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Recovery of induced mutations in M_2 generation of gamma irradiated cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT: A study was carried out on the recovery of induced mutations in the M_2 generation of gamma irradiated cowpea. About 350g of dry cowpea seeds 'IT84S-2246' were equlibrated to 12% moisture content in a dessicator over 65% glycerol for seven days. The sample were irradiated with gamma rays from Cobalt 60 source to a dosage level of 245Gy at the rate of 24.0 Gy/minute. The treated seeds were planted at a spacing of 30 x 30 cm to raise the M_1 generation. At maturity, 150 M_1 plants were harvested according to the branching pattern of cowpea and peduncle by peduncle. Progenies so obtained from M_1 plants were sown peduncle to row at a spacing of 15 x 15cm to produce M_2 plants. Chlorophyll mutations were evaluated at the seedling stage. The frequencies of chlorophyll mutations were computed. Results obtained were analysed by chi-square test. A wide spectrum of chlorophyll mutations ranging from Albina, Maculata, Viridis, Zonata and Marginata were observed. The highest frequency of chlorophyll mutations (0.33) was obtained for M_2 plants that originated from peduncle three(3) of the main branch (1) while the least (0.05) was obtained for M_2 plants that originated from peduncle three (3) of secondary branch five (V). The occurrence of mutation was not significantly different from peduncle to peduncle. This implies that selection of pods for the perpetuation of mutations in subsequent generation could be done from any part of the plant, though the four peduncles originating from the main branch (1) have higher mutation frequencies than secondary branches, and this may confer selection advantage on the former.

Key Words: Cowpea; *Vigna unguiculata*; Mutation; Gamma irradiation.

Introduction

One of the basic principles of mutation induction is that ' a mutation is induced in a single cell and carried only in the progeny of that cell''(Brock and Micke, 1979). When multicellular organs like seed embryo are mutagenised, the treatment imposes a stress whose degree of disruption depends on the type of mutagen, total dose, dose rate and physiological state of the apical cells at the time of exposure. As a result, cells mutate independently and the descendants of each mutated cell may form a sector within chimeric M_1 plants. In which case plants will develop from embryonic tissue composed of genetically different tissues. Thus, the different parts of the meristem have the potential to give rise to a certain part of the mature plant, such plants are referred to as chimeras. Chimerism occur as a result of dissociation or re-combination of genetic composition of cells within active meristems that may be due to the effect of

irradiation. There had been important evidence regarding the role of the apical cells in chimerism. This was based on the fact that sectors of substantial size would never develop as a result of mutation in a single cell if there were no apical initials (Stewart and Dermen, 1970). Each of the sectors was assumed to originate from individual initial cells. Mutations involving ploidy change due to loss of an entire chromosome(aneuploidy), loss of chromosome segment (translocation, inversion or deletion) within an individual initial cell of a shoot apex that is reproducibly stable, will resault in a mericlinal, periclinal or sectorial chimerism. The size of the sector and plant part that will be affected depends on the location of the mutated cell, the number of other non-mutated cells and the ability of mutated cells to compete with the non-mutated cells. A mutated cell within the centre of an actively dividing primodium are likely to have its descendants being displaced. The chances of its inclusion in the meristem and of producing a larger chimeric segment become reduced. Loss of sectorial chimerism is often ascribed to diplontic selection and a consequence of normal growth of the plant apex where the sector originate (Gaul, 1959). For mutations to be expressed, it must be induced in cells that survives with its reproductive ability unimpaired to form detectable tissues that can be located and be propagated.

After many years of studies on induced mutation, the induction and recovery of beneficial mutants for the improvement of crop plants still remain largely a matter of chance (Hermelin et al., 1981) due to:

(a) mutation frequency that is very low (Brock, 1979),
(b) knowledge about possible relation between choice of mutagen and mutation spectrum is not yet established,
(c) some induced mutations are not carried to M_2 because the harvested seeds from chimeric M_1 plants may not derive from mutated sector(s).
(d) Also, the embryo structure of many dicotyledonous crop plants have not been adequately studied.

