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Irradiation-induced variations in M2 populations of Eksotika papaya

(Variasi iradiasi-aruhan dalam populasi M2 betik Eksotika)

Y.K. Chan*, H.K. Lee** and I. Rusna*

Key words: *Carica papaya,* papaya, gamma-irradiation, mutant, ringspot virus disease

Abstract

Mutation breeding of papaya by gamma irradiation was started at MARDI in August, 2000 as a contract research project with the International Atomic Energy Agency (IAEA). Irradiation of seed of 'Eksotika' variety was carried out at MINT using 525 Gy for dry seed and 42.5 Gy for pre-soaked seed previously found to be the most suitable dosages for mass irradiation. A total of 200 M1 plants from each dosage treatment were subsequently raised. M2 seed were obtained by self-pollinating M1 hermaphrodite trees and a population of 1,000 M2 plants was established for studies on variation of 13 traits. Another 1,920 M2 seedlings were raised, inoculated and screened for resistance to papaya ringspot virus disease.

In the M2 populations, wide variability was recorded for many traits. At the seedling stage, low irradiation of 42.5 Gy on pre-soaked seed produced a high number of M2 progenies that were shorter and more vigorous in leaf development than those irradiated at 525 Gy and the control seedlings. The distribution patterns of M2 progenies for nine quantitative traits showed great variation with ranges often exceeding the limits of the control population. There appears to be good prospects in improving Eksotika papaya using irradiation, especially in selecting for more dwarf trees, lower fruit bearing stature, higher total soluble solids in fruit and larger fruit. Several M2 mutants also showed very good resistance to malformed top disease caused by *Cladosporium*. However, no resistance to papaya ringspot virus disease was found in the 1,920 M2 seedlings that were inoculated and screened.

Introduction

Papaya is one of the most important export fruits in Malaysia, grossing annual revenue of RM120 million in 2003. There are two major constraints faced by the papaya industry i.e. the papaya ringspot virus (PRSV) disease and the rapid deterioration of the fruit. At the Malaysian Agricultural Research and Development Institute (MARDI), conventional breeding for tolerance to PRSV resulted in the selection of four elite lines (Chan and Ong 2003). In genetic engineering, the 'Rainbow' variety with PRSV resistance developed by the University of Hawaii in 1998 is the world's first commercial transgenic papaya. Following this success, Southeast Asian countries in the Papaya Biotechnology

*Horticulture Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
**MARDI Station, Pontian, Locked Bag 506, 82000 Pontian, Johor, Malaysia
Authors' full names: Chan Ying Kwok, Lee Hoon Kok and Rusna Isa
E-mail: ykchan@mafc.com.my
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Network coordinated by the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) have embarked on the development of transgenic papayas with coat-protein mediated resistance to PRSV (Krattiger 2000).

Another important disease is the Malformed Top Disease (MTD), caused by *Cladosporium*-thrip complex. It is very damaging especially to the emerging foliage and if left untreated, the leaves will show typical 'shot-hole' symptom and trees will be stunted. The Eksotika and Solo papayas are particularly susceptible to this disease (Chan and Mak 1993).

Development of new papaya varieties with improved performance and disease resistance using irradiation is another approach that can be explored. Irradiation-induced mutation is increasingly used as a complementary tool in plant breeding. It is most appropriate for improving one or two easily identifiable traits in an otherwise well accepted breeding line or commercial variety.

Worldwide, more than 1,500 mutant cultivars of crop plants and ornamentals have been released to growers in the past 30 years (Mohd. Nazir et al. 1998). Eight per cent of agricultural land in China, for example, is planted with mutants of rice, wheat, corn and cotton. In Malaysia, mutation breeding covers a range of crops, notably rice, banana, soybean, mungbean, groundnut, rubber, *Lansium*, mangosteen, legume cover crops and ornamental plants. Several rice mutant varieties such as Muda 2 (short stature), Mahsuri mutant (improved quality) and Manik 817 (short stature glutinous rice) have been released (Mohamad et al. 1990).

For fruits, a new variety of local banana, Novaria, with early fruiting and improved bunch characteristics has been developed using gamma irradiation of in vitro cultured Grand Nain banana (Mak and Ho 1999). There have been no reports, however, on the use of irradiation for improving papaya cultivars particularly in developing resistance to ringspot virus disease, currently the most devastating and threatening to the local industry (Chan and Ong 2003).

