Radiation-Induced Mutation by Using in vitro
Adventitious Bud Technique
in Red Pepper (*Capsicum annuum* L. cv. Yatsufusa)
-Analysis of the variant appeared in M_1 generation

Prapa SRIPICHITT, Eiji NAWATA and Shoji SHIGENAGA

Faculty of Agriculture, Kyoto University, Kyoto, 〒 606

The in vitro adventitious bud technique was used in conjunction with gamma irradiation for the induction of mutation in red pepper. The twelve-day-old seedlings were irradiated by gamma radiation at the exposure doses of 0, 0.5, 0.75, 1.0 and 1.25 krad delivered at the dose rate of 5 krad/h. After irradiation adventitious shoots were induced from the excised cotyledons. The shoots were rooted and the obtained plantlets were grown under greenhouse conditions until maturity. No variant segregation was observed in control plants from both seeds and non-irradiated cotyledon explants whereas visible variants were found among the M_1 plants regenerated from irradiated cotyledon explants at the frequency of 4.4%. The variation spectrum was consisted of plant type, leaf, flower and fruit variation. Rate of chimerism based on visual characters was considerably high, indicating the multicellular origin of adventitious shoot. A number of variants were recovered in the immediate gamma ray treated generation (M_1). Most of the variant characters obtained were transmitted to the M_2 progeny and were considered as mutants. Some of the characters were bred true and many were segregated. Most of the mutated characters were stabilized in the M_3 generation. Three types of the variant phenotypes were lost and the causes were discussed.

KEY WORDS : *Capsicum annuum* L., adventitious shoot, gamma irradiation, frequency of variant, spectrum of variation, chimera.

Introduction

Mutation induction is of great potential value for plant improvement. The combined use of adventitious bud technique and irradiation has been demonstrated as an effective method for generating a high frequency and a broad spectrum of non-chimeric mutants (BROERTJES *et al.*, 1968 ; THOMAS *et al.*, 1979).

Many plant species can be stimulated to form adventitious buds on tissue or organ. It has been revealed that the adventitious buds are originated from only one or a few cells (NAYLOR and JOHNSON, 1937). The plants obtained through adventitious buds after irradiation of the tissue or organ are either normal (non-mutated) or almost exclusively solid (non-chimeric) mutants (BROERTJES, 1969 ; BROERTJES *et al.*, 1976). A higher frequency and a wider spectrum of mutations could be obtained by this method (ROEST *et al.*, 1981 ; VAN HARTEN *et al.*, 1981).

The present study was originally designed to investigate whether the application of in vitro adventitious bud technique in combination with gamma irradiation could lead to the production of high mutation frequency and wide mutation spectrum in red pepper. On the process of the study, we found putative homozygous mutants in the immediate gamma ray treated generation (M_1) unexpectedly. In the present experiment, the plants obtained from gamma-irradiated cotyledon explants (M_1 generation) were scored for the frequency of phenotypic variants, their spectrum as well as the chimerism. The segrega-

tion and inheritance of the variant characters in the subsequent generations (M_2 and M_3) were also evaluated.

Materials and Methods

Plant materials

Selfed seeds from single plant of red pepper cultivar "Yatsufusa" (Fig. 1 a) were used. Yatsufusa has compact and bushy plant type with shortened internodes, and flowers and fruits are born in clusters (fasciculated). Flowers have white petals, purplish blue anthers and colorless filaments and styles. The fruits are set on erect pedicels and elongated in shape with pointed apices. They are green and turn red at mature stage.

Preparation of seedlings

Preparation of seedlings was performed following the procedures as described elsewhere (SRIPICHITT *et al.*, 1987).

Irradiation and culture of cotyledon explants

The twelve-day-old seedlings grown in flasks were irradiated by gamma radiation from a ^{60}Co source at the exposure doses of 0, 0.5, 0.75, 1.0 and 1.25 krad delivered at the dose rate of 5 krad/h according to the radiosensitivity of adventitious shoot formation on cotyledon explants as described previously (SRIPICHITT *et al.*, 1988). After irradiation, trimmed cotyledons with petioles were excised from the seedlings and cultured by the methods as described in the previous study (SRIPICHITT *et al.*, 1988).

