Induced Macromutations in Tomatillo (Physalis ixocarpa Brot).

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Abstract: This experiment was conducted during the two summer seasons; 2008 and 2009. For raising M_1 generation seeds of two Tomatillo cultivars were exposed to six γ -ray treatments; 0, 2, 4, 6, 8 and 10 Kr. The harvested M₁ seeds of each treatment were bulked and used to produce M₂ generation. The seedlings of M₂ progenies were screened for detecting and assessment of chlorophyll mutations. As well, M₂ populations were overlooked to detect the morphological macromutations. The obtained results suggested that, among M_{γ} -seedlings, γ -ray treatments induced four different types of chlorophyll mutation within each tomatillo cultivar. The chlorophyll mutation frequency was relatively higher in 'Cisneros' than in 'Solo' indicating that the former cultivar relatively showed more mutable sensitivity. Xantha chlorophyll mutation recorded the highest frequency followed by albina, chlorina and variegated. The highest chlorophyll mutation frequency in both cultivars was obtained with 10 Kr. Morphological mutation occurance frequency differed according to the genotype, the applied dose and the mutation type. Where, 'Cisneros' tomatillo cultivar scored the highest frequency followed by 'Solo', while, the late-flowering mutant scored the lowest frequency. The trifurcate stem mutant was completely absent in 'Solo' and therefore the number of detected mutant types was nine in 'Cisneros' against eight types in 'Solo'. Giant mutants were observed in the progenies of both treated cultivars within the progenies of 6, 8 and 10 Kr in 'Cisneros' and within 4, 8 and 10 Kr in 'Solo'. Four floral mutants were isolated from M₂ generation. These floral mutants showed four different floral structural deviants; open bud, split corolla, polymorphic flower and grandiflora mutants. Moreover, γ-ray treated progenies in M₂ generation showed some deviants in fruit shape. These deviants include; long, grooved, obconical and large sized fruits.

Key words: Tomatillo, Physalis ixocarpa, Gamma ray, macromutations.

INTRODUCTION

Tomatillo *Physalis ixocarpa*. Brot. commonly known as the husk tomato is a member of Solanaceae family. It belongs to the genus *Physalis* with approximately 80 species cultivated in Mexico and Guatemala and originating from Mesoamerica (Menzel 1951 and Bukasov 1963). Generally, it is used as a remedy for abscesses, coughs, fevers and sore throats; a decoction is used in the treatment of high blood pressure, the fruits act as a laxative and a diuretic, an extract of leaves showed antibiotic activity against Staphylococcus.

The authors are aware that no comprehensive studies were performed by means of mutation breeding on tomatillo. So an attempt was made through this study for inducing mutation in tomatillo. It is known that induced mutation program is the choice of isolating an effective and efficient mutagen will certainly increase the possibility of recovering benefit mutants in the M_2 generation. Among different mutagenic agent gamma irradiation had been used successfully with several plant species for scoring such goals. Various chlorophyll mutants have been isolated by Mahana and Singh (1982) on tomatillo, Rao *et al.* (1991), Kumar *et al.* (2001) on pepper and Sangsiri (2005) on mungbean. Moreover, many morphological mutants were induced after gamma ray treatments by Mahana and Singh (1975), Mahana and Singh (1982), Padmavathi (2005) on tomatillo, Rao *et al.* (1991), Shafik (1992), Kumar *et al.* (2001), Tomlekova (2007) on pepper, Sngsiri *et al.* (2005) on mungbean, and Saito *et al.* (2009) on tomato.

The present work was carried out aiming to study the induced variation in M_2 generation of two tomatillo cultivars by using gamma ray. Measuring the frequency and spectrum of both chlorophyll and morphological mutations recoverable in M_2 generation. The mean performance of the selected mutant plants that showed realized deviants in their characteristics was estimated.

MATERIALS AND METHODS

Seeds representing two tomatillo cultivars namely; 'Cisneros' and 'Solo' were secured through the Courtesy of Vegetables Research Institute Dokki, Giza. Irradiation was performed at the Middle Eastern Regional Radioisotope Center for the Arab Countries, Dokki, Giza by using Co₆₀ source. The adopted treatments were 2, 4, 6, 8, 10 Kr in addition to control treatment.

