5.4 Induction of mutation on mulberry (morus alba l.) by using in vitro techniques in combination with gamma irradiation

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Introduction and Scientific background

The Mulberry (*Morus alba* L.) is one of the important industrial crops in the world with about 1.500.000 ha cultivated, Vo Ta Linh and Pham Dinh Son [1].

In fact, Vietnam is located near China and India, where Mulberry is cultivated and thus many varieties of Mulberry are cultivated. Even though different Mulberry can be grown in Vietnam, their yield is degenerating and has not been improved.

As many vegetatively propagated plants such as Bananas, Potatoes, Sweet potatoes, Carnations, etc ... Mulberry has its seed progeny with high heterogeneity. Further more, its seed progeny brings in too many unwanted characters. Mutation induction and selection of desired traits in combination with in intro techniques for propagated crops offer several advantages over conventional methods. In addition, the mutants are really stable and the avoidance of the chimerism will enhance the efficiency of mutation isolation.

Today, agricultural development policy of the Vietnamese Government focuses on food security and production export. This is the way to increase industrialization and modernization rapidly in Vietnam. Improvement of crops by biotechnology and mutation induction method aims at agricultural development. Our plant improvement and genetics group at Nuclear Research Institute, Dalat, supported by the Vietnam Atomic Energy Commission and Sericultural Research Center, has carried out a research to improve some Mulberry varieties. The objectives hopefully are:

1. To conduct biochemical and physiological analyses of collected Mulberry varieties.
2. To improve techniques for boosting yield and better quality in some Mulberry genotypes by using in vitro technique in combination with gamma irradiation.

1. Material and methods

1.1. Material

Two Mulberry varieties named BauDen (local variety) and VA 186 (imported variety) were used for the research. Generally, these two varieties have heart-shaped leaves with a height from 1.8 m - 2.5 m. BauDen especially can be resistant to diseases. All of the samples were supplied by the Sericultural Research Center.

1.2. Method

1.2.1. Method for research of mutation induction.

Two cuts of Mulberry varieties BauDen and VA 186 with length from 18 cm - 20 cm, bearing 3-5 axillary buds, were treated with gamma rays, Co^{60}. The doses used were 0; 1; 2; 3; 4; 5; 6; 7; 8; 9 and 10 krad. After cuts were irradiated, they were cultivated in the experimental field to investigate the influence of gamma rays on plantlets, color of leaves, etc ... 30 days after cultivation. From parameters obtained, we choose 4 krad to treat a large number of cuts (1000 cuts) for mutation induction.

1.2.2. Method for research in vitro techniques

Using in vitro techniques in this research is to rapidly isolate mutants in irradiated population. Young shoots from irradiated plantlets with length of about 10 -15 cm, bearing 5 - 8 axillary buds, leaves cut off and surface sterilized by alcohol 70%, HgCl 2%0 solution for 3-5 minutes. Then, they were washed at least 5-7 times with sterile water.

The culture media for the two varieties, BauDen and VA 186, are:

- The primary medium: the bazalt inorganic salt medium containing BAP 0.5-2 mg/l
- The shoot multiplication medium: The bazalt medium is MS (Murashige and Skoog, 1962) containing vitamins, 20-30 g sucrose, 6-7g agar; 3-5 mg/l BAP; 1 mg/l Kinetin for shoot proliferation.
- The rooting medium: The temperature of the fluorescent room is $25^0C \pm 3^0C$; light intensity ranges 2000 - 2500 lux and light duration is 10 hours per day and the pH of all media are 5.8.

2. Result and Discussion.

2.1. Result of radiosensitivity on Mulberry.

100 cuts of each variety were treated with gamma rays. After 30 days, we obtained the following results:

Table 1. Frequency and spectrum of variation on BAUDEN

Dose krad / Spectrum	0	1	2	3	4	5	6	7	8	9	10
Curled	0	0	3	2	2	3	5	0	0	0	-
Single - lobe leaf	0	0	1	2	3	3	0	0	0	0	-
Multi - lobe leaf	0	0	0	0	2	2	5	7	5	6	-
Part-albino leaf				1						-	-
Solid - albino leaf	0	0	0	0	0	1	4	6	9	12	-
Aborted shoot	0	0	0	0	0	1	6	8	15	28	-
Early-dividing branch	0	0	0	1	1	2	2	0	0	0	-
Double stem	0	0	0	0	1	1	3	4	5	0	-
Short nodal	0	0	0	0	0	1	1	2	0	0	-
Frequency (%)	0	0	4	6	12	14	26	27	34	46	-
Spectrum	0	0	3	4	6	8	7	5	4	3	-

Table 2. Frequency and spectrum of variation on VA 186

Dose krad / Spectrum	0	1	2	3	4	5	6	7	8	9	10
Curled	0	0	2	2	2	2	0	0	0	0	-
Single - lobe leaf	0	0	1	2	3	4	0	0	0	0	-
Multi - lobe leaf	0	0	0	0	1	2	7	8	8	0	-
Part-albino leaf	0	0	0	1	2	5	0	0	0	0	-
Solid - albino leaf	0	0	0	0		1	4	7	5	10	-
Aborted shoot									16	25	-
Early - dividing branch	0	0	0	1	1		0	0	0	0	-
Double stem	0	0	0	0	1	2	3	5	0	0	-
Short nodal	0	0	0	0	1	1	5	3	0	0	-
Frequency (%)	0	0	3	6	11	17	19	23	29	35	-
Spectrum	0	0	2	4	7	7	4	4	3	2	-

Table 1 and 2 showed that:

- Doses of 2 to 10 krad changed the morphology, growth and colour of leaves of irradiated plantlets.