Production of plantlets

After three weeks' culture, the adventitious shoots developed on each explant were removed from the explant and separated into 2-6 clumps and they were subcultured on MURASHIGE and SKOOG (MS) medium (MURASHIGE and SKOOG, 1962) supplemented with 2 mg/l benzyladenine in a 100 ml erlenmeyer flask to promote shoot elongation. After two weeks' subculture, shoots with the length of 0.5-0.8 cm were separated and transferred to the MS medium supplemented with 0.5 mg/l indoleacetic acid to induce root formation. Complete plantlets could be raised within 2 or 3 weeks after transplanting. Then the plantlets were transferred to small plastic pots (6 cm in diameter) containing vermiculite and kept at 26°C under a 16/8 h photoperiod regime of 3000 lux of fluorescent light. Three weeks later, plantlets were transplanted into plastic pots (20 cm in diameter) containing soil mixture of sand, perlite and commercial compost at the rate of 1 : 1 : 1, and grown under greenhouse conditions until maturity.

Evaluation of variation

The M_1 plants derived from adventitious shoots after irradiation of cotyledon explants were evaluated for the frequency of visible variation, variation spectrum and chimerism.

Analysis of pollen fertility

Pollen fertility was estimated in 5 flowers by staining with 1% acetocarmine. The stained pollen grains were considered fertile whereas shriveled and unstained pollen grains were treated as sterile.

Test of the progenies of M_1 variants

Both solid-looking and chimeric variants in M_1 generation were self-pollinated. Seeds thus obtained were sown and grown under greenhouse conditions. The M_2 progeny of

each variant was examined for its character. If the inheritance was proved, they were considered as mutants. The lines which showed segregation in M_2 generation were tested again to ascertain whether the mutants were bred true in the M_3 generation by the procedures as described above.

Results

Frequency of visible variation in M_1 plants

The frequency of M_1 adventitious plants obtained from irradiated cotyledon explants decreased with the increasing exposure of gamma radiation (Table 1). No variant was observed in the control plants from both seeds and non-irradiated cotyledon explants whereas the relatively high frequency of visible variation was found among the M_1 plants regenerated from irradiated cotyledon explants (average of all dosage : 4.4%). The variation frequency was maximum at 0.75 krad and gradually decreased as the exposure increased to 1.25 krad.

Spectrum of the M_1 variants

The visible variants observed in M_1 generation were classified into four groups : plant type variant ; leaf variant ; flower variant ; and fruit variant (Table 2). A brief description of each group of variant is as follows :

I. Plant type variant

1. Dwarf. The variants were 11-20 cm tall whereas the control plants were 32-68 cm tall. The plants had smaller leaves and less branches than controls (Fig.1 b).
2. Indeterminate growth habit. The plant showed abnormal apical dominance with less lateral branches. The main stem did not terminate in fruits but continued producing fruits until the tenth cluster (Fig.1 c).

II. Leaf variant

1. Viridis. The leaves were yellowish green and becoming nearly normal green with maturity (Fig.1 d). The plants were quite dwarf.
2. Variegated mottled. The branches of these two chimeric variants possessed the yellow or light-green mottles on foliages (Fig.1 e) and immature green fruits.

Table 1. Frequency of variation in M_1 plants regenerated from gamma-irradiated cotyledon explants of red pepper

Exposure dose (krad)	No. of irradiated explants	No. of adventitious plants produced		No. of variant plants	Frequency ¹⁾ of variation (%)
		total	per explant		
Control ²⁾	—	80	—	0	0
0	30	60	2	0	0
0.5	134	162	1.2	6	3.7
0.75	194	141	0.7	8	5.7
1.0	225	113	0.5	5	4.4
1.25	283	82	0.3	3	3.7
(Total)	866	638	0.9	22	4.4

¹⁾ The number of variant expressed as a percentage of the total number of plants produced at each exposure dose.

²⁾ Plants obtained from seeds.

Table 2. Spectrum of variation of the M_1 plants derived from gamma-irradiated cotyledon explants of red pepper

Variant character	No. of variant
Plant type	
dwarf	4
indeterminate growth habit	1
Leaf	
viridis	2
variegated mottled	2
bushy variegated	1
filiform	2
Flower	
yellow anther	1
partial male sterility	2
narrow petal	4
Fruit	
blunt apex fruit	2
oblate fruit	1

3. Bushy variegated. The branches of this chimeric plant showed creamy-white and green mottles on the leaves (Fig. 1 f) and immature fruits.

4. Filiform. The variants were characterized by very elongated and narrow leaves with acuminate tips and undulate, irregular and thickened margin. Flower morphology and fruit setting were normal (Fig. 1 g).