A contract research project between MARDI and the International Atomic Energy Agency (IAEA) on 'Irradiation-induced mutations in papaya with special emphasis on papaya ringspot disease resistance' was signed on 1 August 2000. The objectives were to generate variability in papaya variety Eksotika by gamma irradiation and to screen for variants that have resistance to ringspot virus disease and other commercial agronomic characteristics. It was also aimed at evaluation, description and documentation of induced mutants with traits of horticultural interest and to conserve them in germplasm for the purpose of future breeding and exchange of genetic materials.

This paper reports the progress of the research, focusing on the variation obtained in the M2 populations. The potential of using irradiation for the production of useful mutants and improvement of varieties in papaya is discussed.

Materials and methods

Irradiation of the papaya seed was carried out at the Malaysian Institute for Nuclear Technology Research (MINT). Seed for low dosage irradiation of 10–100 Gy was irradiated in a Gamma Chamber GC4000A (10kCi), while seed for high dosage of 300–1,000 Gy was irradiated in the Shepperd & Associates Model 109-68 Irradiator (24kCi). The radiation source was ^{60}Cobalt.

Mass irradiation of Eksotika seed

Mass irradiation of Eksotika dry seed at 525 Gy (denoted as D525 Gy) was carried out on 13 February 2001 and Eksotika wet seed at 42.5 Gy (denoted as W42.5 Gy) on 23 May 2001. These dosages were recommended following results of sensitivity tests carried earlier by Chan et al. 2002. Each batch consisted of 4,000 seed. A total of 250 irradiated seedlings were raised from each batch.

Evaluation of M2 populations

The M1 population consisting of 500 progenies (250 each from treatments W42.5 Gy and D525 Gy) were sown in polybags on 24 May 2001 at MARDI, Serdang and field planted eight weeks later. The planting distance was 1.8 m between plants and 2.1 m between rows. The experimental plot was irrigated with a drip system. Plant characteristics out of the norm for the Eksotika variety were noted.

M2 seed were prepared by self-pollinating M1 hermaphrodite trees. This was done by placing a small wax envelope over unopened flowers. About 100 M2 seed batches (each batch from one fruit) from the D525 Gy treatment and 125 seed batches from W42.5 Gy treatment were obtained. The M2 seed batches of each treatment were bulked and a random sample of seed was drawn for sowing on 10 June 2002. A total of 500 seedlings from each treatment were raised. At six weeks after sowing, the leaf length and the height to the first cotyledon (indicating dwarf stature) were measured for 200 seedlings each of the W42.5 Gy and D525 Gy treatments compared with 50 control seedlings raised from non-irradiated seed.

The M2 population of 1,000 plants (500 each from W42.5 Gy and D525 Gy treatments) was field planted on 5 August 2002 at Serdang for evaluation of morphological changes and fruit ripening characteristics. For comparison, 50 control trees were planted in the middle of the plot. In field evaluation, 200 trees from each irradiation treatment and all 50 control trees were measured to record the distribution of nine characters in the M2 populations. At the flowering stage, data on days to appearance of first flower, flowering height (distance of flowering node to ground) and sex of flower were recorded. At one year from seed sowing, data on tree height, stem diameter (15 cm from the ground), fruit number and tolerance to malformed top disease (ranking from symptomless '0' to most severe '9') were taken. Five mature fruits from each tree were harvested to record the mean fruit weight, flesh colour and total soluble solids % (TSS). The latter was recorded with a hand refractometer (0–25 ^{o}Brix). Total yield one year after seed sowing of each tree was computed from the product of fruit number and the mean fruit weight.

Screening M2 seedlings for PRSV tolerance

Two batches, each with 960 irradiated seedlings (480 42.5 Gy and 480 525 Gy), and 60 control seedlings were raised in seedling trays on 19 June 2002 at MARDI, Pontian. The seedlings were inoculated at eight weeks from seed sowing i.e. at the 12-leaf stage on 14 August 2002. Two recently fully opened leaves were inoculated by gently rubbing the top surface of the leaves with the index finger dipped in the inoculum. The inoculum was prepared by grinding fresh, infected leaves in 0.01 M phosphate buffer at pH 7.0 containing 0.25% DIECA (sodium diethyldithiocarbamate). Carborundum, mesh 600, to cause injury and aid entry of the virus, was suspended in the buffer before sap inoculation.