However, the low mutation frequency could be compensated for by large mutagenised M_1 population. Thus, collection of all seeds from M_1 plants become too large to be progeny tested in M_2 and a representative sample has to be taken, in which as many mutant sector as possible will be represented. Unlike the monocot cereals where the chimeric pattern is relatively well known (IAEA, 1977), information about leguminous crop species are scarce. Previous study of chimerism in M_1 plants of *Vicia faba*, *Capsicum annuum* and *Linum usitatissimum* by Hermelin et al (1983), had shown that the highest degree of recovery of M_2 mutants from M_1 plants of *Vicia faba* was from the second and or third pod-bearing node(s), while in *C. annuum*, highest mutation frequency was obtained from the fruit at the main bifurcation of the M_1 plant, while in *L. usitatissimum*, recovery of M_2 mutants is higher in later developed part of the shoot. Also, in an experiment conducted by Saccardo (1980), higher mutation frequency was derived from seeds originating from the main stem of M_1 plants of *P. sativum* for chlorophyll and viable mutations. The potential of anthers from different parts of the panicle to induce callus was investigated with rice variety "Taipei 309". The result showed that anthers from basal part of the panicle have higher efficiencies for callus induction than those from middle and top part (FAO/IAEA, 2000). There is the need to improve the knowledge on the selection of pods/seeds from chimeric M_1 dicotyledonous plant species. This will enable the perpectuation of mutants with desirable characters at reduced cost.

The objectives of this study therefore are:

(i) To investigate the frequency of chlorophyll mutations in the M_2 generation of gamma irradiated cowpea seedlings.

(ii) To examine and test if these mutations are confined to few of the branches/peduncles or randomly distributed.

Materials and Methods

Dry cowpea seeds 'IT84S-2246' was used for this study. Seed moisture content was equilibrated to 12% over 65% glycerol. The seeds were irradiated at the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife to a dose of 245 Gy at the rate of 24.0 Gy/minute. The irradiated

seeds were planted at the Teaching and Research Farm, University of Ilorin, to establish M_1 generation. Seeds were sown at a spacing of 30 x 30cm within row and 75cm between rows at the rate of two seeds per hole. All necessary weeed management and protection practices were adopted. At maturity, pods from one hundred and fifty M_1 plants were harvested according to the branching pattern of cowpea peduncles. Progenies of M_1 plants were sown peduncle to row at a spacing of 15 x 15cm. The primary branch/main stem was noted as (I), while secondary branches were noted as II – V from the stem base (Figure 1). The peduncles on each branch were noted as (1, 2, 3 – 6) also from the base of the branch upward (Figure 1). M_2 seedlings were evaluated for chlorophyll mutations according to Gutstafsson classification (Gustafsson, 1940) four weeks after planting. Chlorophyll mutation frequencies were computed on the basis of peduncle positions.

Mutation frequency $= \frac{\text{Number of mutations at M2}}{\text{Number of survived plants at } M_2}$

Chi-square test was used to test the hypothesis that mutation is distributed evenly in all the peduncles, that is, probability of mutation is equal in all peduncles irrespective of position on the stem:

$$X^2_{K-1,1-a} = \Sigma \frac{ni - E(ni)^2}{E(ni)}$$

Where E(ni) is the expected value while (ni) is the observed value. The null hypothesis is that mutations are equally distributed, and can be recovered at the same frequency in all the peduncles, that is

$H_o = P_1 = P_2 = P_3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots P_{16} = 1/16$

The alternative hypothesis is that mutations are not equally distributed and cannot be recovered at the same frequency in all the peduncles.

$H_A = P_1 \pm P_2 \pm \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots P_{16} \pm 1/16$

Reject null hypothesis when

$X^2_{K-1.1-\alpha}$, X^2 Calculated

Do not reject null hypothesis when
$X^2_{K-1,1-\alpha} > X^2$ Calculated

Results and Discussion

Chlorophyll mutations were recorded as presented in Table 1. The surviving plants showed a wide spectrum of chlorophyll mutations, which ranged from Albina, Maculata, Viridis, Zonata and Marginata (Table 1). Total number of chlorophyll mutations identified was 517. Maximum number of Maculata types was observed in all the branches and peduncles followed by Viridis, both of which are viable. This is contrary to the findings of Subramanian (1980), where he observed maximum number of non-viable chlorophyll mutations (Xantha and Albina) in *Vigna radiata*, *V. trilobata* and *V. aconitifolia*. This finding confirms the specificity of different mutagens on various *Vigna* species to produce different types of mutations. With the exception of Albina where chlorophyll pigments and carotenoids are not formed at all, other chlorophyll mutations identified would confer viability to the plants identified with such mutations, since such seedling would regain normal coloration as growth progressed. Chlorophyll mutation frequencies ranged from 0.05 to 0.33 (Table 2). Generally, higher chlorophyll mutation frequencies were obtained for plants originating from the main stem (Branch 1), while the secondary branches recorded quite low mutation frequencies. The highest frequency (0.33) was obtained for M_2 plants that originated from the main stem (Branch 1) peduncle three (3) while the least (0.05) was obtained from branch (v) peduncle three (Figure 1). This implies that the earlier formed main stem and peduncles originating from it

Table 1: Phenotypic classification of chlorophyll mutations in M_2 gamma irradiated cowpea seedlings.