III. Flower variant

1. Yellow anther. The branch bore flowers with yellow anthers and poor fertility.

2. Partial male sterility. The anthers of these plants were reduced in size and shrunk. The percentage of pollen fertility in variant plants was low (<50%). The

fruits were very small compared to the controls.

3. Narrow petal flower. The plants bore small flowers with narrow petals (Fig. 1 h). The petals were greenish-white compared to the white petals of controls.

IV. Fruit variant

1. Blunt apex fruit. The fruits were elongated in shape with blunt apex (Fig. 1 i). They were chimeras producing both blunt and pointed apex fruits in each cluster.

2. Oblate fruit. The fruits were short and round in shape with blunt and partially pointed apex (Fig. 1 j). Fruit base was not entirely enclosed by calyx. The percentage of pollen fertility was about 50% and seed-set was low.

It was shown that the variation spectrum was fairly wide and consisted of almost all the visible characters of the plant. The broadest variation spectrum was found to occur in leaf variation. In some cases, more than one phenotypically identical variants were observed and they were derived from both the same and different explants as well as from the explants subjected to different exposure doses.

Inheritance of variant characters to M_2 selfed progenies

The segregation of the variant characters in the M_2 progenies are shown in Table 3. All of the dwarf variants bred true for their characters in the M_2 generation. The M_2 progenies of the indeterminate plant type variant showed segregation of 39 normal plants and 2 variants.

The progenies of viridis plants segregated into normal and viridis plants. The viridis plants had normal green cotyledons with true leaves turning yellowish-green. Some of these plants died before flowering. The segregation of the normal, surviving viridis and dead viridis in M_2 generation fitted well for the 1 : 2 : 1 ratio (variant no. 268 : $X^2=0.091$, 2 d. f., $0.95 < P < 0.99$; variant no. 269 : $X^2=0.333$, 2 d. f., $0.75 < P < 0.90$). The M_2 progenies of the variegated mottled segregated into variegated and normal plants. All of

Fig. 1 a) A normal plant of *C. annuum* cv. Yatsufusa
 b) Dwarf plant
 c) Indeterminate plant
 d) Viridis plant
 e) A variegated mottled plant showing chimeric branches with yellow or light-green mottles on foliages (arrow)
 f) A branch of bushy variegated plant having creamy-white and green mottles on the leaves
 g) Filiform plant
 h) Narrow petal flower (right) and normal flower (left)
 i) A cluster of blunt apex fruit
 j) A cluster of oblate fruit
 k) An M_2 seedling of bushy variegated plant

Table 3. Segregation of the variant characters in M_2 selfed progenies of plants regenerated from gamma-irradiated cotyledon explants of red pepper

Variant no.	Variant character	No. of M_2 progenies observed	Segregation normal : variant
098	dwarf	27	0 : 27
110	do	34	0 : 34
124	do	18	0 : 18
130	do	30	0 : 30
570	indeterminate	41	39 : 2
268	viridis	22	6 : 16 ¹⁾
269	do	27	8 : 19 ²⁾
156	variegated mottled	29	2 : 27
615	do	68	24 : 44
217	bushy variegated	75	9 : 66
273	filiform	28	28 : 0
613	do	30	30 : 0
079	yellow anther	27	27 : 0
167	partial male sterility	20	20 : 0
295	do	34	34 : 0
176	narrow petal	29	0 : 29
177	do	32	0 : 32
219	do	24	0 : 24
220	do	27	0 : 27
190	blunt apex fruit	26	7 : 19
619	do	19	4 : 15
446	oblate fruit	36	30 : 6

¹⁾ Five of 16 variant plants were lethal.

²⁾ Six of 19 variant plants were lethal.

the 27 variegated seedlings of variant no.156 had yellow cotyledons and died before the development of true leaves. Among the 44 variegated seedlings of variant no.615, 40 seedlings had yellow cotyledons and the other 4 had normal green cotyledons but the younger leaf blades were yellow particularly at the area near the petiole. These four variegated plants could survive until maturity and the selfed seeds were obtained. The bushy variegated segregated into 9 normal and 66 variegated plants. The variegated plants were distinguishable at an early stage by the white streak along the midrib on the upper surface of the cotyledons and the stem were white with varying degree of purple streaks. The true leaves were creamy-white with green mottles (Fig.1k). All the variegated plants died after having 3-4 leaves.

The M_2 progenies of both filiform variants were all normal. Similarly, all of the M_2 progenies of yellow anther variant had purplish-blue anthers of control plants. No segregating variant was detected from the partially male sterile variants.