Plant disease severity was scored from 0 (disease-free) to 5 (terminal shoot yellowing with leaf mottling and mosaic and small water-soaked spots on petioles and stem) to 9 (severe infection with plants near death). Diseased plants were removed while symptomless ones were cut back for re-growth, and the new shoots that emerged after three weeks were re-inoculated. This process was repeated twice to reduce the risk of selecting 'escapes' from the disease. This method has been successful for selecting elite PRSV tolerant papaya lines at MARDI (Chan and Ong 2003).

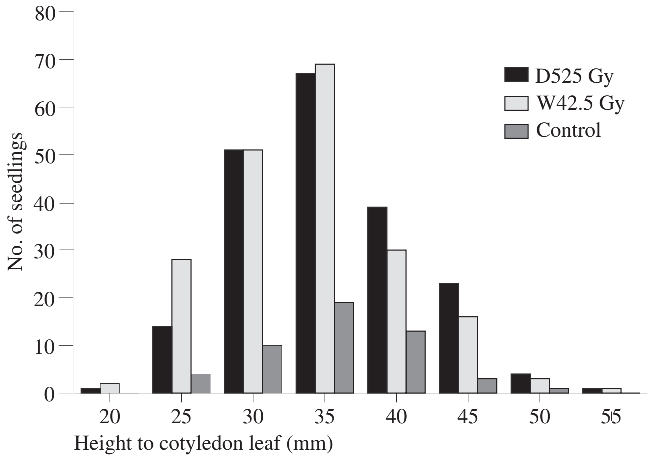

Figure 1. Variation in height of M2 seedlings

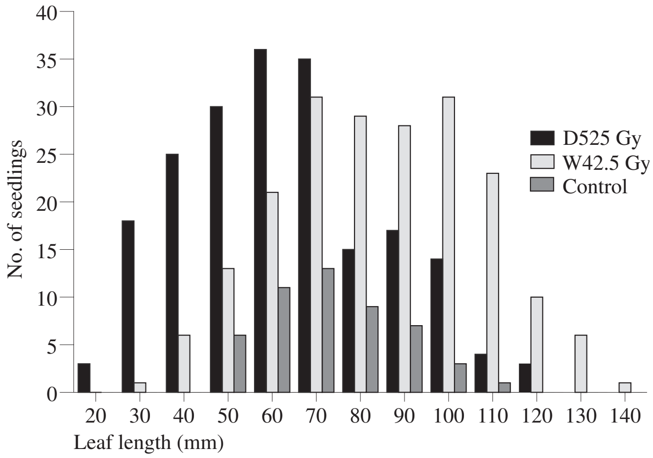

Figure 2. Variation in leaf length of M2 seedlings

Results and discussion

Juvenile characters

Data on height to first cotyledon and leaf length of 6-week-old M2 seedlings grown from W42.5 Gy and D525 Gy- treated seeds are presented in *Figures 1* and *2* respectively.

In *Figure 1*, taking the control to represent the normal distribution, it can be seen that the distribution for irradiated seedlings, regardless of dosage, was skewed towards the lower heights, perhaps more so for the W42.5 Gy treatment. This implies that more dwarf-stature progenies might be obtained in the M2 population compared with non-irradiated control seedlings.

In *Figure 2*, leaf length as an indicator of vigour shows that the low dosage treatment produced more progenies with longer leaves than the high dosage treatment. Taking the control as the normal distribution, it is evident that the distribution of D525 Gy progenies was skewed towards shorter leaf length while that of W42.5 Gy was skewed towards longer leaf length. It can be concluded, therefore, that the low irradiation dosage at 42.5 Gy was more desirable for producing a higher frequency of M2 progenies that were dwarf and have good vigour as expressed in longer leaves.

The effects of irradiation on M2 seedling height and vigour have been reported by Chan and Lam (2003). The control row was uniform in plant height, but there was considerable variation in the irradiated treatments. In general, the M2 seedling populations were taller and more vigorous, particularly those from the W42.5 Gy treatment. In the D525 Gy treatment, the effect of a higher irradiation dosage was evident with more seedlings showing very stunted growth.

