus*, *R. moluccanus*, *R. rosifolius*, *R. pyrifolius* and *Rubus* sp. (Surya et al., 2015). Raspberries of Indonesian mountain forest is highly known by its potential as fresh fruits, beverage raw materials, and medicinal plants (Normasiwi & Surya, 2016). Utilization and development of wild rasp-

berry as a fruit crop becomes a CBG programme. An improvement of fruit quality was conducted by three steps, i.e. selection, mutation breeding, and cultivation. At present, we are using four species of *Rubus* (*R. fraxinifolius*, *R. rosifolius*, *R. chrysophyllus* and *R. lineatus*) in the fruit breeding, selection, and cultivation programmes.

Mutation is an approach for species improvement and development. Several mutagen were used in the mutation breeding programme, such as physical and chemical mutagens (Medina et al., 2004), to improve and develop a *Rubus* species. In order to domesticate and develop wild raspberries from Indonesian Mountain Forests, this activity is aimed to improve the genetic variance of wild raspberry for cultivation purposes through mutation breeding.

METHODS

The research was conducted in the laboratory and greenhouse of Cibodas Botanical Garden-Indonesian Institute of Sciences (LIPI), West Java, in 2014-2015. Plant material were used in this research are seeds from four species of wild raspberries of CBG collection i.e. *R. rosifolius*, *R. chrysophyllus*, *R. fraxinifolius* and *R. lineatus*. Seeds were obtained from mature fruit. Induction of mutation *Rubus* spp. was divided into two different types of mutagens, i.e. oryzalin and gamma rays.

R. fraxinifolius and *R. rosifolius* seeds were subjected to chemical mutagen treatments by oryzalin. Each of this species obtained different concentration of oryzalin. *R. fraxinifolius* used three concentration of oryzalin (50 µM, 100 µM, and control) and two levels of time to submersion of seeds (6 and 24 hours). In other hand, *R. rosifolius* used eleven concentration of oryzalin (0 µM, 10 µM, 20 µM, 30 µM, 40 µM, 50 µM, 60 µM, 70 µM, 80 µM, 90 µM, 100 µM) and three levels of time to submersion of seeds (1, 6, 24 hours). The difference in this treatment based on the availability of seed and number of seeds when the study was conducted.

Application of gamma rays irradiation of *Rubus* spp. had been conducted at the Center for the Application of Isotopes and Radiation Technology, BATAN. Gamma rays with six doses (100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, and control) were subjected to the seed of *R. lineatus*, while seeds of *R. chrysophyllus* used eleven doses of gamma ray (50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy)

Rubus spp's. seeds were germinated on wet tissue paper in a petridish. Percentage of germina-

tion was recorded every weeks for three months. Moreover, around 12 weeks after germinate, the seedlings of *Rubus* were carefully removed from petridish. The root were cleaned from remaining tissue paper. Afterward, the seedlings were transferred to small individual pots (10x10 cm), which containing of rice husk and compost (1:1).

Statistical analysis was used to analyze the data. Referring to the procedure of data analysis from Gomez & Gomez (1995), all the data were expressed as the mean.

RESULTS AND DISCUSSION

Mutation breeding on *Rubus fraxinifolius*

Chemical mutagen, oryzalin, was subjected to the seed of *R. fraxinifolius*. Five combinations of oryzalin concentration and submersion time, i.e. 0 μM in 0 hour (control), 50 μM in 6 hour, 50 μM in 24 hour, 100 μM in 6 hour, and 100 μM in 24 hour, were applied to seeds. Seed that had been treated by oryzalin was germinated in a petridish on wet tissue paper. Moreover, Figure 1 shows that germination percentage and survival of germination of *R. fraxinifolius* seed treated by oryzalin was higher than control. The highest of germination percentage was showed by the combination of 24 hours of submersion with 50 μM of oryzalin (75.76 %) and 6 hours of submersion with 100 μM of oryzalin (75.76 %). It showed that the higher levels of oryzalin concentration and the longer of submersion time were related to a germination percentage of *R.*

fraxinifolius seed. In the other hands, the percentage of survival germination after transplanting was showed by the combination of 6 hours of submersion with 50 μM of oryzalin (92.59 %) and 24 hours of submersion with 100 μM of oryzalin (92.59 %). Furthermore, Figure 2 shows the variation of leaf morphology in *R. fraxinifolius* treated by oryzalin.