The narrow petal character was transmitted to the M_2 progenies. Both normal and blunt apex fruit segregants were observed among the M_2 progenies of blunt apex fruit variants. The M_2 progenies of the oblate fruit plant segregated into 30 normal and 6 oblate fruit plants.

Table 4. M_3 progenies of the M_2 variant segregants obtained from the M_1 variants

Variant no.	Variant character	No. of M_3 progenies observed	Segregation
			normal : variant
570	indeterminate	21	0 : 21
615	variegated mottled	27	0 : 27
190	blunt apex fruit	24	0 : 24
619	do	26	0 : 26
446	oblate fruit	33	25 : 8

Inheritance of variant characters in M_3 selfed progenies

The variant segregants in M_2 variants were self-pollinated and the seeds thus obtained were sown into M_3 progenies. All the variant characters were stabilized in the M_3 generation except the oblate fruit trait which still segregated into normal and oblate fruit plants (Table 4).

Rate of chimerism

Among the 22 variants obtained, 6 were visually identified as chimeras. In addition to them, an indeterminate plant was classified as chimeric variant based on segregation data.

Discussion

It is of interest that a number of variants were recovered in the immediate gamma ray treated generation (M_1). Similar observation has been demonstrated in *Nicotiana glauca* (DE NETTANCOURT *et al.*, 1971), rice (SHOME and BHADURI, 1982) and tomato (EVANS and SHARP, 1983).

Various types of morphological variants were identified based on the phenotypic characterization. In most cases, the variants obtained here were similar to those which were reported to occur spontaneously or after mutagenic treatment of the seeds of *Capsicum* spp. Many of them are classified as single recessive gene mutants. These included dwarf (LIPPERT *et al.*, 1965), variegated mottled (LIPPERT *et al.*, 1964), bushy variegated (COOK, 1962), filiform (COOK, 1961), yellow anther (DESHPANDE, 1939), male sterility (DASKALOFF, 1968) and blunt apex fruit mutants (DESHPANDE, 1933). The viridis is a lethal mutant caused by a single semidominant gene mutation (KORMOS and KORMOS, 1956). The oblate fruit trait is reported to be controlled by a single dominant gene (PETERSON, 1959), but the variants found in the present experiment seemed to be controlled by another mechanism. The other two variants, indeterminate plant type and narrow petal flower, were first discovered in the present study. Further research is necessary to clarify the genetic basis of the inherited variant characters.

Most of the variants observed in the present study were inherited to the M_2 progenies. Two of the variant characters, dwarf and narrow petal flower segregated no normal progenies. There is a possibility that those two characters are controlled by single recessive genes and that the recovery of homozygous recessive variants in M_1 generation took place, although participations of chromosome aberrations should be also considered. Further study must be needed. Several others, such as indeterminate, variegated mottled, bushy variegated and blunt apex fruit, were segregating in M_2 generation.

The selfed seeds obtained from M_2 variants segregated no normal progenies in M_3 generation. Their segregation ratios in M_2 must be examined in relation to their chimeric nature and genetic basis. Segregation in M_2 progenies shown by viridis variants was seemed to be caused by single semi-dominant gene mutations. The oblate fruit trait which was reported by PETERSON (1959) was a single dominant mutant. However the comparable variant found in the present experiment showed the segregation of normal and variant plants until M_3 generation.

Three variant characters, namely filiform, yellow anther and partial male sterility were not transmitted to the M_2 progenies. In all cases, the variant phenotypes might be due to participation of chromosomal aberrations which might be selected out in M_2 . One possibility of the disappearance of the filiform leaf trait might be the periclinal chimera of the variant plants. Mutation occurred in the L_1 tissue in which leaf shape was expressed, while the L_2 and L_3 layers remained normal and produced functionally reproductive organ. The selfed seeds thus obtained gave rise to plants having normal leaf shape. Loss of yellow anther character in M_2 progenies might have been resulted from the chimeric condition of the M_1 variant and the very low percentage of seed germination (28%) of the M_2 seeds. It is assumed that seeds with homozygous recessive genotype could not germinate and that only the plants with purplish blue anther were observed. Male sterility in genus *Capsicum* is controlled by at least one recessive nuclear gene (DASKALOFF, 1968) or by the interaction between the sterile cytoplasm and a nuclear gene (PETERSON, 1958). The expression of male sterility is often influenced by the ambient temperature (PETERSON, 1958, TATLIOGLU, 1985). The change from partial sterility of M_1 variant to full fertility in M_2 progenies may be caused by the change in temperature. Partial sterility might have been expressed under cool condition in early spring when the M_1 variants began to flower and fertility was restored under warm condition in mid summer when the M_2 progenies were raised.