Field characters

Plant vigour – tree height and stem diameter Irradiated trees were skewed towards shorter height (around 100–110 cm) compared with the control (median around 130 cm) *(Figure 3)*. There may be potential in selecting for more dwarf trees from the M2 populations. For stem diameter there was not much difference in distribution between irradiated and control trees *(Figure 4)*.

Fruiting characteristics In general, flowering time of trees irradiated at 42.5 Gy was delayed with a median of about 320 days compared with trees irradiated at 525 Gy and the control which have a median of about 280 days *(Figure 5)*. There seems to be little prospect in selecting for earliness in flowering in the M2 populations. For flowering height, the two irradiation treatments and the control showed similar medians at just over 90 cm. However for the 42.5 Gy treatment, several trees with very low flowering heights (50–60 cm) were found *(Figure 6)*. These may be useful mutants for getting short-bearing stature to facilitate fruit harvest.

Sex segregation Eksotika papaya segregates into 2 hermaphrodites: 1 female in normal populations and this is indicated in the control *(Table 1)*. In the case of irradiation-derived M2 populations, a small number of male trees emerged. Irradiation had caused the hermaphrodite gene M^H to mutate into the male gene M. The change was more significant in populations irradiated at the higher 525 Gy dosage.

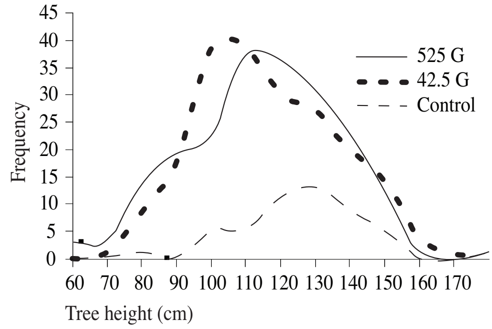

Figure 3. Distribution of M2 population for tree height

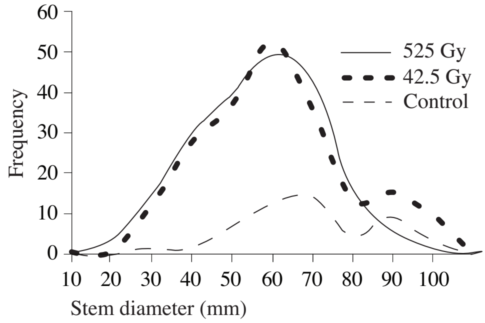

Figure 4. Distribution of M2 population for stem diameter

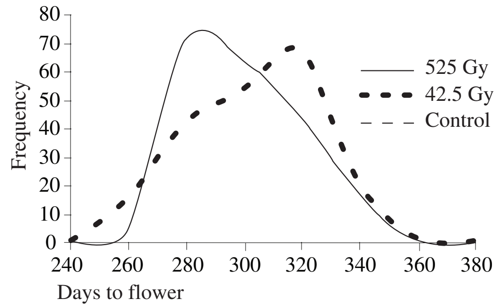

Figure 5. Distribution of M2 population for days to flower

Yield components and yield Irradiated trees showed quite a high frequency of sterility (non-fruit bearing) compared with the control *(Figure 7)*. There was little difference in the distribution for fruit weight *(Figure 8)* for all three populations.

Table 1. Sex segregation in M2 population

	Hermaphrodite		Female		Male		Total
	Number	%	Number	%	Number	%	
42.5 Gy	257	64.1	143	35.7	1	0.2	401
575 Gy	229	65.1	119	33.8	4	1.1	352
Control	35	70.0	15	30.0	0	0.0	50