For raising M_1 -generation 150 viable dried seeds from each cultivar were exposed to the adopted γ -ray treatments; **0**, **2**, **4**, **6**, **8 and 10 Kr.** Seed were sown in plastic trays at the rate of one seed per cell on 13th March 2008. Plantlets aged 30 days were transplanted in the field on 13th April 2008. The trial layout was **R**andomized Complete Block Design with three replicates; each experimental plot comprised eight treatments for each cultivar.

Bulked seeds representing the progenies of each M₁ irradiated plants were sown on 15th March 2009 for raising M₂-generation. Chlorophyll mutation assessment was done within 10-20 days from sowing. Seedlings were screened and examined to sort out chlorophyll mutations. Normal plants were counted and the chlorophyll mutants were detected and classified according to the system described by Gustafsson (1940). Chlorophyll mutations frequencies were calculated according to the formula suggested by Gaul (1960).

During the growing season, the treated progenies were carefully examined for detecting the morphological macromutations. The selected macromutants candidates were phenotyped morphologically based on the following categories: stem and branches habit, yielding ability, flower structure and fruit shape. The macromutantion frequency was estimated according to Gaul's formula (1960) previously mentioned. Data were recorded on all selected macromutant plants for their given trait(s) that typified it beside their fruit yield/plant (g).

RESULTS AND DISCUISSION

Chlorophyll Mutation Frequency and Description:

During the life span M² generation, various numbers of individuals bearing abnormal characters appeared within the M2 treated progenies of each investigated cultivar. These plants were considered off types with qualitative that showed sharp deviation in macromutations. Macromutants were either distinguished in the seedling stage and those were referred as chlorophyII mutants or at flowering and mature stage and they were referred as morphological mutantes.

The detected chlorophyll mutant types were; **albina** that showed cotyledons and leaves formed without chlorophyll, this type of mutation is lethal. Albina seedling survived for less than 2 weeks after germination. The second type was **Xantha** that have orange yellow to light yellowish leaves and survived for only 2-3 weeks after germination. **Variegated** showed variegated yellow –green leaves and finally **Chlorine** which showed pale green yellowish colored leaves that may turned to normal green leaves during short period.

The frequency and spectrum of chlorophyll mutations of the two tomatillo cultivars are given in Table (1) and Figure (1). Data revealed that the total mutation frequency was relatively higher in 'Cisneros' 1.208% than in 'Solo' 1.0% indicating that the former cultivar relatively showed more mutable sensitivity. The highest chlorophyll mutation frequency in the two cultivars was obtained at 10 Kr where it amounted to 0.405 and 0.316% for 'Cisneros' and 'Solo' cultivars, respectively.

Results presented in Table (1) demonstrated that, among M_2 -seedlings, γ -ray treatments induced four different types of chlorophyll mutations within each tomatillo cultivar. Two of these mutants were lethal; albina and xantha and two were viable; chlorina and varigata. The total frequencies of the lethal types were 1.025% (0.409% + 0.616%) for cv. 'Cisnerose' and 0.874% (0.316% + 0.558%) in 'Solo'. The viable types were remarkably less frequent than the lethal ones, being; 0.183% (0.117% + 0.066%), 0.126% (0.084% + 0.042%) for 'Cisneros' and 'Solo' tomatillo cultivars, respectively. Gamma rays induced different types of chlorophyll mutations in M_2 were reported by Mahana (1982) on *physalis ixocarpa* and Kumar (2000) on pepeer.

Xantha type recorded the highest frequency followed by albina, chlorina and variegated ranked in descending order within the treated progenies of the two cultivars, (Table, 1). In this respect, the total chlorophyll mutation frequencies were 0.409%, 0.616%, 0.117% and 0.066% for 'Cisneros' and 0.316%, 0.558%, 0.084% and 0.042% for 'Solo'. The frequency of chlorophyll mutation recoverable in M₂ generation may be taken as a good indicator for the frequency of mutations with less expression and it could be the most reliable measure for evaluating the mutagenically induced genetic alterations (Mesken and Van der Veen, 1968 and Nssar *et al.* 1994).