The recovery of homozygous recessive mutations of red pepper in the M_1 generation must be proved by further examinations. Presently we consider the possibility that two identical recessive mutations were simultaneously induced in both alleles of a locus in the homologous chromosome or that a mutation occurred in one allele followed by mitotic recombination. Another possibility is the various types of chromosome aberrations. Further investigations must be directed to this field.

Many of the mutants detected were chimeras which were observable as mixed phenotypes in M_1 plant, indicating that adventitious shoots were derived not only from single cells but also from multiple ones. This observation is not in agreement with the previous studies in *Chrysanthemum* (BROERTJES *et al.*, 1976), *Begonia* (ROEST *et al.*, 1981) and potato (VAN HARTEN *et al.*, 1981). In these cases, a very low rate of chimerism was observed among the in vitro mutants produced after irradiation, suggesting the unicellular origin of the plants. It has been manifested that chimerism was observed in adventitious plants regenerated from callus culture of tobacco (SACRISTAN and MELCHERS, 1969), anther and stem internode culture of tomato (SREE RAMULU *et al.*, 1976) and protoplast culture of potato (SREE RAMULU *et al.*, 1984). Therefore, the in vitro adventitious shoots may originate from multiple cells as well as from single ones.

Evaluation of the frequency and spectrum of variation in this experiment should be conducted by progeny test on all the M_1 plants produced after irradiation.

Acknowledgments

Authors wish to express their thanks to Dr. Hiroshi HASEGAWA, Radiation Center of Osaka Prefecture for his valuable suggestion and kind help in providing us the irradiation facilities. They are also grateful to Dr. Tadashi ASAHIRA and all members of Laboratory of Vegetable and Ornamental Horticulture, Kyoto University, for allowing us to use many facilities throughout this experiment.

Literature Cited

- BROERTJES, C. 1969. Mutation breeding of *Streptocarpus*. Euphytica 18 : 333~339.
- , B. HACCUS and S. WEIDLICH 1968. Adventitious bud formation on isolated leaves and its significance for mutation breeding. Euphytica 17 : 321~344.
- , S. ROEST and G. S. BOKELMANN 1976. Mutation breeding of *Chrysanthemum molifolium* Ram. using in vivo and in vitro adventitious bud techniques. Euphytica 25 : 11~19.
- COOK, A. A. 1961. Inheritance of mutant-1 phenotype in the pepper. J. Hered. 52 : 154~158.
- 1962. Studies of a foliage variegation in pepper. Proc. Amer. Soc. Hort. Sci. 81 : 390~395.
- DASKALOFF, S. 1968. A male sterile pepper (*C. annuum* L.) mutant. Theor. Appl. Genet. 38 : 370~372.
- DE NETTANCOURT, D., P. DIJKHUIS, A. J. G. VAN GASTEL and C. BROERTJES 1971. The combined use of leaf irradiation and of the adventitious bud technique for inducing and detecting polyploid, marker mutations and self-compatibility in clonal population of *Nicotiana glauca* Link and Otto. Euphytica 20 : 508~520.
- DESHPANDE, R. B. 1933. Studies in Indian chillies. (3) The inheritance of some characters in *Capsicum annuum* L. Indian J. Agric. Sci. 3 : 219~300.
- 1939. Studies in Indian chillies. (5) Inheritance of anther colour and its relation to colour in petal and node in *Capsicum annuum* L. Indian J. Agric. Sci. 9 : 185~192.
- EVANS, D. A. and W. R. SHARP 1983. Single gene mutations in tomato plants regenerated from tissue culture. Science 221 : 949~951.
- KORMOS, J. and J. KORMOS 1956. Data on the genetics of chlorophyll deficiency. Plant Breed. Abst. 26 : 640.
- LIPPERT, L. F., B. O. BURGH and A. A. COOK 1964. Three variegated seedling mutants in the pepper. J. Hered. 55 : 79~83.
- , ——— and P. G. SMITH 1965. Gene list for the pepper. J. Hered. 56 : 30~34.
- MURASHIGE, T. and F. SKOOG 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15 : 473~497.
- NAYLOR, E. and B. JOHNSON 1937. A histological study of vegetative reproduction in *Saintpaulia ionantha*. Amer. J. Bot. 24 : 673~678.
- PETERSON, P. A. 1958. Cytoplasmically inherited male sterility in *Capsicum*. Amer. Nat. 92 : 111~119.
- 1959. Linkage of fruit shape and color genes in *Capsicum*. Genetics 44 : 407~419.
- ROEST, S., M. A. E. VAN BERKEL, G. S. BOKELMANN and C. BROERTJES 1981. The use of an in vitro adventitious bud technique for mutation breeding of *Begonia* × *Hiemalis*. Euphytica 30 : 381~388.
- SACRISTON, M. D. and G. MELCHERS 1969. The caryological analysis of plants regenerated from tumorous and other callus cultures of tobacco. Mol. Gen. Genet. 105 : 317~333.
- SHOME, A. and P. N. BHADURI 1982. Responses of excised embryos of rice (*Oryza sativa* L.) to X-rays. Theor. Appl. Genet. 61 : 135~139.
- SREE RAMULU, K., M. DEVREUX, G. ANCORA and U. LANERI 1976. Chimerism in *Lycopersicon peruvianum* plants regenerated from in vitro cultures of anthers and stem internodes. Z. Pflanzenzüchtg. 76 : 299~319.
- , P. J. DIJKHUIS and S. ROEST 1984. Genetic instability in protoclonal lines of potato (*Solanum*