Frequency and spectrum of variation increase at high doses.

- At 4 and 5 krad, plantlets reduced growth 30%.
- At 9 and 10 krad, all of irradiated plantlets were dead.
- The are not many differences on frequency and spectrum of variation in BauDen and VA 186.

According to Kamra [2], parameters such as shoot growth inhibition or shoot length reduction and finally somatic variation observed are requirements, for determining the mutation induction dose. This is point out in Gunkel [3], Konzak, et. al [4] and Mike, et al. [5].

From the results obtained, we choose 4 krad and irradiated 1000 cuts of each variety. After 40 days cultivating, all shoot of each plant were cultured and subcultured from M_1V_1 to M_1V_4 for the selection and isolation (the data not presented here). All none and irradiated explants in M_1V_4 were acclimatized in green house. After 60 days in acclimatization condition, more than 90% explants have survived and grown well; the height of which is about 25-30 cm. We transplanted them in the field for selection and isolation.

2.2. Results of selection and isolation on Mulberry

Table 3. Frequency and spectrum of mutant on BAUDEN

Spectrum	Number of mutants	Frequency $f^{\%} \pm m^{\%}$	Control
Deformed leaf	169	0.013 ± 0.004	-
Single-lobe leaf	204	0.016 ± 0.006	-
Solid-albino leaf	112	0.009 ± 0.002	-
Small leaf	144	0.011 ± 0.003	-
Big leaf	192	0.015 ± 0.002	-
Thick leaf	165	0.013 ± 0.002	-
Short nodal	129	0.010 ± 0.003	-
Multi branch	156	0.012 ± 0.002	-
Semidwarf	174	0.013 ± 0.002	-
Total number of testing clones	12654		
Total number of mutants	1645	0.13	

Table 4. Frequency and spectrum of mutant on VA 186

Spectrum	Number of mutants	Frequency $f^{\%} \pm m^{\%}$	Control
Deformed leaf	185	0.016 ± 0.004	-
Single-lobe leaf	214	0.018 ± 0.002	-
Solid-albino leaf	98	0.008 ± 0.003	-
Small leaf	126	0.011 ± 0.004	-
Big leaf	215	0.018 ± 0.01	-
Thick leaf	106	0.009 ± 0.006	-
Short nodal	198	0.017 ± 0.001	-
Multi branch	175	0.015 ± 0.002	-
Semidwarf	231	0.020 ± 0.003	-
Total number of testing clones	11558		
Total number of mutants	1452		

Table 3 and 4 showed that:

Nine mutation spectrum occurred in M_1V_4 with 4 spectrum: big leaf mutant, thick leaf mutant. Multibranch mutant and semidwaf mutant are valuable. According to Aloowalia [6], Amano [7], Fujita el al. [8], Han Ming Zhai et al. [9] and Lacey et al. [10], mutant obtained in M_1V_4 are usually stable. They can be used for cultivating as new clones, or as resources for hybriding. From their research, some mutants on Potatoes, Apples and mulberry were created.

In 3 year, from 1996-1998, we investigated and evaluated mutants on morphology, yield in testing fields.

2.2. Results of testing on mutants

Table 5. Results of testing on mutants

Clones	Height (cm)	No. of leave/ branch	No. of leaves/tree	S of leaves (D x L) cm	Weight of 100 leaves (gr)	Total of branch/ tree	Yield (ton/ha/ year)
Control (BauDen)	180 ± 25	12 ± 3	130 ± 10	13.1 x 8.8	119.5 ± 10	12.5 ± 3	16.5 ± 5
B - 18 (Semidwarf)	155 ± 25	15 ± 3	128 ± 10	13.5 x 9.1	122.4 ± 10	14.8 ± 3	177 ± 5
Control (VA 186)	220 ± 25	12.5 ± 3	140 ± 10	15.5 x 11.2	135.6 ± 10	14.5 ± 3	20.5 ± 5
VA - 12 (Big leaves)	218 ± 25	13.1± 3	145 ± 10	16.8 x 12.1	140.7 ± 10	14.8 ± 3	22.8 ± 5
VA -93 - 5 Thick leaves)	220 ± 25	13.5± 3	142 ± 10	16.5 x 11.8	150.8 ± 10	15.1 ± 3	24.5 ± 5

Table 5 show that:
Mutated clones with thick and big leaves had some better characters than control group. These valuable characters have been stable.
Today, they are cultivated in DonDuong, LamHa, DucTrong, BaoLoc, districts of LamDong Province.

3. Conclusion.

From 1993-1999, we have carried out the research "Induction of mutation Mulberry (*Morus alba* L.) by using in vitro techniques in combination with gamma irradiation" and obtained 11 mutated clones: B93-1, B93-2, B93-3, B-16, B-17, B-18; from BanDen variety and VA93-5, VA93-8, VA-15, VA-18 from VA 186 variety, 3 mutated clones: B-18, VA-12 and VA93-5 in the obtained mutants are valuable and now farmers of LamDong province are cultivating as new clones in their fields.

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