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Short Comminication

Radiosensitivity test on two varieties of Terengganu and Arab used in mutation breeding of roselle (*Hibiscus sabdariffa* L.)

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A study was carried out in a greenhouse to determine the effective doses for mutation breeding of roselle during the year 2006/2007. Seedling height was the parameter recorded at 2, 3, 4 and 5 weeks after sowing of M_1 seeds of 2 varieties Terengganu and Arab. The results indicated that increasing doses of gamma irradiation from 0 to 1200 Gy in steps of 100 Grey (Gy) caused increasing physiological effect on seedling height. There was a progressive increase in seedling height from 2 until 5 weeks after sowing. However, at week 2 and 3 after sowing there was no significant difference between seedling heights. The LD_{50} values determined from regression analyses for Terengganu based on seedling height were 754, 821.4, 761.7 and 766.7% at 2, 3, 4 and 5 weeks after sowing, respectively and the LD_{50} values for Arab were 773.8.%, 804.1.%, 704.2 and 708.3% at 2, 3, 4 and 5 weeks, respectively. 2 weeks after sowing appears to be the most appropriate time for data collection. The LD_{50} values at 2 weeks were not significantly different from 3, 4 and 5 weeks after sowing. The LD_{50} values for Terengganu and Arab determined at 2 weeks were 754 and 773.8%, respectively.

Key words: Roselle, mutation breeding, seedling height, $LD_{50,}$ radiosensitivity, dosimetry, *Hibiscus sabdariffa*, gamma irradiation.

INTRODUCTION

Roselle is a relatively new crop in Malaysia. It was introduced in 1990s (Mohamad et al., 2002a). The crop is believed to have useful properties. Amongst others roselle can be used as pro-health beverage and as a medicinal plant (Mohamad et al., 2005).

There are 2 varieties currently available for growers to cultivate, “Terengganu” and “Arab”. Since conventional hybridization in roselle is practically not possible, mutation breeding of these accessions is important to generate new traits for breeding purposes so as to develop improved selections and increase crop productivity (Mohamad et al., 2003).

Past research activities in mutation breeding on roselle have reported plant height as an important trait to be improved upon for optimizing yields of roselle (Mohamad et al., 2005). Similar studies undertaken by Chauhan et al., (1992), Narasimba and Bhalla (1998) revealed similar findings on the cultivars Aruna and pigeon pea, respectively.

Further research under taken at UKM revealed that increasing doses caused strong physiological effect on seedling heights when measurements were taken at 2 weeks after sowing (Mohamad et al., 2005) and no substantive effects on percentage (%) germination was reported. Since dosimetry work on roselle have reported for optimum doses at 2 weeks after sowing, it is importance for other dates to be evaluated to determine the most critical time for physiological effect for different roselle varieties using physical and chemical mutagens.

This study was therefore carried out to determine the optimum doses and time for physiological effects on seedling height in mutation breeding of two roselle varieties (Terengganu and Arab).

MATERIALS AND METHODS

An experiment using variety Terengganu (accession No. 6) and variety Arab (accession No. 21) were used for mutation breeding. In

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Table 1a. Mean seedling height (cm) of Terengganu and Arab at 2, 3, 4 and 5 weeks after sowing.

Treatment (Gy)	Variety Terengganu				Variety Arab			
	Seedling height (cm) Weeks after sowing				Seedling height (cm) Weeks after sowing			
	2	3	4	5	2	3	4	5
0	7.3	11.3	17.6	17.7	6.4	9.2	17.9	17.9
100	7.2	10.9	17.9	17.9	6.0	9.0	16.2	16.2
200	7.0	10.3	17.0	17.1	5.9	8.5	15.2	15.2
300	6.5	9.8	15.2	15.3	4.8	8.0	13.7	13.8
400	6.0	8.9	13.8	13.9	4.4	7.6	12.0	12.1
500	5.2	8.6	12.0	12.1	3.6	7.0	10.9	11.0
600	4.3	7.5	10.0	10.2	3.3	6.6	8.6	8.6
700	3.5	5.7	8.6	8.6	3.0	5.5	7.5	7.6
800	3.3	4.8	6.7	6.9	2.8	4.2	6.5	6.5
900	3.1	4.6	6.5	6.6	2.4	3.2	5.6	5.7
1000	2.9	4.4	6.0	5.9	2.0	3.1	5.3	5.3
1100	2.6	4.3	5.6	5.5	1.8	3.1	5.1	5.1
1200	2.5	4.2	5.4	5.5	1.8	3.0	5.0	5.1
Mean	**4.7**	**7.3**	**10.9**	**11.0**	**3.7**	**6.0**	**10.0**	**10.0**

Table1b. Means separations of 2 roselle varieties at 2, 3, 4 and 5 weeks after sowing.