Type of chlorophyll mutations	Branch/peduncle number															
	I_1	I_2	I_3	I_4	I_5	I_6	II_1	II_2	III_1	III_2	III_3	IV_1	IV_2	V_1	V_2	V_3
Albina	–.	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–
Maculata	106	80	92	42	13	9	6	9	10	10	10	9	2	6	4	1
Viridis	4	13	53	15	–	3	3	–	3	1	3	2	2	–	1	1
Zonata	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Marginata	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Xantha	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total mutations	111	95	147	57	13	12	9	9	13	11	13	11	4	6	5	2

Table 2: Frequency of chlorophyll mutations in M_2 gamma irradiated cowpea seedlings.

	Branch/peduncle number															
	I_1	I_2	I_3	I_4	I_5	I_6	II_1	II_2	III_1	III_2	III_3	IV_1	IV_2	V_1	V_2	V_3
No. of plants assessed	480	360	440	190	120	100	90	92	75	68	59	60	65	70	53	40
No. of chlorophyll mutations	111	95	147	57	13	12	9	9	13	13	13	11	4	6	5	2
Observed chlorophy frequency	0.23	0.26	0.33	0.3	0.1	0.12	0.1	0.1	0.2	0.16	0.22	0.18	0.06	0.1	0.1	0.05

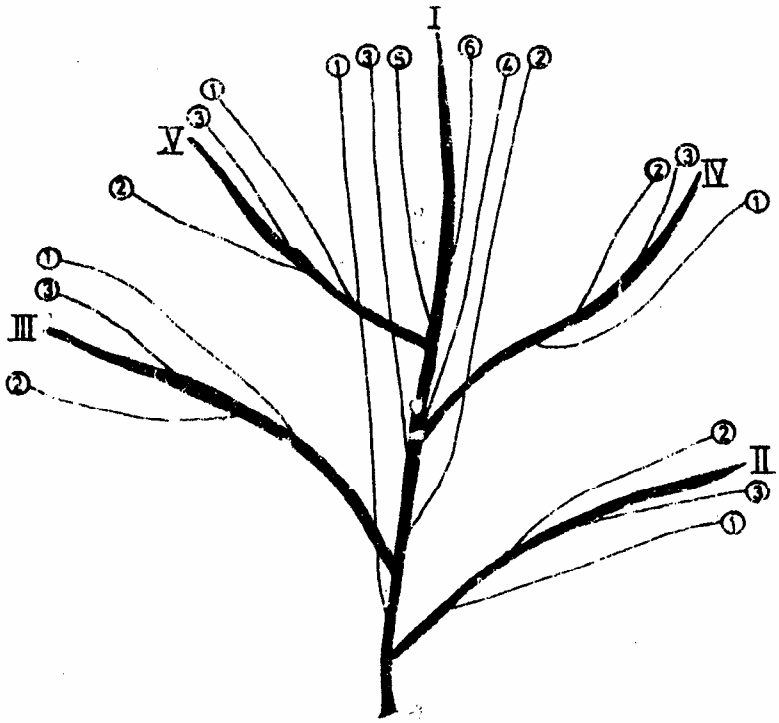

Fig. 1: The system used for labeling branch and peduncle position on M_1 cowpea plants.

has the highest probability of expressing mutation and hence more probable site for recovering mutations. This is in agreement with the findings of Saccardo (1981) on *Capsicum annum*, where he discovered that the first appearing fruits carried higher mutation frequencies than the later appearing ones. However, from chi-square test, the tabulated chi-square value (25), is greater than the calculated value (0.68). This implies that there is no significant differences in the distribution of mutations among peduncles of cowpea plant, though the higher frequencies observed for peduncles originating from the main stem may confer selection advantage on them.

References

Brock, R.D. (1971). The role of induced mutations in plant improvement. *Ibid.* 11: 181 – 196.