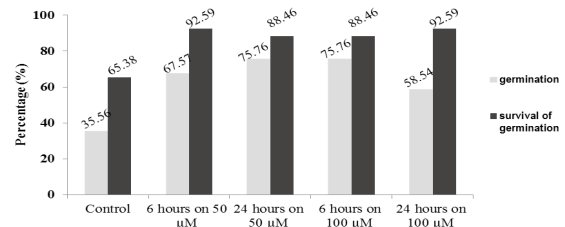


Figure 1. The effect of submersion time and doses of oryzalin on the germination percentage of *Rubus fraxinifolius*

Mutation breeding on *Rubus lineatus*.

Physical mutagen, gamma ray with six doses (0, 100, 200, 300, 400, 500), were subjected to the seed of *R. lineatus*. The result shows that the seeds of *R. lineatus* on doses 0, 100, 200 and 400 gray were still able to germinate. The highest percentage germination was showed in doses 100 gray (Figure 3).

Mutation breeding on *Rubus lineatus*

Physical mutagen, gamma ray with eleven doses (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500), were subjected to the seed of *R. chryso-*

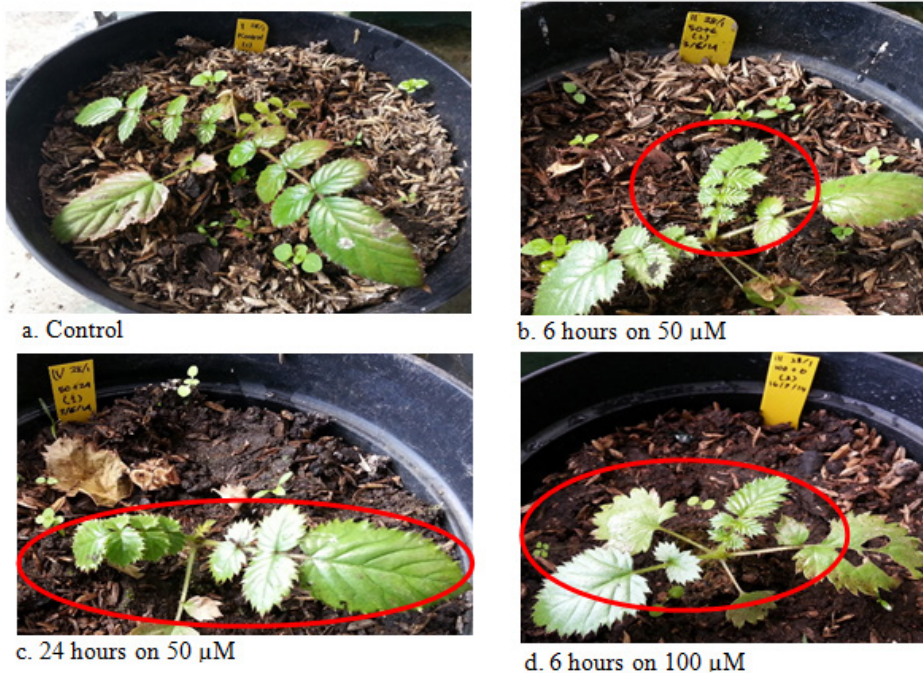


Figure 2. The leaf morphology variation of *R. fraxinifolius* after treated by oryzalin

phyllus. Except on the doses of 500 gray, germination percentage increased after treated by gamma ray. The highest of germination percentage was showed on 50 gray (Figure 4).

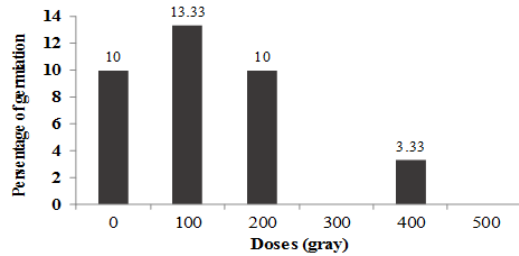


Figure 3. The effect of gamma ray irradiation on the germination percentage of *Rubus lineatus*

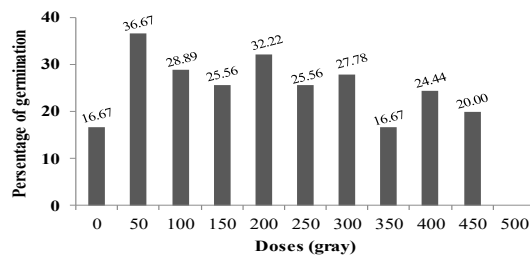


Figure 4. The effect of gamma ray irradiation on the germination percentage of *Rubus chrysophyllus*