The present results proved that gamma ray treatment is efficient for inducing high frequency and wide spectrum of chlorophyll mutations. Some of these mutants can be used in fundamental biological research as

they have good marker genes which find expression during early growth stages. Similar chlorophyll mutations were isolated by many investigators (Mahana (1982) on *Physalis ixocarpa*, Rao *et al.* (1991) and Shafik (1992) on pepper, Sangsiri *et al.* (2005) on mung bean). Kumar *et al.* (2000) reported that, chlorrina, xantha and Albina appeared more frequently relative to other chlorophyll mutations. Inheritance of these mutations in different generations revealed that each of them was monogenic and recessive in nature.



Fig. 1: a photograph of chlorophyll mutation types, the normal seedling (A), albina (B), chlorine (C), variegated (D), xantha (E).

Table 1: frequencies and spectrum of chlorophyll mutation in M₂ generation of two tomatillo cultivars treated with gamma rays.

Dose Kr.	Seedlings Number		Total %			
		Albina	Xantha	Chlorina	Varigated	
			'Cisneros'		-	
0	592	0.00	0.00	0.00	0.00	0.00
2	572	0.056	0.113	0.000	0.023	0.192
4	520	0.072	0.079	0.000	0.000	0.151
6	440	0.000	0.077	0.011	0.017	0.105
8	320	0.121	0.168	0.040	0.026	0.355
10	230	0.160	0.179	0.066	0.000	0.405
Total	2674	0.409	0.616	0.117	0.066	1.208
			'Solo'			
0	560	0.000	0.000	0.000	0.000	0.000
2	580	0.041	0.044	0.000	0.000	0.085
4	500	0.000	0.12	0.057	0.015	0.192
6	464	0.061	0.099	0.000	0.000	0.16
8	360	0.076	0.144	0.000	0.027	0.247
10	270	0.138	0.151	0.027	0.000	0.316
Total	2734	0.316	0.558	0.084	0.042	1.000

Morphological Macromutations:

The obtained morphological mutations were classified into nine different types. These mutant types could be classified according to their deviants in plant height, developing number of branches, flowering date, stem habit, flowers and fruits shape. The various types of macromutions that gained in the M₂ generation and their frequencies at different gamma-ray doses are presented in Table (2) and Fig (2). It is evident that morphological mutation frequency differed according to the cultivar (genotype), the applied mutagen dose and the mutation type. Concerning the first variable, 'Cisneros' tomatillo cultivar scored the highest frequency 1.211% then 'Solo' 0.921%. These results substantiate again the higher sensitivity of 'Cisneros' for the mutagen. As to the mutation type, the giant mutant scored the highest frequencies in both cultivars being 0.316% and 0.225% for 'Cisneros' and 'Solo', respectively. Meanwhile, the late-flowering mutant scored the lowest frequency being; 0.077% and 0.072%, respectively. Trifurcate stem mutant was completely absent in 'Solo' and therefore the number of mutant types was nine in 'Cisneros' against eight types in 'Solo'. This proves that 'Solo' tomatillo cultivar was more mutable sensitivity.

Mean Performance and the Descriptions of Different Selected Macromutants: Giant Mutants:

This type was observed in both cultivars with 6, 8 and 10 Kr in 'Cisneros' and with 4, 8 and 10 Kr in 'Solo' (Table, 2). The main stem and branches grew much taller than the control. The height of giant plants in 'Cisneros' ranged between 250.6 and 270.3 cm with an average of 260.4 compared with an average of 157.2

Table 2: frequencies of macromutations in M₂ generation of two tomatillo cultivars treated by Gamma rays.