Brock, R.D. (1976). Mutation plant breeding for seed protein improvement. Seed Protein Improvement in Cereals and Grain Legumes. Vol. 1, p. 43 – 55, IAEA, Vienna.

Brock, R.D. and Micke, A. (1979). Economic aspects of using induced mutation in plant breeding. In: "Induced Mutations for Crop Improvement in Africa". TECDOC, Series No. 222: 19 – 32, IAEA, Vienna.

Chakrabarti, S.N. and Sen, S. (1976). Indian Journal of Genetics and Plant Breeding. Vol. 36, 2: 162 – 259.

Davies, D.R. (1981). Chimerism in Irradiated Dicotyledonous plants. In: "Chimerism in Irradiated Dicotyledonous plants." TEC, DOC Series, No. 289: 13 – 17, IAEA, Vienna.

Dellaert, L.M.W. (1981). The use of induced mutations in improvement of seed propagated crops. In: "Induced variability in plant breeding". Proc. International Symposium, Wageningen, Pudoc. Pg. 14 – 18.

FAO/IAEA (2000). Effect of spikelet position on rice anther culture efficiency. Plant Breeding and Genetics Newsletter, IAEA, Vienna, 5: 17.

Gaul, H. (1959). Studies on diplontic selection after X-irradiation of barley seeds. In: "Effect of Ionizing Radiations on Seeds". Pg. 117 – 138, IAEA, Vienna.

Gustafsson, A. (1940). The mutation system of the chlorophyll apparatus, Lunds Universitet Arsskrift NF Avd 2, Vol. 36, 11: 1 – 40.

Hermelin, T.; Brunner, H.S.; Daskalov, H. and Nakai, H. (1981). Chimerism in M_1 Plants of *Vicia faba*, *Capsicum annum* and *Linum usitatissimum*. In: "Chimerism in Irradiated Dicotyledonous Plants". TEC.DOC. Series No. 289: 35 – 42, IAEA, Vienna.

Holm, G. (1954). Chlorophyll Mutations in Barley. Acta Agric. Scand. 4: 457 – 471.

IAEA (1977). Manual on mutation breeding. Second edition, Technical Report Series No. 119. IAEA, Vienna.

Kundu, S.K. and Singh, D.P. (1982). Gamma ray induced variability for quantitative characters in black bean plant. Breeding Abstract, vol. 52(1).

Micke, A. and Domini, B. (1982). Use of induced mutations in improvement of seed propagated crops. In: "Induced Variability in Plant Breeding". Proc. Eucarpia Symp. Wageningen, Netherlands, p. 2 – 9.

Monti, L.M. (1965). Chimera formation in Pea plants raised from mutagen treated seeds. Proc. Symp. On the mutational process, Prague, p. 217 – 222.

Muller, A.J. (1965). The Chimeral structure of M_1 plants and its bearing on the determination of mutation frequencies in *Arabidopsis*. Induction of Mutations and Mutation Process, Praha, p. 46 – 52.

Osone, K. (1963). Studies on the developmental mechanism of mutated cells induced in Irradiated rice seeds. Japan journal of breeding, 13: 1 – 13.

Saccardo, F. (1980). Pea improvement by pollen irradiation. In: "Induced Mutations for Improvement of Grain Legume Production". TEC. DOC. Ser. No. 234: 113 – 115, IAEA, Vienna.

Saccardo, F. (1981). Gametophyte Irradiations in some dicotyledonous crop species. In: "Chimerism in Dicotyledonous Crop Plants". TEC.DOC. 289 Series No. 289: 31 – 32, IAEA, Vienna.

Saccardo, F. (1988). Gamete Irradiation for gene and chromosome mutations in higher plants. In: "Improvement of Grain Legume Production Using Induced Mutations." IAEA, Vienna, pg. 111 – 123.

Maluszynski, M.B.; Ahloowalia, B.; Ashri, A.; Nichterlein, K. and Zanten, L. (1999). Induced mutations in rice breeding and germplasm enhancement. In: "Proceedings of the 19th session of the International rice commission. FAO, Rome, pp. 194 – 204.

Stewart, R.N. and Dermen, H. (1970). Determination of number of mitotic activity of shoot apical initial cells by analysis of mericlinal chimeras. Amer. J. of Botany, 57: 816 – 826.

Subramanian, D. (1980). Effect of Gamma radiation in Vigna. Indian Journal of Genetics and Plant Breeding. Vol. 40, 1: 187 – 193.