Chemical mutagen, oryzalin with eleven doses (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100), were subjected to the seeds of *R. rosifolius*. Three levels of time (1, 6, 24 hours) were used to submersion the seed of *R. rosifolius*. The results showed that the highest of germination percentage was 98%, while the seeds of *R. rosifolius* was soaked 24 hours in 30 μ M of oryzalin (Table 1). Moreover, after a year growth in a petridish, all germinated seeds were transplanted onto the pot in the plastic house. Base on survival percentage of *R. rosifolius* mutants, Table 2 shows that on 14 days after transplanting, mutants treated by higher concentration of oryzalin is more adap-

table. The lowest survival percentage was mutant of *R. rosifolius* treated in 10 μ M of oryzalin.

Oryzalin and gamma rays affected the seed germination of *R. fraxinifolius*, *R. lineatus*, *R. chrysophyllus*, and *R. rosifolius*. The results showed that the increasing concentration of oryzalin was able to rise up the percentage germination of *R. fraxinifolius* (Figure 1) and *R. rosifolius* on 6 and 24 hours of submersion time (Tabel 2). The results seems unusual because generally the percentage of germination decreased as the concentration of mutagens increased. The reduction in germination may be due to genetic and physiological proses inhibited by the mutagens resulting in cell maturity (Umavathi & Mullainathan, 2014). On the other side, Roychowdhury & Tah (2011) reported that increasing the doses of colchicine were able to increase the germination percentage of *Dianthus caryophyllus*. Their result was in line with our experiment, which is the increasing concentration of oryzalin was able to increase the percentage germination of *R. fraxinifolius* and *R. rosifolius*. Furthermore, the range of gamma rays doses tested did not significantly affect the germination percentage of *R. lineatus* and *R. chrysophyllus* seeds. It is slightly different with certain studies performed on seeds of several species such as *Withania samnifera* (Bharathi et al., 2013), *Citrus jambhiri* (Sharma et al., 2013), *Cajanus cajan* (Ari-raman et al., 2014) that a gradual decrease in seed germination from lower doses to higher doses of gamma rays were observed. In the other hand, Giovani et al. (2015) reported that the effect of gamma rays at 50, 100 and 200 Gy were able to increase seed germination of *Rosa hybrida*. Their results are in line with our experiment, which was the seed germination of *R. lineatus* increase in 50, 100, 150, 200, 250, 300, 400, 450 Gy and *R. chrysophyllus* in 100 Gy.

Mutation induction is a part of domestication programme, in order to utilizing and cul-

Table 1. The effect of submersion time and doses of oryzalin on the percentage of *Rubus rosifolius* germination

Submersion Time	Doses (μ M) (%)										
	0	10	20	30	40	50	60	70	80	90	100
1 hours	0	12	0	32	0	0	0	8	20	0	54
6 hours	4	14	56	22	58	38	93.33	14	78	74	54
24 hours	68	40	6	98	70	24	36	0	0	58	0

Table 2. Survival percentage of *R. rosifolius* mutants on 14 days after transplanting.

Doses (μ M) (%)	0	10	20	30	40	50	60	70	80	90	100
Survival	50	37.50	60	73.53	85.71	85.71	92	100	94.74	100	100

tivating wild species. Mutagens have the ability to cause biological damage and it can be measured based on the seed germination and survival reduction in M1 generation (Umavathi & Mullainathan, 2014). However, the percentage of seed germination was not always decreased with increasing doses/concentration of gamma rays and oryzalin in wild raspberries. In general, these results show that the mutagens have affected on seed germination. Moreover, small doses indicated that the mutagens have exerted a stimulus effect on seed germination, but in some cases the increasing doses have exerted an inhibitor effect.

Furthermore, oryzalin and gamma rays were able to affect the seed germination of wild raspberries. Mutagenic agents, such as oryzalin and gamma rays, with certain doses may be used to enhance seed vigor and increase the percentage of germination of wild raspberries

CONCLUSION

A preliminary study of mutation breeding is an effort to develop wild raspberry in Cibodas Botanical Garden. Both of mutagens, oryzalin and gamma rays affected the germination of *Rubus* sp. Moreover, less than 100 µM of oryzalin with 6 hours submersion time and less than 60 µM of oryzalin with 24 hours submersion time were the recommendation to treat the *Rubus*'s seeds by oryzalin mutagen. In the other hand, the effective doses of gamma rays for *Rubus*'s seeds were less than 300 Gy.

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