Dose Kr.	Plants number	Macromutation frequency %							Total %
		Ts.	D.	G.	HB.	EF.	LF.	S.	
				'Cisneros	,				
0.0	565	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	493	0.000	0.064	0.000	0.000	0.078	0.000	0.000	0.142
4	437	0.063	0.050	0.000	0.102	0.000	0.037	0.000	0.252
6	377	0.000	0.000	0.081	0.045	0.000	0.041	0.000	0.167
8	245	0.000	0.000	0.165	0.067	0.000	0.000	0.092	0.324
10	180	0.022	0.047	0.069	0.000	0.125	0.000	0.063	0.326
Total		0.085	0.161	0.315	0.214	0.203	0.078	0.155	1.211
				'Solo'					
0.0	553	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	524	0.000	0.057	0.000	0.000	0.000	0.000	0.000	0.057
4	485	0.000	0.038	0.027	0.079	0.043	0.000	0.02	0.207
6	391	0.000	0.000	0.000	0.067	0.044	0.025	0.000	0.136
8	270	0.000	0.000	0.12	0.000	0.000	0.000	0.083	0.203
10	192	0.000	0.000	0.078	0.052	0.097	0.047	0.044	0.318
Total		0.000	0.095	0.225	0.198	0.184	0.072	0.147	0.921

Details; Trifurcate stem (Ts.), Dwarf (D.), Giant (G.), Heavy branching / high yield (H.B.), Early flowering (E.F.), Late flowering (L.F.), Sterile (S.).

cm for the control (Fig, 2C). In case of 'Solo' the height of giant mutant ranged between 244.2 and 256.8 cm compared with an average of 138.3 cm for the control. This mutation exhibited the highest frequency in relation to other macromutants in the cultivars, being 0.315% and 0.225% for 'Cisneros' and 'Solo' tomatillo cultivars, respectively (Table, 2). Kumar *et al.* (2001) classified the morphological mutants according to plant height as stunted, dwarf, semi-dwarf and tall on *Capsicum annuum*, Shafik (1992) on *Capsicum annuum*. Similar types of mutants were obtained by Al- Rubeai (1982), El-Sgai (1993) on *Phaseolus vulgaris* Harb (1981), and Nofal and Mohamed (1989) on many legume crops.

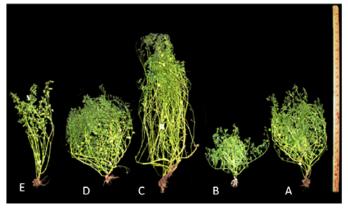


Fig. 2: a photograph of normal tomatillo plant (A), dwarf (B), giant (C), heavy branching and high yield (D), sterile (E), in M₂ generation of 'Cisneros' cultivar.

Dwarf Mutants:

This type showed shortened stem and branches. The majority of these plants showed normal flowers and fruits where they have smaller number of fruits. These mutants were observed with 2, 4 and 10 Kr in 'Cisneros' and with 2, 4 Kr in 'Solo'. Plant height of dwarf plants ranged between 55.8-67.7 cm in 'Cisneros' with an average of 61.7 cm, while the average height of the control was 157.2 cm. The corresponding range values for 'Solo' were 47.4 to 53.6 cm with an average of 50.5 cm compared with 138.3 cm for 'Solo' (Fig, 2B). It is worthy to mention that, the frequencies of this mutant type were 0.161% for 'Cisneros' and 0.095% for 'Solo'. Comparable dwarf mutant types were obtained by utilizing various γ -ray doses in different crop plants; Shafik (1992), Kumar *et al.* (2001) and Honda (2006) on pepper. Saito *et al.* (2009) on tomato recorded that gamma ray induced many morphological deviants attributes among them dwarf plants.

Heavy Branching and High Yielding Mutants:

This mutant type was easily demonstrated among the M₂ plants by having abnormally higher number of branches. The plants were taller mainly due to the outstanding increase in length of their branches. The stem

and branches were thicker and carrying high number of leaves and fruits compared to their controls (Fig, 2D). This mutant type was occurred with relatively high frequency in both cultivars being; 0.214 and 0.198% for 'Cisneros' and 'Solo', respectively (Table, 2).