Weeks after sowing	Variety Terengganu	Variety Arab
2	4.7 b	3.7 b
3	7.3 ab	6.0 ab
4	10.9 a	10.0 a
5	11.0 a	10.0 a

Means followed by the same letter are not significantly different at 5% level

radiosensitivity tests, 100 seeds of each sample for each variety were subjected to gamma irradiation from ^{60}Co Source at UKM facility using doses from 100 to 1200 Gy in steps of 100 Gy and a control, involving no irradiation (0).

Seeds were planted at a spacing of 5 cm between and within rows under greenhouse conditions in plastics containing a mixture of peat about 4 to 6 cm thick. The experiment was laid out in 3 replications.

Seedling heights were recorded on weekly basis from 2 to 5 weeks after sowing to measure the physiological effects and to evaluate their usefulness in estimating the optimum doses required for mutation breeding of roselle. Linear regression analysis was used to estimate the optimum LD_{50} doses for the 2 roselle varieties using seedling height data as standard measure of physiological effect. The determination of the optimum doses was based on Gaul, Osborne and Lunden (Mohamad et al., 2005).

Data analysis was done using the MINITAB package used by the University, Kebangsaan Malaysia.

RESULTS

The results of the experiment indicated that increasing doses of gamma irradiation caused severe effects on the plant development as indicated by a significant reduction of the seedling height for the two varieties Terengganu and Arab when data were recorded at 2, 3, 4 and 5 weeks after sowing as shown in Tables 1a and b.

The tables show progressive reductions in seedling heights (cm) with increasing doses of gamma irradiation from 0 to 1200 Gy. However, seedling heights increased from 2 to 5 weeks after sowing although there was no significant difference between 3, 4 and 5 and also between 2 and 3 weeks after sowing for both varieties. Seedling height for Terengganu and Arab range from 2.5 - 17.9 and 1.8 - 17.9, respectively. The averages were 4.7, 7.3, 10.9 and 11.0 for Terengganu and 3.7, 6.0, 10.0 and 10.0 for Arab at 2, 3, 4 and 5 weeks, respectively.

Table 2 shows the LD_{50} and R^2 values determined based on seedling height. LD_{50} values determined for Terengganu were 754, 821.4, 761.7 and 766.7 and for Arab were 773.8, 804.1, 704.2 and 708.3. These values were not significantly different at 2, 3, 4 and 5 weeks, respectively for the 2 varieties.

DISCUSSION

The more important factors in producing variability in plants are hybridization, recombination and mutation. However the naturally occurring mutation rate is too low for practical application in most crops (Donini and Sonnino, 1998). Therefore physical and chemical mutagen

Table 2. LD_{50} for Terengganu and Arab based on seedling height at 2, 3, 4 and 5 weeks.

Variety	Weeks after sowing	Regression equations	R^2 (%)	LD_{50} (Gy)
Terengganu	2	y=7.54-0.005x	95.4	754.0
	3	y=11.50-0.007x	95.7	821.4
	4	y=18.28-0.012x	96.5	761.7
	5	y=18.40-0.012x	96.0	766.7
Arab	2	y=6.19-0.004x	95.9	773.8
	3	y=9.65-0.006x	96.0	804.1
	4	y=16.90-0.012x	95.3	704.2
	5	y=17.00-0.012x	95.3	708.3

have proven useful application for increasing the frequency of mutations and variation (Chauhan et al., 1992, Mohamad et al., 2005). In this regard mutation breeding is considered complementary to the conventional method.

Previous work done in radiosensitivity study demonstrated that seedling height seems appropriate as a standard measure of the effect of gamma irradiation on the growth and development of crops (Sinha and Chowdhury, 1991; Datta, 1995). This finding was also confirmed with the crop, roselle. Sinha and Chowdhury (1991), reported that 20Krad (200Gy) treated seeds of pigeon pea (*Cajanus cajan*) produced mutants lines that were semi-dwarf in M_2 generation and in M_3 generation segregated into dwarf, semi-dwarf and tall plants whilst work done on *Thrichosanthes anquina* L was induced by 30 Krad (Datta, 1995). These findings were later confirmed by Narasimba Chary and Bhalla (1998) who worked on different varieties of pigeon pea and concluded that optimum doses vary between and within crop species. This research also established that for Terengganu and Arab as different optimum doses were obtained for the 2 different varieties of roselle. The optimum dose determined for Terengganu was 754% and for Arab 773.8%. These doses reported in this study may be of importance in selection of mutant lines that are valuable for further breeding of roselle and techniques used could be applicable to other crops such as rice, cassava and sweet potato.

Conclusion

Increasing doses of gamma irradiation caused severe physiological damages on seedling height. The optimum dose determined for mutation breeding of Terengganu was 754.0% and for Arab 773.8% at 2 weeks after sowing.

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