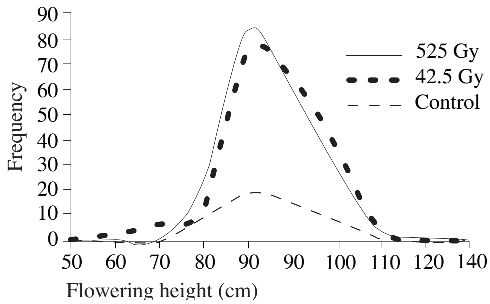

Figure 6. Distribution of M2 population for flowering height

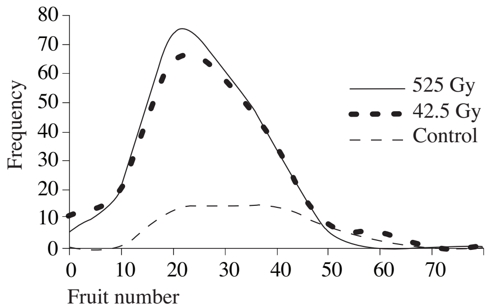

Figure 7. Distribution of M2 population for fruit number

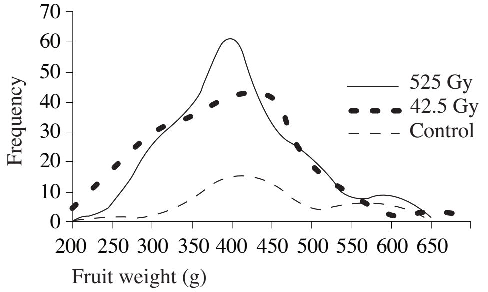

Figure 8. Distribution of M2 population for fruit weight

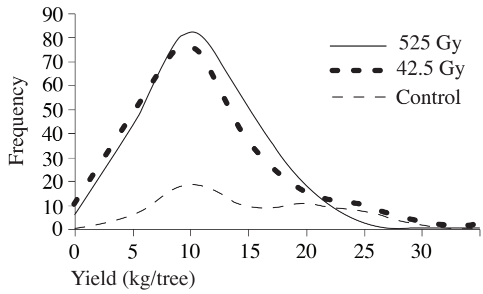

Figure 9. Distribution of M2 population for yield

However, trees derived from the low dosage irradiation showed a wider distribution with some fruit exceeding 650 g. The general pattern for yield distribution is rather similar for irradiated trees and the non-irradiated control *(Figure 9),* suggesting that irradiation may not be effective for increasing yield.

Fruit quality For total soluble solids, the distribution of the irradiated populations exceeded the upper limits of 15.5% of the control, reaching as high as 16.5% *(Figure 10)*. This is rather unusual for Eksotika papaya which has a TSS range from 13–15%. There is good prospect for increasing TSS% by selection from the M2 populations. For flesh colour *(Table 2)*, the control population is very stable with all the trees bearing fruit with orange-red flesh. However, for the irradiation-derived trees, a fairly high number of unattractive yellow-orange types had surfaced. Further, it is very surprising to find the emergence of yellow flesh types, ranging from 0.4% for 525 Gy treatment to 1.0% for 42.5 Gy treatment. Yellow flesh is controlled by a dominant gene (Storey 1969) and is usually not favoured by local consumers. Irradiation

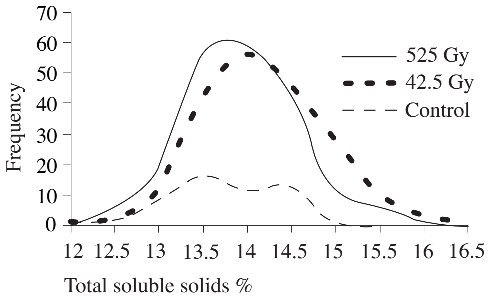

Figure 10. Distribution of M2 population for total soluble solids

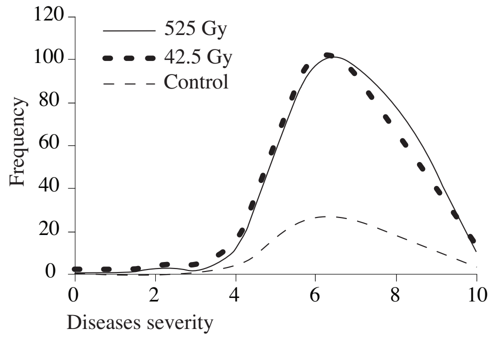

Figure 11. Distribution of M2 population for malformed top disease

Table 2. Flesh colour segregation in M2 population

	Orange-red		Yellow-orange		Yellow		Total
	Number	%	Number	%	Number	%	
42.5 Gy	333	84.9	55	14.1	4	1.0	392
575 Gy	280	97.5	6	2.1	1	0.4	287
Control	50	100.0	0	0.0	0	0.0	50

Table 3. Results of screening of M2 population for PRSV resistance

	Number and % of surviving symptomless plants									
	Pre-inoculation		1st inoculation		2nd inoculation		3rd inoculation		4th inoculation	
	No.	%	No.	%	No.	%	No.	%	No.	%
Batch 1										
42.5 Gy	480	100	109	22.7	48	10.0	8	1.7	2	0.4*
575 Gy	480	100	348	72.5	82	17.1	5	1.0	0	0.0
Control	56	100	45	80.3	6	10.7	0	0.0	0	0.0
Batch 2										
42.5 Gy	480	100	7	1.5	0	0.0				
575 Gy	480	100	36	7.5	0	0.0				
Control	60	100	0	0.0	0	0.0				

*The two plants were field planted, but subsequently succumbed to PRSV

appears to have caused a rare mutation from the normal recessive red gene in the Eksotika papaya.