- tuberosum* L. cv. 'Bintje'): new types of variation after vegetative propagation. Theor. Appl. Genet. **68**: 515~519.
- SRIPICHITT, P., E. NAWATA and S. SHIGENAGA 1987. In vitro shoot-forming capacity of cotyledon explants in red pepper (*Capsicum annuum* L. cv. Yatsufusa). Japan. J. Breed. **37**: 133~142.
- , ——— and ——— 1988. Effects of exposure dose and dose rate on in vitro shoot-forming capacity of cotyledon explants in red pepper (*Capsicum annuum* L. cv. Yatsufusa). Japan. J. Breed. **38**: 27~34.
- TATLIOGLU, T. 1985. Influence of temperature on the expression of cytoplasmic male sterility in Chives (*Allium schoenoprasum* L.). Z. Pflanzenzüchtg. **94**: 156~161.
- THOMAS, E., P. J. KING and I. POTRYKUS 1979. Improvement of crop plants via single cell in vitro-an assessment. Z. Pflanzenzüchtg. **82**: 1~30.
- VAN HARTEN, A. M., H. BOUTER and C. BROERTJES 1981. In vitro adventitious bud techniques for vegetative propagation and mutation breeding of potato (*Solanum tuberosum* L.). II. Significance for mutation breeding. Euphytica **30**: 1~8.

組織培養下での不定芽形成を利用した，トウガラシ (*Capsicum annuum* L.)
の放射線突然変異誘発— M_1 世代に見られた変異体の解析

Prapa SRIPICHITT・縄田栄治・重永昌二

(京都大学農学部，京都市，〒606)

突然変異誘発効率の検討を目的として，トウガラシ幼植物にガンマ線照射を行った後，組織培養下で形成された不定芽に由来する植物体を育成した。播種12日後の苗に，照射線量 0, 0.5, 0.75, 1.0, 1.25 krad のガンマ線を線量率 5 krad/hr で照射した。照射後，子葉培養組織から形成された不定芽を，発根させた後温室内で育成した。種子及び照射を行わなかった子葉培養組織に由来する植物体には，全く変異が認められなかったが，照射を行った子葉培養組織から得られた植物体には，高い頻度(4.4%)で可視変異が認められた(Table 1)。変異スペクトルは比較的広く，草型，葉，花，果実についての変異が見られた (Table 2, Fig. 1)。キメラ発生率は相当高く，不定芽が単一の細胞のみに由来するのではなく，複数の細胞に由来する場合もある事が示唆された。見られた変異体の殆どが，一遺伝子性形質によるものであった。 M_1 世代で見いだされたこれらの変異形質の次代 (M_2) への伝達を調査したところ， M_2 世代で固定した形質と分離した形質が見られた (Table 3)。そして，それらの殆どが M_3 世代で固定した (Table 4)。3種類の変異形質が M_2 世代で消失し，その原因について考察した。ガンマ線照射を行った世代 (M_1) の不定芽培養植物に多くの変異が見いだされたことは，突然変異育種においてこの方法が有効であることを示唆している。