It is realized that most of the obtained heavy branching/high yield mutants were selected within plants of the irradiated progenies by 4, 6 and 8 Kr in 'Cisneros' and by 4, 6 and 10 Kr in 'Solo'. The average number of branches of 'Cisneros' cultivar ranged between 19.7 to 22.6 with an average amounted to 21.2 as compared with 7.71 branches for their respective control. In case of 'Solo' mutants the number branches ranged between 16.8 to 19.7 with an average of 18.3 as compared with an average of 7.66 branches for the 'Solo' cultivar. Moreover, the average number of fruits in 'Cisneros' ranged between 324.6 to 378.6 fruits compared with 143.7 fruits for the original 'Cisneros' cultivar. The corresponding recorded number of fruits/plant for 'Solo' ranged between 286.9 to 315.7 fruits as compared with an average of 135.5 fruits for the control. Moreover, plants of the heavy branching/high yielding mutant type exhibited high averages of fruit yield/plant. Since the average fruit yield per plant were 3544.6 g as compared with 1422.4 g for 'Cisneros' cultivar, and 2992.4 g compared with 1327.8 g for 'Solo' cultivar. Similar heavy branches high yielding mutants were reported in M₂ generation after γ-ray treatments in many solanaceae plants, Bansal (1973), Shafik (1992) in *Capsicum*, Mahna and Rashmi Garg (1989) in *Petunia*. Many resembling types were detected by several researchers in many legume i.e., Sanaev and Ob'edkina (1977), Nofal and Mohamed (1989) and El-Sgai (1993).

Early Flowering Mutants:

Plants of this type were selected within the progenies of 2 and 10 Kr γ -ray treatments in 'Cisneros' and with 4, 6 and 10 Kr in 'Solo'. The frequencies of this mutant type were 0.203% for 'Cisneros' and 0.184% for 'Solo' (Table, 2). It is obvious that number of days elapsed from sowing till the appearance of the first flower in the mutant plants ranged between 40 to 44 days in 'Cisneros' and 46 to 48 days in 'Solo'. The corresponding recorded values of the controls were 50 and 55 days in the two cultivars. Therefore, the early flowering mutants showed earliness averaged 6 to 10 days compared with their respective controls. Earliness of flowering and fruit setting of *C. annum* in M_2 were reported by Hanumanthappa (1974), Daskalov (1975), Zeerak (1990) and Shafik (1992). While Esipova and Veselovskij (1965) reported different early ripening mutants in tomato varieties treated by γ -ray.

Late Flowering Mutants:

Plants of such mutant type showed normal morphological performance during the vegetative growth period. These mutants were observed in M_2 generation among treated progenies of 4 and 6 Kr in 'Cisneros' and 6 and 10 Kr in 'Solo' this mutant represents the lowest frequency among the selected macromutants in both cultivars, being 0.078% in 'Cisneros' and 0.072% in 'Solo'. The mutant plants showed the first flower after 60 to 62 days, and 63 to 65 days for 'Cisneros' and 'Solo', respectively. The corresponding number of days for control plants was 50 and 55 days. Therefore, these mutants were delayed by 10 to 13 days in the two cultivars. The late flowering mutants were reported by many previous investigators among them Shafik (1992) and Kumar (2001) on pepper and Saito *et al.* (2009) on tomato.

Sterile Mutants:

This type of mutant was observed among the treated progenies of 8 and 10 Kr in 'Cisneros' and 4, 8 and 10 Kr in 'Solo'. Relative to the normal tomatillo plants, the plants of such mutant carried remarkable lower number of branches with lower number of leaves and flowers without fruit set formation (Fig, 2F). The frequency of this mutant was moderate being; 0.155 for 'Cisneros' and 0.147% for 'Solo' Table (2). Rao and Suvartha (2006) reported many male sterile mutants in M_2 generation after 30-70 Kr γ -ray treatments in tomato, Shafik (1992) in pepper, El-Sgai (1993) in *Phaseolus vulgaris*.