Resistance to malformed top disease The distribution for infection to malformed top disease of M2 irradiated populations compared with control is shown in *Figure 11*. The majority of trees in the three populations succumbed to this disease. For the control, there were no trees having a disease rating lower than 4, but several trees from the irradiated populations have resistant ratings of 0–1. There is very good prospect in selecting mutants in the M2 population for resistance to MTD.

PRSV tolerance The results of the screening are shown in *Table 3*. In the first batch inoculated on 14 August 2002, a high number of seedlings did not show symptoms and these were cutback, allowed to re-shoot and were re-inoculated on the emerging new shoots. This was repeated three times to eliminate escapes until the fourth inoculation when only two seedlings were symptomless. These were then field planted for M3 seed. In the second batch inoculated on 18 December 2002, there were far fewer escapes, indicating an improvement in the inoculation technique, probably due to better skills of the workers. By the second inoculation, there were no surviving symptomless plants. In conclusion, of a total of 1,920 irradiation-derived M2 seedlings screened, only two were selected. However, when these two were field planted for M3 seed, they too succumbed to PRSV under field-challenge conditions. Irradiation, therefore, did not seem to be successful for developing PRSV resistance in Eksotika papaya.

Conclusion and recommendation

Wider variability was generally observed in the irradiation-derived M2 populations for many traits. Because of this, there are good prospects in improving Eksotika papaya, especially for more dwarf trees, lower fruit bearing stature, higher total soluble solids in fruit and, very importantly, for resistance to malformed top disease. However, no PRSV- resistant selections were made from screening 1,920 M2 seedlings.

M3 seed were obtained by self-pollination of selected mutants with superior traits. These will be planted for further evaluation for stability of the desired characteristics and selection of improved mutants.

Acknowledgement

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Abstrak
Pembiakbakaan mutasi bagi betik melalui iradiasi gama telah dimulakan di MARDI pada bulan Ogos 2000 dalam projek penyelidikan kontrak dengan International Atomic Energy Agency (IAEA). Iradiasi benih varieti Eksotika telah dijalankan di MINT dengan menggunakan dos yang sesuai untuk mass-iradiasi iaitu 525 Gy bagi biji benih kering dan 42.5 Gy untuk biji benih yang telah direndam. Sebanyak 200 pokok M1 dari setiap rawatan dos disediakan. Benih M2 telah dihasilkan dengan cara pendebungaan sendiri dari pokok hermafrodit M1 manakala 1,000 pokok M2 telah disediakan untuk tujuan mengkaji variasi terhadap 13 ciri. Sebanyak 1,920 biji M2 lagi ditanam, diinokulasikan dan disaring untuk menilai ketahanan pokok terhadap virus bintik cincin.

Dalam populasi M2, variasi luas direkodkan bagi banyak ciri. Pada peringkat percambahan benih, iradiasi yang rendah iaitu 42.5 Gy menghasilkan banyak progeni yang lebih pendek dan daun yang lebih subur berbanding dengan benih yang dipancarkan iradiasi 525 Gy dan anak pokok kawalan. Corak pengagihan progeni M2 untuk sembilan sifat kuantitatif menunjukkan variasi yang besar dengan julat yang melebihi populasi kawalan. Ini menunjukkan bahawa ada peluang yang baik untuk pembaikan betik Eksotika dengan menggunakan teknik iradiasi terutamanya, dalam pemilihan pokok yang bersaiz kecil, berbuah pendek, kandungan larutan pepejal tinggi dan buah yang lebih besar. Beberapa mutan M2 menunjukkan ketahanan yang baik terhadap penyakit *Cladosporium.* Walau bagaimanapun tiada ketahanan terhadap virus bintik cincin dijumpai dalam 1,920 anak pokok M2 yang telah diinokulasi dan disaring.

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