Trifurcate Stem Mutants:

This mutant was observed in 'Cisneros' among treated progenies of 4 and 10 Kr with frequency of 0.085% (Table, 2). The normal stem plants is bifurcation where the stems deviated to two branches but stem of mutant plants was trifurcate at first forking with swollen nodes, which gave characteristic appearance to the mutant (Fig, 3B). Similar mutation was detected by Mahana and Singh (1982) on *Physalis ixocarpa* and Matsukura *et al.* (2007) on tomato.

Floral Mutations:

Four floral macromutants were isolated from M_2 generation of *Physalis ixocarpa* Brot. These mutants were classified to four different structural deviants; open bud mutant, split corolla mutant, polymorphic flower mutant and grandiflora mutant. These mutants were observed in 'Cisneros' among treated progenies of 2, 6, 8 and 10

Kr and in 'Solo' among 4, 8 and 10 Kr. A brief description for the different floral macromutant types are

given below:-



Fig. 3: a photograph of normal tomatillo plant (A) the abnormal branching mutant one, (B) in M₂ generation of 'Cisneros' cultivar.

Open Bud Mutant:

This mutant type showed early opened buds with early style projecting from the bud and corolla showed slow rate of growth with a very short tube. Stamens which in normal flowers occur in the center and surrounded the ovary closely were placed wide apart with exposed ovary projecting above all the floral parts. After pollination the fruit developed rapidly and since the ovary was protruding, the enlarged calyx failed to enclose it. The calyx occurred as a cap-like structure at the base of fruit giving curious look to the mutant as compared to the control in which the fruit was completely enclosed by the enlarged calyx. Fig (4B). The abnormalities in flower structure may show an important value in breeding behavior of the plant. Open bud mutant in tomatillo was previously reported by Mahana and Singh (1975), Mahana and Singh (1982) on tomatillo.

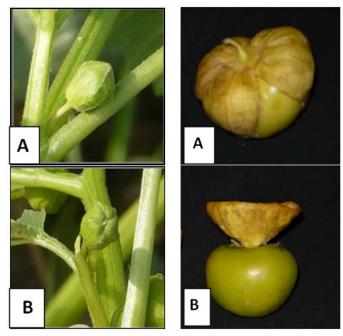


Fig. 4: a photograph of normal tomatillo plant (A) the abnormal open bud (B) in M₂ generation of 'Cisneros' cultivar.

Polymorphic Flower Mutant:

The polymorphic flowers showed six to seven petals while the normal tomatillo flowers have five petals (Fig, 5). I was reported that this type of mutants was monogenic and recessive in nature with genetic importance in the study of the inheritance of such trait. So that, the seeds of such mutant plant were collected for further investigations. The abnormalities in floral structure were early reported in *Physalis ixocarpa* Bort by Padmavathi (2005).

Split Corolla:

The corolla of this variant was apparently free from one another but united at the base giving a pseudopolypetalous appearance (Fig, 5). Split corolla mutant was reported in tomatillo plant by means of mutagens by Padmavathi (2005).

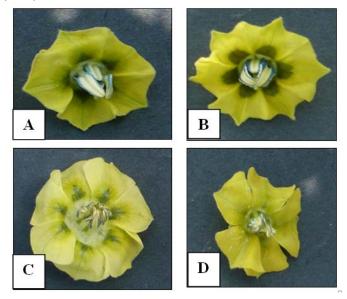


Fig. 5: a photograph of normal tomatillo flower (A) the abnormal polymorphic flowers, (B) six petals, (C) seven petals, (D) split corolla in M₂ generation of 'Cisneros' cultivar.

Grandiflora flower:

The plants of such mutant type were robust with profusely branched bearing large leaves and flowers, the flowers were dark yellow with distinct dark brown spots on the corolla throat, (Fig, 6B). The fruits and seed size of such mutants were slightly bigger compared to the control, Padmavathi (2005).



Fig. 6: a photograph of normal tomatillo flower (A) and the abnormal Grandiflora flower (B) in M₂ generation of 'Cisneros' cultivar.

Fruit Shape Mutants:

Gamma ray treated progenies in M_2 generation showed some deviants in fruit shape. These deviants include; long fruits, grooved fruits, obconical fruits and large sized fruits. These mutants were observed in 'Cisneros' among the treated progenies of 8 and 10 Kr and among 'Solo' in 6 and 8 Kr. Different fruit shape mutants were isolated after gamma ray treatments in M_2 generation by many researchers *i.e.*, Matsukura *et al.* (2007), Satio *et al.* (2009) in tomato and by Mahana and Singh (1982) in tomatillo. The description for the different fruit shape mutants (Fig. 7 and 8) are given below:-

Long Fruit:

The fruits of such mutant type were comparably large in size, oblong in shape since, fruit length was relatively longer than fruit width. The average oblong capitates fruit weight was 9.7 g. Which is comparably the same weight g control plant.

Grooved Fruit:

The grooved fruit mutants were isolated from 4 and 8 kr γ -ray treated progenies in both cultivars. These fruits are larger and heavier. Moreover, fruit surface topography characterized by five deep grooves. Similar grooved fruit type was early reported where ascorbic acid was estimated to be on the higher side, which is an added utility to its fruit value (Mahana and Singh 1974).

Obconical Fruit:

The obconical fruit mutants were isolated from 6 kr treated progeny in 'Cisneros'. The obconical fruits mainly characterized by pointed tips and showed comparatively the same control size and weight. It is worthy to mention that this mutant type was observed within M₂-progenies of the two cultivars.

Large Sized Fruit:

Many plants showed large sized fruits. These fruits were observed in the progenies of 2, 4 and 8 Kr, treated progenies of 'Cisneros' The muted plants exhibit large fruits with an average of 27.6 g as compared with 9.9 g for the control(Fig 8B). The average number of fruits per plant was relatively lower compared with the original cultivar.

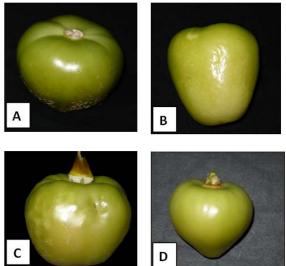


Fig. 7: a photograph of normal tomatillo fruit (A) control, (B) long fruit, (C) Grooved fruit and (D) Obconical fruit in M₂ generation of 'Cisneros' cultivar.

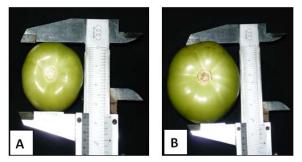


Fig. 8: a photograph of normal tomatillo fruit (A) and (B) Larg sized fruit in M₂ generation of 'Cisneros' cultivar.

During the life span of M_2 -generation, various numbers of individuals bearing abnormal characters appeared within the M_2 -treated progenies of each investigated cultivar. These plants were considered off types with qualitative nature that showed sharp deviation in their morphological given character (s), and therefore they were considered as macromutations. Macromutants were either distinguished in the seedling stage and those were referred as chlorophyll mutants or at flowering and mature stage and they were referred as morphological mutants.

It could be concluded that gamma-rays is an efficient tool for inducing high frequency of chlorophyll as well as morphological mutations. The absence of both mutation types in M_1 and their appearance in M_2 generation might be attributed to one of these two assumptions; 1) the induction of mutants, each of which was controlled by one or few number of recessive genes, in the M_1 and their segregation in a homozygous state in the M_2 . 2) the induction of mutants, in M_1 , each of which was governed by a number of genes, every gene had a small effect and the accumulation of such genes in one plant as a result of segregation in the second generation. It is worthmentioning to note that some of these mutants have undergone simultaneous changes in a number of characters. Such multiple phenotypic changes have often been observed following mutagenic treatments (Brock, 1965 and 1970; Emery *et al.*, 1965; and Gregory, 1968). It seems unlikely that, the mutation of a single gene could pleiotropically affect such diverse set of characters. More probably a number of genes has been mutated either within a linked gene complex or distributed more widely over the genome.

Detailed studies of the new induced morphological mutants in M_3 concerning their behavior, performance and their economic and/or breeding value will be considered. The results of these studies will be reported in other publication.

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